



Examining the relationship between vitamin D levels and *Helicobacter pylori* infection and its effect on the hypothalamic-pituitary-adrenal axis in dyspeptic patients

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ABSTRACT

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The purpose of this study was to determine whether or not *Helicobacter pylori* (*H. pylori*) infection affects the hypothalamic-pituitary-adrenal (HPA) axis and to evaluate the association between vitamin D levels with *H. pylori* infected and eradicated patients. The glucagon stimulation test (GST) was used to assess the HPA gland axis. An *H. pylori* infection was diagnosed based on the rapid urease test and histology. All infected patients received triple eradication therapy. Three months after the treatment, ¹⁴C urea breath test was reinstated, and GST was repeated in patients who were negative for *H. pylori*. Of the 43 patients, 20 (46%) were found to have a lower mean value of peak cortisol than normal responses to the GST in the *H. pylori*-infected subjects. In 29 subjects, *H. pylori* infection was successfully eradicated. Twelve (41%) out of 29 subjects were defined as having a blunted GST response and 17 (59%) subjects had normal HPA axis response to GST in the *H. pylori*-eradicated subjects. A significant correlation between low 25-hydroxyvitamin D₃ (25(OH)D₃) level and low peak cortisol response to GST was identified in the *H. pylori*-infected subjects. After *H. pylori* eradication, the positive correlation between 25(OH)D₃ level and high peak cortisol response to GST was also identified. Mean 25(OH)D₃ levels were lower in the *H. pylori*-infected subjects than in the *H. pylori*-eradicated subjects. Our results indicate an increased prevalence of blunted glucocorticoid response to GST in patients with infected *H. pylori*. Vitamin D deficiency is also common in the *H. pylori*-infected subjects and associated with blunted glucocorticoid response. These findings suggest that *H. pylori* eradication increases the cortisol response to GST. Vitamin D supports adrenal/cortisol production whereby a deficiency can result blunted glucocorticoid response to GST in patients with infected *H. pylori*.

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1. Introduction

Helicobacter pylori (*H. pylori*) is a type of the bacteria that chronically infects more than 50% of the human population (Montecucco and Rappuoli, 2001). The prevalence of *H. pylori* infection is 80% in adult population in Turkey (World Gastroenterology Organisation, 2011). *H. pylori* induces infiltration in the gastric mucosa and stimulates the secretion of inflammatory cytokines, such as interleukin IL-8, tumor necrosis factor (TNF)- α , IL-6, IL-1 β , IL-12, and interferon (IFN)- γ (Peek et al., 2010). The levels of these inflammatory cytokines decrease when *H. pylori* infection is eradicated

(Ando et al., 2006).

Recently, in one of the most interesting reports on vitamin D by Guo et al. (2014), has shown that vitamin D has antimicrobial effect against *H. pylori*. Guo et al. (2014) found that vitamin D plays an important role in gastric mucosa homeostasis and host protection from *H. pylori* infection. Besides, its effect on bone metabolism, vitamin D may reduce inflammatory markers such as CRP, TNF- α , IL-6 and IL-18 and the level of anti-inflammatory cytokine IL-10 may increase (Izquierdo et al., 2012).

Table 1. Comparison of infected patients and eradicated patients in terms of clinical and laboratory characteristics.

Parameter	Infected patients (n=43)	Eradicated patients (n=29)	p
Age (year)	30.58±8.79	31.0±9.28	0.75
BMI (kg/m ²)	25.2±4.2	25.1±4.2	0.9
Total cholesterol (mg/dl)	164.2±46.2	164.5±39.9	0.8
LDL-C (mg/dL)	105.6±33.7	104.5±32.9	0.6
Trygliceride (mg/dl)	111.7±62.1	103.3±42.1	0.4
HDL-C (mg/dL)	41.2±10.0	39.1±7.9	0.7
Vitamin D (ng/ml)	14.7±8.5	20.5±10.0	0.002
Basal cortisol (µg/dl)	12.8±5.1	11.9±4.8	0.1
ACTH (pg/ml)	20.6±11.5	19.3±11.9	0.04

BMI: Body mass index; **LDL-C:** Low-density lipoprotein cholesterol; **HDL-C:** High-density lipoprotein cholesterol; **ACTH:** Adrenocorticotropic hormone

Vitamin D in the endocrine system has significant immunomodulatory properties and impaired vitamin D action might contribute to an immune dysregulation, eventually leading to adrenocortical inhibition or insufficiency, i.e., Addison's disease (Pani et al., 2002). Evidence suggesting a relationship between vitamin D, stress, and cortisol is also limited, but in vitro evidence suggests vitamin D affects hypothalamic-pituitary-adrenal (HPA) axis functioning and glucocorticoid receptors in the hippocampus (Obradovic et al., 2006).

H. pylori is one of the most commonly studied infectious agents triggering an autoimmune response (Montecucco and Rappuoli, 2001). Cortisol, an endogenous glucocorticoid hormone produced by the adrenal gland, plays a role in host defense factors (Filaretova et al., 2004). Koşan et al. (2008) detected lower 24-h urine cortisol output (a marker of body cortisol levels) in cases with *H. pylori* colonization when compared to *H. pylori* negative patients. They also demonstrated that there is a negative association between endogenous cortisol levels and *H. pylori* colonization (Filaretova et al., 2004). To our best knowledge, there is no study published to examine the relationship of HPA-axis in patients infected with *H. pylori*.

