

Analyzing Determinant Factors for Pathophysiology of Functional Dyspepsia Based on Plasma Cortisol Levels, IL-6 and IL-8 Expressions and *H. pylori* Activity

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ABSTRAK

Latar belakang: berbagai faktor penentu diduga berperan dalam patofisiologi dispepsia fungsional, salah satunya adalah stres psikologis yang dapat meningkatkan kortisol, mengubah proses inflamasi dan memengaruhi aktivitas *H. pylori*. Sampai saat ini belum ada penelitian yang menentukan faktor penentu mana yang dominan diantaranya. Tujuan penelitian ini untuk menemukan faktor mana yang dominan di antara kadar kortisol plasma, ekspresi IL-6, IL-8 dan aktivitas *H. pylori* sebagai faktor yang mempengaruhi patofisiologi dispepsia fungsional. **Metode:** penelitian potong lintang ini dilakukan pada penderita sindrom dispepsia di Rumah Sakit Umum M. Djamil, Padang, Sumatera Barat, Indonesia. Pasien dibagi menjadi dua kelompok yakni kelompok stres dan kelompok non-stres berdasarkan kriteria pada kuesioner DASS 42. Penilaian ekspresi inflamasi (IL-6 dan IL-8) dan aktifitas *H. pylori* dilakukan dengan pemeriksaan imunohistokimia pada jaringan biopsi mukosa lambung dan kadar kortisol plasma diukur dari sampel darah perifer. Data kemudian dianalisis dengan menggunakan regresi logistik multivariat biner. **Hasil:** terdapat 80 orang penderita dispepsia fungsional, dengan rerata umur 38,9 tahun. Kadar kortisol plasma pagi hari meningkat bermakna pada kelompok yang mengalami stres. Ekspresi IL-6 dan IL-8 lebih tinggi pada kelompok non stress dibandingkan dengan kelompok stress, namun tidak bermakna secara statistic (IL-6: 73,28 (SB 16,60) vs. 72,95 (SB 19,49); dan IL-8: 18,45 (SB 17,32) vs. 14,80 (SB 12,71) (stres). *Helicobacter pylori* pada kelompok stres lebih aktif karena reaksi Ag-ab menginvasi submukosa. Faktor yang dominan berperan pada dispepsia fungsional adalah kadar kortisol plasma sore hari. **Kesimpulan:** banyak faktor dapat menjadi faktor determinan dari kerusakan mukosa lambung. Kadar kortisol plasma sore hari merupakan faktor yang dominan.

Kata kunci: dispepsia fungsional, stress, kortisol plasma, IL-6, IL-8, *H. pylori*.

ABSTRACT

Background: there are many determinant factors that may play roles in pathophysiology of functional dyspepsia. One of them is psychological stress that can increase plasma cortisol levels, alter inflammation process and affect *Helicobacter pylori* activity. No study has been conducted to find out the dominant factor among them. This study aimed to find the dominant factor among plasma cortisol levels, IL-6 and IL-8 expressions and *H. Pylori* activity, as the determinant factors in the pathophysiology of functional dyspepsia. **Methods:** a cross-sectional study was conducted in 80 patients with dyspepsia syndrome at M. Djamil General Hospital,

Padang, West Sumatera, Indonesia. The patients were categorized into two groups, i.e. the stress and non-stress group, which were identified using DASS 42 questionnaire criteria. The inflammatory expressions (IL-6 and IL-8 expressions) as well as *H. pylori* activity were determined using immunohistochemistry of gastric biopsy specimens; while plasma cortisol levels was measured from peripheral blood samples. Data were analyzed using binary multivariate logistic regression. **Results:** there were 80 patients with functional dyspepsia with mean age of 38.9 years old. The morning cortisol levels was found significantly higher in the stress group. Higher IL-6 and IL-8 expressions were found in patients of non-stress group compared to those in the other group (IL-6: 73.28 (SD 16.60) vs. 72.95 (SD 19.49); and IL-8: 18.45 (SD 17.32) vs. 14.80 (SD 12.71)); although statistically not significant. There was greater *Helicobacter pylori* activity in the group with psychological stress compared to those in the non-stress group since there was antigen-antibody reaction invading the submucosa. The dominant determinant factor was the afternoon plasma cortisol levels. **Conclusion:** many factors can become the determinant factors for gastric mucosal damage; however, our study has demonstrated that the dominant factor is afternoon plasma cortisol levels.