In this context, it is interesting to speculate whether or not *H. pylori* infection affects the HPA axis. It is also of interest to evaluate the association between 25(OH)D₃ levels with *H. pylori* infected and eradicated patients, specifically examining its effect on the HPA axis.

2. Materials and methods

Subjects with dyspeptic symptoms who attended the gastroenterology outpatient clinic were included in the study. The exclusion criteria of the patients were as follows: Age younger than 18 and older than 45; subjects with hypertension, diabetes mellitus, known coronary artery disease, thyroid disease, cerebrovascular disease, renal or hepatic insufficiency, smoking, cancer, systemic or local infection, or prior history of gastric surgery; pregnant or lactating women; subjects on glucocorticoid or vitamin D therapy; and subjects who used supplemental vitamins or nonsteroidal anti-inflammatory drugs within the last 12 weeks prior to the study. Patients treated with antibiotic, bismuth salts, H₂ receptor blockers or proton-pump inhibitors in the previous three months were also excluded. We did not provide any drugs such as vitamin

D supplements or multivitamins to correct the vitamin D levels in *H. pylori* patients. *H. pylori* was considered present when both of the biopsy-based invasive test results, namely the histopathology test and the rapid urease test (RUT), were positive. Subjects who were positive for both RUT and histology were given eradication therapy for 14 days. Three months after the treatment, a ¹⁴C urea breath test (UBT) and the *H. pylori* stool antigen test (HpSA) were reinstated (when both of these tests were negative), and blood sampling was repeated in patients who were negative for *H. pylori*. This study took place from February to November 2014. Each subject provided written and informed consent, and research protocols were approved by the ethical committee of our institution.

All patients with dyspepsia infected with *H. pylori* took the study drugs (n=43). Six patients refused to participate in the glucagon stimulation test (GST) again, and eight patients were discarded because of ¹⁴C UBT and HpSA positivity. Twenty-nine patients completed the study. The HPA axis was studied before (n=43), and a comparable proportion of patients completed the study (n=29) three months after the treatment.

Endoscopy and histology

During the endoscopy, two antrum biopsies and one corpus biopsy for histological evaluation were obtained as well as one antrum biopsy and one corpus biopsy for the rapid urease test were obtained from each patient. The RUT was performed using the manufacturer's instructions (Ballard Medical Products, Draper, UT), and the results were interpreted at 1 hour and 24 hours after sampling. The biopsy specimens were processed for histopathological examination according to the standard procedure. Hematoxylin-eosin staining and a special staining for *H. pylori* (i.e., Giemsa staining) were performed. The same pathologist examined all the biopsy samples. If *H. pylori* infection was observed, the bacterial density was scored semi-quantitatively on an ordinal scale (ranging from 1 to 3) by the pathologist.

¹⁴C urea breath test

After overnight fasting, patients swallowed 37 kBq (1 µCi) of an encapsulated form of ¹⁴C-urea/citric acid composition (Helicap TM Noster System, Stockholm, Sweden) in 25 ml of water. Breath samples were collected with a special dry cartridge system (Heliprobe TM BreathCard TM; Noster System) after 10 min. Patients exhaled gently into the cartridge mouthpiece until the indicator membrane changed its color from orange to yellow. The breath card was inserted into a special small desktop Geiger-Müller counter (Heliprobe TM Analyzer; Noster System) and its activity was counted for 250 s. The results were expressed both as counts per min (cpm) and as grade (0, not infected, <25 cpm; 1, equivocal, 25-50 cpm; 2, infected, >50 cpm), as suggested by the manufacturer, according to the counts obtained from the cartridges.

Stool antigen tests

All subjects gave stool specimens six weeks after the end of the eradication therapy. Stool specimens were stored at -20 °C before analysis. The exclusion criteria of the stool samples were diarrhea, inadequate amount, and delayed delivery of

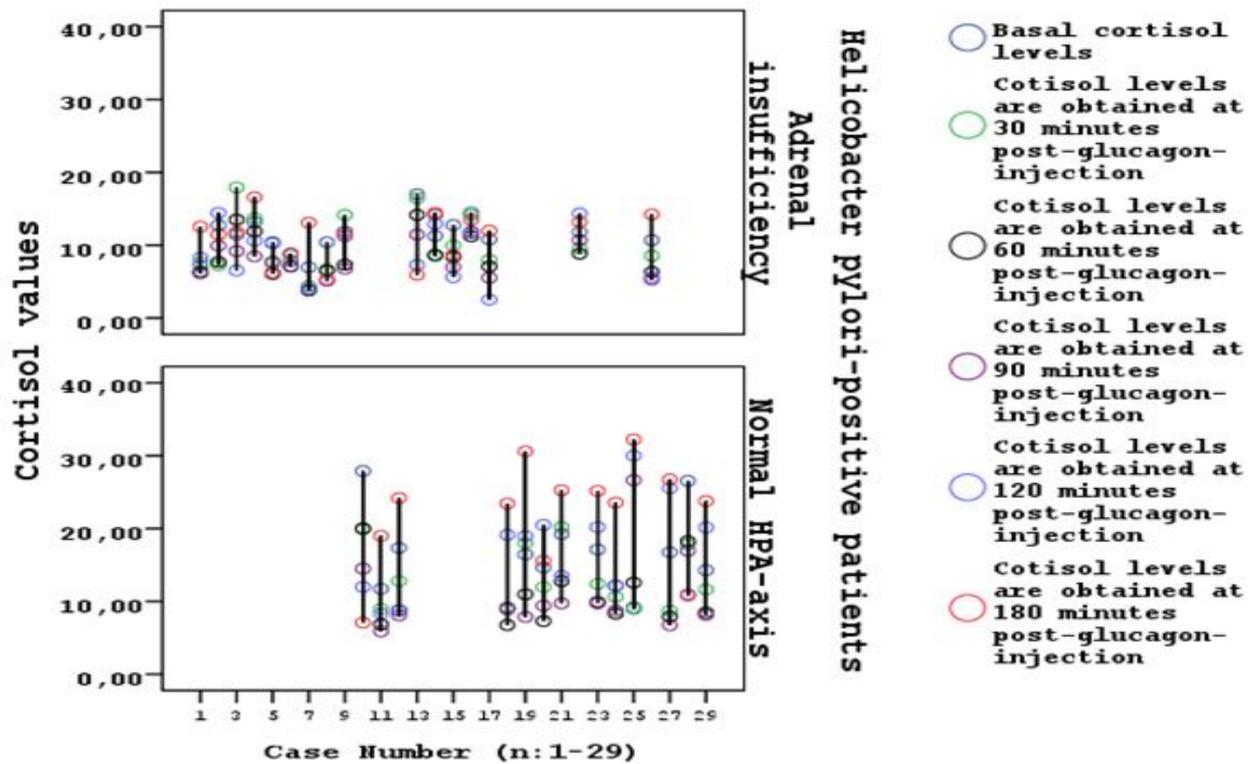


Fig. 1. The adrenal response to the glucagon stimulation test (GST) for evaluation of the hypothalamic-pituitary-adrenal (HPA) axis in *H. pylori* positive patients.

the samples after collection. The Genx *H. pylori* CARD Test (Genx Bioresearch GOSB Teknopark A.Ş. Gebze, Kocaeli, Turkey) is a rapid immunochromatographic assay test that uses a monoclonal anti-*H. pylori* antibody on a strip for the detection of *H. pylori* infections in stool specimens. The test was performed according to the manufacturer's instructions. The test result was read after 10 minutes. A positive test result was indicated by the appearance of a red band in the zone marked C (control line) and a red band in the zone marked T (result line). The sample was considered negative when only one red band appeared across the central window in the zone marked C. If no colored bands appeared or only one band appeared in the T zone, the result was regarded as invalid, and if an inconclusive result was obtained, the test was repeated with a new strip.

Glucagon stimulation test

The glucagon stimulation test and determination of basal cortisol levels were performed in all patients during the morning hours (8-9 am) when they were in fasting states. 43 *H. pylori* patients underwent GST (GlucaGen Hypokit, Novo Nordisk, Denmark; 1 mg or 1.5 mg i.m. in subjects >100 kg). Basal serum cortisol levels were determined before glucagon administration and after 30, 60, 90, 120, 150 and 180 min of glucagon administration (Bilgic et al., 2010).

Parameters of blunted cortisol responses to GST

Basal adrenal function was evaluated by the measurement of early morning levels of serum cortisol and plasma ACTH. According to the GST, impaired response is accepted at a peak cortisol level <18 µg/dL (500 nmol/L) (Inder and Hunt, 2002;

Bilgic et al., 2010; Guran et al., 2015). If the cut-off level was accepted as 9.5 µg/dL or 10.7 µg/dL the sensitivities of the glucagon stimulation test would be increased up to 98.1 and 94.5% respectively (Karaca et al., 2011).

Laboratory Assessment

Serum glucose, total cholesterol, LDL cholesterol, triglycerides, and HDL-cholesterol levels were measured using Randox enzymatic kits in the Roche-Hitachi Modular system. LDL cholesterol was calculated by the Friedewald equation method. Serum baseline 25(OH)D₃ levels were measured by the high-performance liquid chromatography (HPLC) method using an Agilent 1200 Liquid Chromatograph.

Eradication regimen

For a 14-day period, all infected patients received 40 mg pantoprazole twice-daily, 1 g amoxicillin twice daily, and 500 mg clarithromycin 500 mg twice daily.