Keywords: functional dyspepsia, stress, plasma cortisol, IL-6, IL-8, *H. pylori* activity.

INTRODUCTION

The pathophysiology of functional dyspepsia has yet to be established and there are many determinant factors that may induce symptoms of functional dyspepsia (FD). Psychological stress is one of those factors.¹ However, its role is still controversial and difficult to prove.²

The prevalence of functional dyspepsia in Indonesia is increasing each year, which was 1.9% in 1988, 3.3% in 2003 and 5% in 2010 of all patient who visited the primary health care units. So many factors could determine and affect the pathophysiology of functional dyspepsia. Darwin et al³ found that functional dyspepsia could be affected by psychological stress, which at first seemed to have no correlation with IL-6 expression. Yet, there are some evidences that it may correlate by increasing *Helicobacter pylori* activity.

Psychological stress may induce various changes in our body including the enhanced role of cortisol hormone through the HPA axis and induction of the release of inflammatory mediators as well as affecting *Helicobacter pylori* activity.⁴⁻⁶ Murni⁷ conducted a study to evaluate histopathologic profile of gastric mucosa in patients with functional dyspepsia (FD), by comparing patients in the depressive group and non-depressive group. The study found that microscopical damage was more severe in patients of depressive group than non-depressive group as indicated by larger

acute inflammatory area, more severe epithelial damage, worse inflammatory infiltration, more severe degree of metaplasia and more severe atrophy of gastric mucosa.

It is assumed that *H. pylori* can induce up-regulation of inflammatory mediators such as IL-6 and IL-8 that exacerbates FD symptoms.⁶ Psychological stress may affect IL-6 expression through HPA axis pathway.⁷ Moreover, IL-8 is a chemokine in gastric mucosa and its activation is induced by *H. pylori* virulence.⁸ We conducted a study to evaluate the association of psychological stress with plasma cortisol levels, IL-6 and IL-8 expressions as well as *H. pylori* activity in patients with functional dyspepsia and to find out the dominant determinant factor among them.

METHODS

This was an observational study using a cross-sectional design, conducted between May 2015 and May 2017 at Primary Health Care Unit and Outpatient Clinic of M DJamil General Hospital, Padang, West Sumatera, Indonesia. The study protocol had been approved by the Ethical Committee on Healthcare Research, Faculty of Medicine, Andalas University on May 25th 2015 (ethical study number: 081/KEP/FK/2015).

Eighty patients aged 18-65 years with symptom of dyspepsia were included in our study. Subject with ALARM symptom such as

patients with bleeding or patients with weight loss were excluded. Patients with chronic diseases (e.g. diabetes, hypertension, liver cirrhosis, inflammatory bowel syndrome, renal failure, etc), pregnancy or who were using contraceptive pills, NSAID and steroids were also excluded. Patients were selected using consecutive sampling. Psychological stress was identified by using Depression Anxiety and Stress Scale (DASS 42) criteria. Gastric biopsy was performed to evaluate the inflammatory marker (IL-6 and IL-8) and *H. pylori* activity.

Plasma Cortisol Levels

Samples obtained from patients' serum were examined using Elecsys Cortisol Reagent Kit and Electrochemiluminescence Immunoassay system (ECLIA) on Roche Elecsys 1010/2010 device with modular analytcs E 170. The normal morning serum was determined as 4.30 to 22.40 µg/ dL (i.e. the normal limit for adult at the age of 18 years) and the normal limit for evening serum cortisol levels were determined as 3. 09 to 16.66 µg/dL (i.e. for adult subjects aged 18 year).

Histopathological Assessment

Using immunohistochemistry (IHC), we evaluated the gastric tissue specimens obtained from antrum and fundus. Fixation was performed using paraffin block and the specimens were stained using IHC technique to examine IL-6 and IL-8 expressions as well as the *H. pylori* activity.

Immunohistochemistry Procedure

Antigen detection in tissues and cells was performed as an initial step including binding primary antibody to its specific epitope. The primary antibody was bound to a secondary antibody and then the step was followed by using an enzyme-labeled polymer or the polymer may be applied directly to have direct binding with the primary antibody. The bound primary antibody was detected by an enzyme-mediated colorimetric reaction.