Table 2. Comparison of pre and post eradication laboratory characteristics of the eradicated patients

Parameter	Before eradication (n=29)	After eradication (n=29)	P
Vitamin D (ng/ml)	15.1±8.9	20.5±10.0	0.007
Basal cortisol (µg/dl)	13.5±4.9	11.9±4.8	0.1
ACTH (pg/ml)	22.7±12.3	19.3±11.9	0.1
Low Adrenal response (<18 µg/dl)	16/29	12/29	0.1
Low Adrenal response (<10 µg/dl)	7/29	4/29	0.1

BMI: Body mass index; **LDL-C:** Low-density lipoprotein cholesterol; **HDL-C:** High-density lipoprotein cholesterol

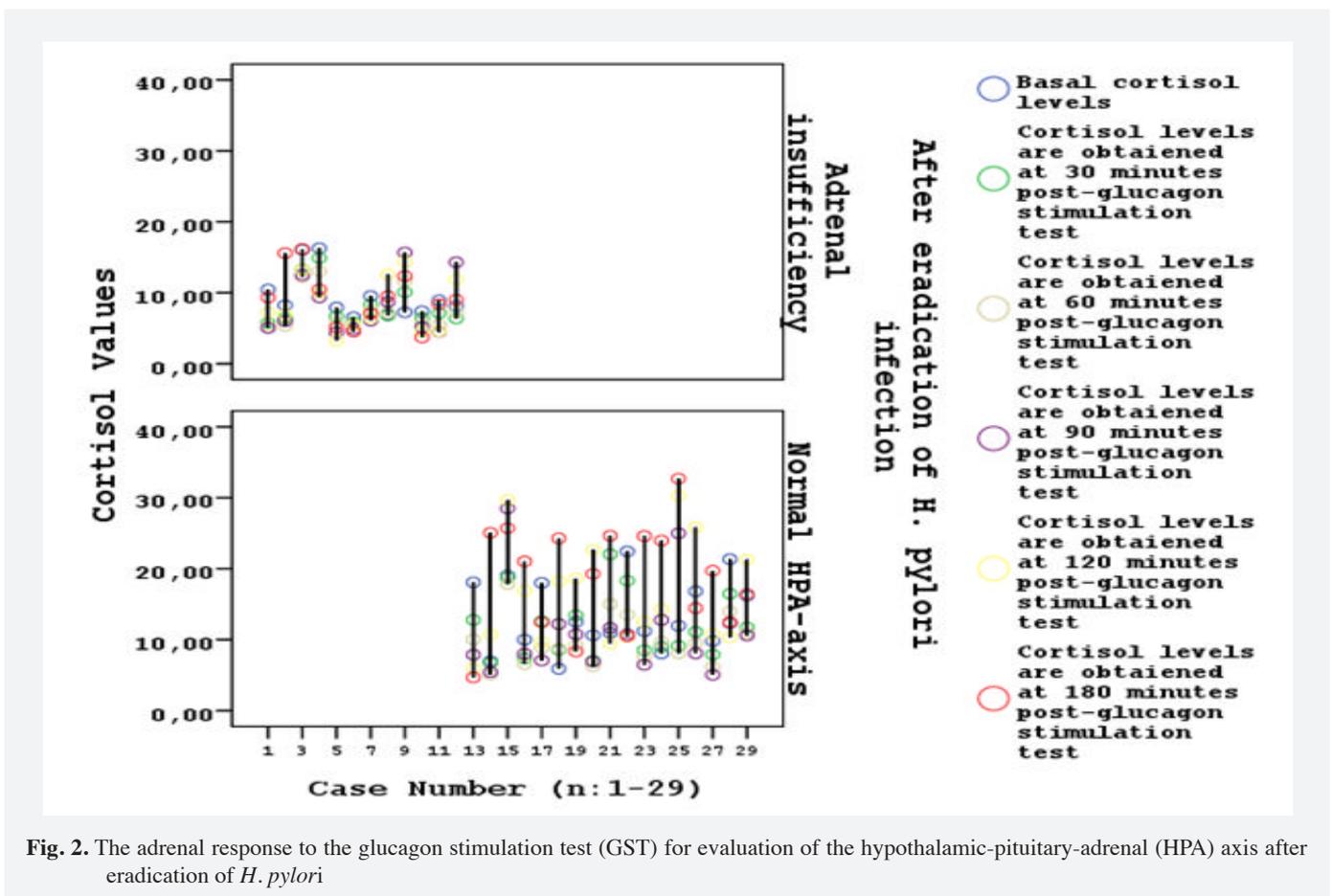


Fig. 2. The adrenal response to the glucagon stimulation test (GST) for evaluation of the hypothalamic-pituitary-adrenal (HPA) axis after eradication of *H. pylori*

Adrenal function tests

Serum cortisol concentrations were measured by chemiluminescent enzyme immunoassay (Immulate, Diagnostic Products Corp., Los Angeles, CA, USA) with an intra-assay coefficient variation (CV) of 5.7% and an interassay CV of 7.8%. Normal blood cortisol levels are 5-23 $\mu\text{g/dl}$ in our laboratory. Plasma ACTH concentrations were analyzed by a two-site sequential chemiluminescent immunoassay (Immulate, Diagnostic Products Corp., Los Angeles, CA, USA). The lower and upper limits of detection for the assays were 5 and 1.250 pg/ml, respectively. The ACTH assay had an intra-assay CV of 6.7-9.5% and an interassay CV of 6.1-10.0%.

Statistical analysis

The results are presented as mean \pm SD. The descriptive statistics were calculated using frequencies and percentages. The crosstabs and Chi-square test was used to compare the categorical parameters. The Spearman's rho correlation was used to determine the relationships between parameters. The Wilcoxon signed rank test was used for the comparison of treatment effects on variables. Statistical analysis was carried out with Statistical Package of Social Science (SPSS), version 15.0 (SPSS Inc., Chicago, IL). A p value of <0.05 was considered statistically significant.

3. Results

The study group consisted of 43 *H. pylori* infected patients (23 males [M]/20 females [F]). The mean age of the patients was 30.58 \pm 8.79 (18-54 yr). The mean age of the M and F was 30.58 \pm 8.79 (18-54 yr) and 30.35 \pm 9.18 (20-54 yr) (respectively; $p>0.05$). Table 1 summarizes the clinical and

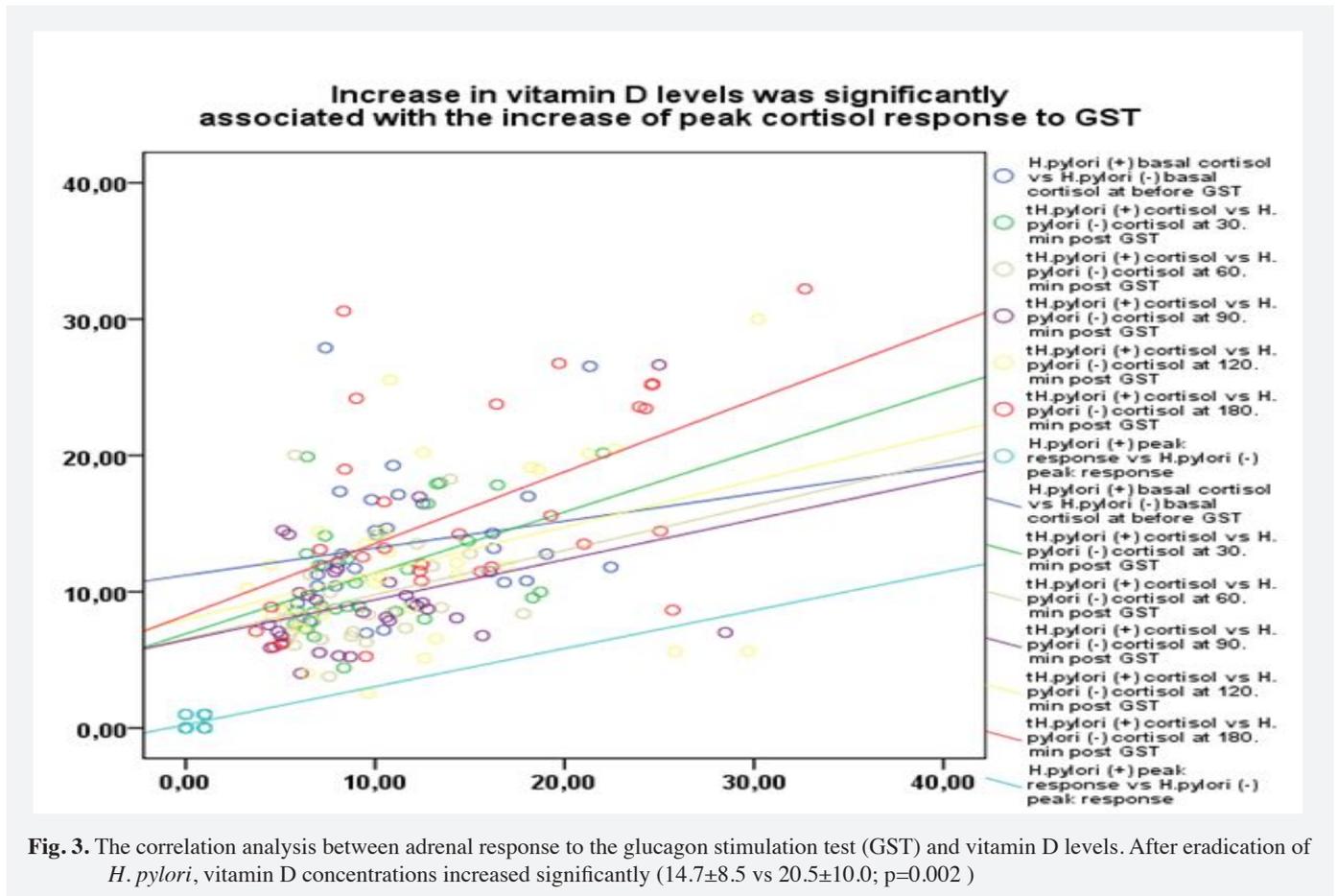
laboratory characteristics of the patients before ($n=43$) and after *H. pylori* ($n=29$) eradication. The endoscopic diagnosis of the patients ($n=43$) in the study included esophagitis in 1 (2.3%), gastric ulcer in 1 (2.3%), duodenal ulcer in 2 (4.6%), antrum and corpus gastritis in 32 (74.4%), pangastritis in 3 (6.9%), gastroduodenitis in 4 (9.3%).