Statistical Analysis

Distribution of each variable was evaluated using univariate analysis and presented in charts and graphs. The association between each variable of psychological stress and non-stress group was performed by Chi-square test; t-test

was used for numerical data. The determinant factors were evaluated using multivariate analysis with binary dichotomy.

RESULTS

Our study was conducted for approximately two years on 80 out of 105 participating patients. Subjects were categorized into two groups (i.e. stress and non-stress groups) with 40 subjects in each group. The mean age of subjects in the stress group was 35.60 years which was lower than those in non-stress group (42.15 years); however, the difference was not statistically significant.

In **Table 1**, there is no difference in subject characteristics for both groups except for the dyspepsia score. The subjects in the stress group had higher score than those in the non-stress group indicating that they had more severe dyspepsia although endoscopy findings were similar for both groups.

Table 2 shows that the morning and afternoon serum cortisol levels were significantly higher in patients of the stress group than those in the non-stress group.

Table 1. Subject characteristics

Variables	Stress (n=40)	Non-stress (n=40)
Age (years), mean (SD)	35.60 (10.58)	42.15 (12.79)
Gender		
- Male	14	18
- Female	26	22
Occupational status		
- Working	20	23
- Not working	20	17
Educational Level		
- Lower	30	28
- Higher	10	12
Income Level		
- Low	9	12
- Moderate	22	19
- High	9	9
Dyspepsia score		
- Mild	10	7
- Moderate	23	31
- Severe	7	2
Endoscopic finding		
- Hyperemia	27	25
- Normal	13	15

Table 2. Comparison of patients with functional dyspepsia based on plasma cortisol levels, IL-6 and IL-8 expression and *H. pylori* activity between both of groups with and without psychological stress

Group	Non-stress (mean)	SD	Stress (mean)	SD	P
Morning Cortisol	15.79	7.65	24.03	12.18	0.001
Afternoon Cortisol	6.43	4.53	12.42	9.25	0.000
IL-6	73.28	16.70	72.95	19.49	0.94
IL-8	18.45	17.32	14.80	12.71	0.29

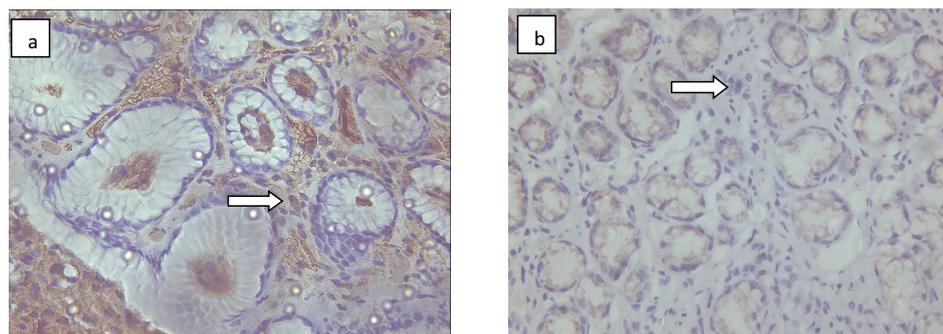


Figure 1. Immunohistochemistry of IL-6 (a) and IL-8 (b) expression found in subjects of the stress group.

Figure 1 shows that the expressions IL-6 and IL8 are similar. However, IL-8 tends to be non-reactive or to have minimal expression.

In **Table 3**, we can see *H. pylori* activity in gastrointestinal mucosa in both groups, however higher activity was found in subjects of the stress group than those in the non-stress group.

Table 4 demonstrates that there was a higher percentage of *H. pylori* activity in in gastric mucosa in subjects of the stress group compared to the non-stress group, although it was not statistically significant ($p=0.27$).

Table 3. Distribution of patients with functional dyspepsia based on *H. pylori* activity in gastrointestinal mucosa

Group	Positive n (%)	Negative n (%)	Total
Non-stress	26 (65.0)	14 (35.0)	100.0
Stress	31 (77.4)	9 (22.5)	100.0

Table 4. Distribution of functional dyspepsia patients based on activity of *H. pylori* in gastric mucosa

Group	None n (%)	Epithelial n (%)	Sub-mucosa n (%)	P
Non-stress	14 (60.9)	7 (51.5)	9 (37.5)	0.27
Stress	9 (39.1)	16 (48.5)	15 (62.5)	

Figure 2 shows a sign of *H. pylori* activity in the interstitial tissue, which was deeper than epithelial layer and was not usually found in normal condition.