Evaluation of hypothalamic-pituitary-adrenocortical axis in *H. pylori* positive patients

A glucagon stimulation test was performed on all patients and cortisol levels were determined. Of the 43 patients in the study, 20 (46%) were found to have a lower mean value of peak cortisol-with a cortisol cut-off value of 18 $\mu\text{g/dL}$ -than normal responses (54%) to the glucagon stimulation test (GST) in the *H. pylori*-infected subjects. Figure 1 shows the adrenal response to the GST for evaluation of HPA-axis in *H. pylori* positive patients ($n=29$; Fig. 1). Of the 29 subjects whose *H. pylori* infection was successfully eradicated, there were 16 patients with an impaired HPA axis and 13 patients were defined as having a normal HPA axis (Table 2). The sufficient cortisol responses (≥ 10 $\mu\text{g/dL}$) suggested by Karaca et al. (2011) were achieved in 22 of the 29 *H. pylori* (+) subjects during glucagon stimulation test (Table 2).

Correlations before eradication of *H. pylori*

Of the 43 patients, 20 (46%) were found to have a lower mean value of peak cortisol than normal responses to the GST in the *H. pylori*-infected subjects. Before eradication, Spearman's correlation analysis showed that adrenal response to the GST was significantly positively correlated with 25(OH) D_3 ($n=43$; $r=0.442$, $p=0.003$) and 25(OH) D_3 levels positively correlated with 120 m cortisol ($n=43$; $r=0.408$, $p=0.007$), and 180 m



cortisol ($n=43$; $r=0.414$, $p=0.006$). Before eradication, low adrenal response to GST ($n=20$) was significantly positively correlated with low $25(\text{OH})\text{D}_3$ levels ($n=20$; $r=0.427$, $p=0.021$). Spearman's correlation analysis before eradication showed that plasma low adrenal response to GST and lipid values were not correlated with variables in the group.

Evaluation of the HPA axis after eradication of *H. pylori* ($n=29$)

A glucagon stimulation test was performed on 29 patients and cortisol levels were determined after eradication of *H. pylori*. There were twelve patients (ten males, two females, Table 2, Fig. 2) with an impaired HPA axis. Before eradication, 13 patients (13/29) were defined as having a normal HPA axis, after eradication, 17 patients (17/29) were defined as having a normal HPA axis (Table 2, Fig. 2). The sufficient cortisol responses ($\geq 10 \mu\text{g/dL}$) suggested by Karaca et al. (2011) were achieved in 25 of the 29 *H. pylori* (+) subjects during glucagon stimulation test (Table 2).

Evaluation of the ACTH and $25(\text{OH})\text{D}_3$ levels after eradication of *H. pylori* ($n=29$)

Plasma ACTH concentrations decreased significantly after eradication (Table 1; 20.6 ± 11.5 ($n=43$) vs. 19.3 ± 11.9 ng/ml ($n=29$); $p=0.049$). Although ACTH levels showed a slight trend to be decreased in eradicated patients ($n=29$) than infected patients ($n=29$), the difference did not reach significance (22.7 ± 12.3 vs. 19.3 ± 11.9 , $p=0.1$). Following the eradication of *H. pylori*, $25(\text{OH})\text{D}_3$ concentrations increased significantly (Table 1; $n=43$, 14.7 ± 8.5 vs 20.5 ± 10.0 ; $p=0.002$). After eradication of *H. pylori* $25(\text{OH})\text{D}_3$ concentrations increased significantly ($n=29$; 15.1 ± 8.9 vs 20.5 ± 10.0 ; $p=0.002$).

Correlations after eradication of *H. pylori*

After eradication, Spearman's correlation analysis showed that the increased adrenal response to GST was correlated with high $25(\text{OH})\text{D}_3$ levels ($r=0.368$, $p=0.049$), and positively correlated with 0 m ($r=0.469$, $p=0.010$), 30 m ($r=0.519$, $p=0.004$), 60 m ($r=0.377$, $p=0.044$), 120 m ($r=0.536$, $p=0.003$), and 180 m cortisol ($r=0.628$, $p<0.001$).

Correlations after eradication of *H. pylori* between peak cortisol response to GST and $25(\text{OH})\text{D}_3$ levels

Increase in $25(\text{OH})\text{D}_3$ levels was significantly associated with the increase of peak cortisol response to GST in study group of eradicated subjects (Fig. 3). However, in the comparison, the lipid values were not different after eradication ($p>0.05$). No statistically significant difference was found between lipid levels in *H. pylori*-infected patients and eradicated subjects (Table 1).

4. Discussion

Our hypothesis was that an association between low $25(\text{OH})\text{D}_3$ levels and *H. pylori* infection with the disturbance of the HPA axis and inadequate cortisol production can lead to hemodynamic instability through impaired adrenal-glucocorticoid function. The effectiveness of the axis in patients with *H. pylori* infection with respect to cortisol secretion remains unclear. Our results showed that *H. pylori* positive patients may at high risk of subclinical cortisol deficiency. We assessed the HPA axis by a GST. Twenty of 43 (46%) patients had an impaired response to the GST. With the combination of these tests (cutoff limit for 500 nmol/L), only 23 subjects (54%) showed normal responses. After eradication of *H. pylori*, a GST was performed again

on 29 patients for which cortisol levels were determined. There were twelve patients (28%) with impaired HPA axes. Therefore, 17 patients (68%) were defined as having normal HPA axes. If the cut-off level was accepted as 9.5 µg/dL or 10.7 µg/dL the sensitivities of the glucagon stimulation test would be increased up to 98.1 and 94.5% respectively. The peak cortisol response of 10 µg/dL to glucagon stimulation was found to be appropriate as a lower cut-off in this study. The sufficient cortisol responses (≥ 10 µg/dL) suggested by Karaca et al. (2011) were achieved in 22 of the 29 *H. pylori* (+) subjects during glucagon stimulation test. After eradication, 25 of the 29 *H. pylori* (+) subjects were achieved (≥ 10 µg/dL) in during glucagon stimulation test. ACTH, is secreted to activate the HPA axis. Cortisol inhibits the secretion of ACTH (negative feedback) to normalize the HPA axis. When cortisol levels fall, ACTH levels normally rise. Therefore, generally, ACTH and cortisol are thought to be indicators of the condition of the HPA axis. An elevated ACTH level is consistent with primary adrenal insufficiency (Del Rey et al., 2007). In our study, plasma ACTH concentrations decreased significantly after eradication, which was consistent with adrenal insufficiency.

H. pylori is one of the most commonly studied infectious agents that is proposed as triggering an autoimmune response (Montecucco and Rappuoli, 2001). It is reported that *H. pylori* may be associated with diseases of organs other than the digestive system, including the blood system and idiopathic thrombocytopenic purpura (ITP), circulatory, renal, endocrine, nervous, and dermal systems (Gasbarrini, and Franceschi, 1999). *H. pylori* infection, which have direct cytotoxic and proinflammatory effects, may affects brain-gut axis (Alvarez-Arellano and Maldonado-Bernal, 2014). From this point, it is not surprising that *H. pylori* may affect the pituitary gland and HPA axis.

On the other hand, host defense mainly involves the action of the innate immune system via neutrophils and lymphocytes. Vitamin D stimulates the activation of innate immune system that functions through cells like macrophages and monocytes (Wang et al., 2004; Korf et al., 2014). With regard to infectious diseases, recent studies have demonstrated that vitamin D regulates the expression of specific antimicrobial peptides in immune cells (Wang et al., 2004; Guo et al., 2014; Korf et al., 2014) explaining at least in part the mechanisms underlying the anti-infectious properties of vitamin D. The study by Guo et al. (2014) has clearly shown that vitamin D has an effect on antimicrobial activity against *H. pylori*. A researcher also suggests that vitamin D may mediate positive glucocorticoid effects on cardiovascular disease (Ahmed, 2013). Vitamin D is an efficacious modulator of the deleterious cardiovascular responses induced by glucocorticoid excess (Ahmed, 2013). In our study, a significant correlation between low 25(OH)D₃ level and low peak cortisol response to GST was identified in the *H. pylori*-infected subjects. After *H. pylori* infection was successfully eradicated, a significant correlation between high 25(OH)D₃ level and increased adrenal response to GST were still identified. Mean 25(OH)D₃ levels were lower in the *H. pylori*-infected subjects compared to *H. pylori*-eradicated subjects. Plasma 25(OH)D₃ level that is below < 20 ng/ml is described as vitamin D deficiency (Holick et al., 2011). In the current study, we found vitamin D deficiency to be significantly more prevalent among *H. pylori* infected

patients compared to eradicated subjects. As expected, we found very low 25(OH)D₃ levels (14.7±8.5) in our clinical setting among *H. pylori* infected patients. 25(OH)D₃ levels increased simultaneously with rising peak cortisol responses to the GST in *H. pylori*-eradicated subjects. The enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD-1) synthesizes active cortisol from inactive cortisone, and controls local adipose tissue glucocorticoid concentrations and intracellular availability of glucocorticoids. In addition, Morris and Zemel (2005) demonstrated that 25(OH)D₃ upregulates 11β-HSD-1 expression and cortisol release in adipocytes and they emphasized that 25(OH)D₃ might have a potential role in cortisol synthesis.

One may speculate that this observation (i.e., a high percentage of blunted cortisol responses) may be a result of proton-pump inhibitors treatment. However, it has been reported that a 60 mg dose of omeprazole may have showed inhibition of both the basal levels and adrenocorticotropic hormone (ACTH)-stimulated levels (Dowie et al., 1988), lansoprazole 30 and 60 mg (Dammann et al., 1993) and rabeprazole 20 mg (Dammann et al., 1999) showed no effects of either drug on cortisol synthesis. In contrast, lansoprazole and rabeprazole increased plasma ACTH and cortisol levels (Katagiri et al., 2006). Thus, according to our study, pantoprazole may not affect either basal or ACTH-stimulated serum cortisol levels (Dammann et al., 1994).