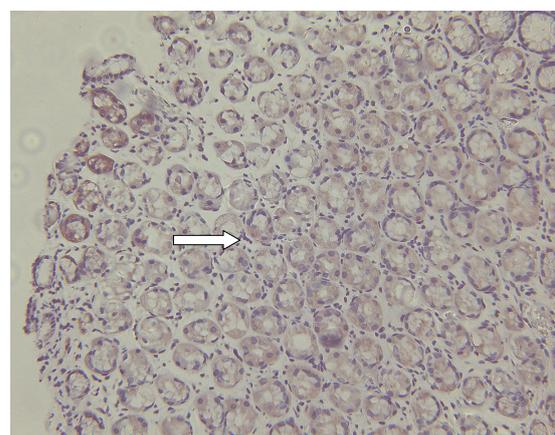


Figure 2. *Helicobacter pylori* activity in gastric mucosa of patients with functional dyspepsia

DISCUSSION

The association of psychosocial stress and psychological factors with clinical manifestations of functional dyspepsia can be explained scientifically. Genetic factors, stressful situation in early life, environmental factors such as losing

a family member, abuse and other negative factors may affect psychological development. They also may affect the ability in adapting to psychosocial problems later in life.

In gastrointestinal tract, some changes may occur through the Brain-Gut-Axis mechanism including changes in gastric and intestinal motility, mucosa immunity and visceral hypersensitivity. Stress and psychological problems may affect the balance of autonomic nervous system, central and peripheral nervous system, immunity system and body hormonal function (i.e. psycho-neuro-immuno-endocrine functions). Those changes may induce the development of signs and symptoms of functional dyspepsia. The mechanism will bring further effect on gastrointestinal tract, secretion, motility, vascularization and reduce pain onset, which may lead to deteriorating health and living activities.⁹⁻¹¹

Our study demonstrated that there was an increase in morning plasma cortisol levels in the subjects of stress group (24.03 (SD 12.18) µg/dL), which was significantly different from those in the non-stress group (15.79 (SD 7.65) µg/dL, $p < 0.005$). Contrasting results were found for the afternoon plasma cortisol levels, in which the levels were found within normal limits for both groups. Nevertheless, the afternoon plasma cortisol levels in subjects of the stress group were higher than those in the non-stress group (12.42 (SD 9.25) vs. 6.43 (SD 4.53) µg/dL, $p < 0.005$).

A study conducted by Murni¹² has also demonstrated that afternoon plasma cortisol levels in patients with psychosomatic disorder was higher than normal, although statistically insignificant (163.09 (SD 130.29) nmol/L vs. 112.69 (SD 86.46) nmol/L). Depressive patients in the study also had higher morning plasma cortisol levels than the normal healthy subjects (328.92 (SD 172.98) nmol/L vs. 188.84 (SD 103.14) nmol/L, $p < 0.05$).¹¹

Actually, high morning plasma cortisol levels is a normal phenomenon found in about 77% at the population, which is known as the Cortisol Awakening Response (CAR). It occurs due to increased reactivity of the HPA axis in the morning and the reaction is separated from the cortisol circadian rhythm. The Cortisol

Awakening Response is superimposed with the peak of cortisol secretion in early morning.

The Association of Psychological Stress with IL-6 and IL-8 Expressions

Our study showed that there was lower IL-6 expression in subjects of the stress group compared to the non-stress group. Similarly, subjects in the non-stress group had higher IL-8 expression. However no significant difference was found in both groups.

IL-6 (interleukin-6) is the first-line cytokine that plays a role in the central process of systemic inflammation. It is both pro- and anti-inflammatory agent that will activate inflammation through activating and proliferating lymphocytes, differentiating B cells, increasing the number of leukocytes and inducing the response of acute-phase protein in the liver. IL-6 (22-27kD) is produced by various cells in immune system including monocytes, mesothelial cells, fibroblast, adipocytes and lymphocytes that account for response to physiological stimulus, such as TNF α , IL-1 β , bacterial endotoxins, physical exercise, oxidative stress as well as psychological stress.^{12,13}

Plasma soluble IL-6 is a form of free IL-6. After binding with IL-6R, the receptor of IL-6, in the circulation it can stimulate the hypothalamus-pituitary-adrenal (HPA). Stress will affect IL-6 expression through the HPA-axis pathway. Numerous studies have demonstrated that individuals with low stress levels have improved immune system.^{7,12}