Our findings corroborate the hypothesis that gastric ulceration, together with the enlargement of the adrenal glands, is indeed one of the key elements of Hans Selye's general adaptation syndrome model (1936), which maintains that stress is a major cause of disease because chronic stress causes long-term chemical changes (Murison and Milde, 2007). In adrenal hypertrophy, if one thinks there is no glucocorticoid deficiency or no ACTH increase as a response to stress, then further examination is required (Harvey and Sutcliffe, 2010).

The GST is a simple and safe test to perform. In addition, glucagon is relatively inexpensive. Studies demonstrate the comparability of the GST to the insulin tolerance test (ITT) in assessing the HPA axis (Kappy et al., 2006; di Iorgi et al., 2010; Simsek et al., 2015). GST might be an appealing test if it can rule out adrenal deficiency obviously, since it assesses GH and cortisol reserves at the same time as ITT (Kappy et al., 2006; di Iorgi et al., 2010; Simsek et al., 2015). GST has been widely used to assess the HPA axis. In accordance with these reports, we used the GST to check that the *H. pylori* patients were producing high enough cortisol levels.

Although, there is increased prevalence of glucocorticoid deficiency in patients infected with *H. pylori* by GST response, it is very surprising that there are no differences in basal cortisol levels pre and post eradication whereas ACTH levels were slightly statically decreased after eradication. Random measurements of plasma cortisol are very often not helpful in identifying patients with hypoadrenalism, and dynamic stimulation tests are preferred. Although, various levels of early morning cortisol have been proposed to predict the integrity of the HPA axis, the basal plasma cortisol level is not a reliable test to assess HPA axis function (Hagg et al., 1987; Erturk et al., 1998). Morning serum cortisol concentrations of <150 nmol/L (5 µg/dL) are highly suggestive of adrenal insufficiency, whereas cortisol concentrations >500 nmol/L

(18 µg/dL) rule out the diagnosis and cortisol levels between 150 and 500 nmol/L are recommended for evaluation by dynamic testing such as GST (Oelkers, 1996; Pereira and Bevan, 2008).

Basal cortisol secretion occurs in a circadian rhythm, and feedback inhibition plays a critical role in the regulation of the axis by ACTH level and the degrees of increased ACTH determine the severity of adrenal impairment (Klose et al., 2005). Therefore, plasma ACTH levels are often used with clinical signs in diagnosis of adrenal insufficiency (Oelkers, 1996).

H. pylori-infected patients exhibit blunted cortisol responses to GST prior to eradication, but following *H. pylori* eradication, twelve patients still exhibited blunted cortisol responses. The effects of *H. pylori* on HPA axis may presumably more complex than it is thought to be. Firstly, a too short interval between *H. pylori* infection and hormonal evaluation may result lower response to GST in eradicated subjects. Secondly, our results, similarly to those described in the literature, suggest that 1 mg glucagon saturates the ability of the corticotrope may be submaximally stimulated by this dose (Tenenbaum et al., 2014). Our results showed a high prevalence of hypovitaminosis D in eradicated subjects, identifying 51% of patients with serum vitamin D concentrations lower than 20 ng/ml. This hypovitaminosis may contribute to low response to GST in eradicated subjects (Obradovic et al., 2006). Finally, lasting stresses may associate with blunted cortisol responses in eradicated twelve patients (Kunz-Ebrecht et al., 2003). This study took place from February to November 2014. While summer and eradication of *H. pylori* may last into October and November, the vitamin D synthesized throughout the summer and eradication of *H.*

pylori might be sufficient to compensate. It is also interesting that of the twelve patients with blunted cortisol responses to GST, the majority are males (n=10). This suggests that males are more susceptible to adrenal insufficiency in our cohort. Our results were in line with previous studies which peak cortisol response to GST was found to be higher in females (Rao and Spathis, 1987; Simsek et al., 2015).

There are a number of limitations of our study. One of them is the limited number of subjects. The other limitation is the lack of an ITT, which is the gold standard for assessing the HPA axis. However, we performed our study using a GST, which is a reliable alternative to an ITT for the diagnosis of glucocorticoid insufficiency. IL-8, TNF- α , IL-6, IL-1 β , IL-12, and IFN- γ production could be performed on the participants to determine a correlation between these markers and the risk of subclinical adrenal insufficiency in patients with *H. pylori*. Unfortunately, we were unable to evaluate the measuring salivary cortisol levels at intervals over a 24-hour period.

In conclusion, our results indicate an increased prevalence of blunted glucocorticoid response in patients with *H. pylori*. This may be useful in screening these patients for glucocorticoid deficiency, especially under conditions of stress. There was a high prevalence of 25(OH)D₃ insufficiency in the *H. pylori*-infected subjects. Here, we demonstrated a link between vitamin D insufficiency and low response to GST in the *H. pylori*-infected subjects. Vitamin D supports adrenal/cortisol production whereby a deficiency can result blunted glucocorticoid response to GST in patients with infected *H. pylori*. Therefore, further longitudinal studies are required to clarify the relationship between vitamin D and HPA-axis, particularly in individuals with *H. pylori* infection.

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