Psychological stress can stimulate gastrointestinal immune response and aggravate inflammatory symptoms. Although the mechanism of how stress can stimulating such response cannot be explained clearly, it has been suspected that the aggressive response of commensal bacteria may play a role, in which may lead to abnormality in gastric and intestinal function when an individual endures psychological stress. Numerous studies have provided evidences of increased permeability of the mucosa. Therefore, the microorganisms that causing such abnormality can be determined. Hence, stress can trigger increased permeability of gastrointestinal mucosa, which may facilitate microorganisms and their antigens trespass the

epithelial barrier and results in activation of mucosal immune response.¹⁴

Our study has demonstrated some differences between both groups. For example, IL-6 and IL-8 expressions were more readily observable in the non-stress group than the stress group, although, the difference was not significant. Other factors have been predicted to play a role in developing mucosal balance including the neuronal factor that can suppress IL-6 and IL-8 expressions. Another presumption suggests that *H. pylori* infection may induce inflammatory response that can affect IL-6 and IL-8 expressions in the non-stress group.

In the gastrointestinal mucosa, homeostasis of the immune system and autoimmune mechanism play important roles in maintaining mucosa stability by regulating T-cells activity through several mechanisms including: 1) Controlling infection by activating IL 17, IL-17F, IL-21 and IL-22 produced by Th17; 2) Protecting against parasites by activating IL-4, IL-5 and IL-13 produced by Th2; 3) Protecting against intracellular microbiota by regulating IFN; 4) Regulating immune system by activation of IL-10, IL-35, TGF β , which are produced by Treg.¹⁵

Chronic stress can alter microbiota composition and increase IL-6 expression, which indicates immune system activation. Cortisol does not only affect the hormonal pathway, but also neuronal pathway. Vagal nerve can activate noradrenaline secretion that may cause altered microbiota composition and maintain the balance of immune system in the mucosa. Some neurotransmitters may affect the composition of microbiota including serotonin, GABA, etc.

The altered microbiota can influence the nerves and stimulate the brain to reduce inflammatory process. Emotion can also be affected by the feedback mechanism of some neurotransmitters, tryptophan metabolism and spinal cord. Such homeostasis process allows distinct expression of IL-6 and IL-8. In our study, chronic stress in patients with functional dyspepsia does not increase IL-6 and IL-8 expressions since there is another mechanism that covers the inflammatory response.

The Association of Psychological Stress with *Helicobacter pylori* Activity

In our study, we attempted to evaluate the correlation between stress and *H. pylori* activity. We found positive *H. pylori* infection in subjects of both groups. The positive presentation is more visible in the stress group than non-stress group (77.5% vs. 65%). Overall, about 71.25% our patients with functional dyspepsia had been infected by *H. pylori*. The rate is much higher than the world's prevalence, which is about 30%. The result depends on what kind of the procedure used in the study for identifying *H. pylori* infection. The best technique or the gold standard is gastric mucosa biopsy, which had been performed in our study.

Our study also observed some differences between both groups regarding *H. pylori* infection. In individuals with psychological stress, higher *H. pylori* activity was found compared to those in the non-stress group. Based on **Table 4** which presents the activity of *H. pylori*, higher activity involving submucosa was found in the stress group compared to the non-stress group (62.7% vs. 37.5%). It indicates that stress can stimulate *H. pylori* activity to be more aggressive to penetrate the epithelial and involve the submucosa that subsequently will cause more severe mucosal damages.

Murni⁷ has investigated the role of depressive mood on *H. pylori* infection by using gastric mucosa biopsy. The study suggested that patients with depressive mood had greater *H. pylori* infection and more severe histopathological feature.

The role of *H. pylori* in the pathophysiology of functional dyspepsia is still controversial. Activation of the immune system will enhance inflammation and stimulate the release of inflammatory mediators and chemotaxis factor such as IL-6, IL-8, IL-1 β , TNF α , IL-10 and etc. It will accelerate the inflammatory response of gastric mucosa. It also can cause macroscopic and microscopic damage of gastric mucosa.

Toll-like receptor-2 (TLR-2) is a main receptor that can identify *H. pylori* infection and its inflammatory process. Activating the receptor will cause further activation of

NF- κ B, caspase and interferon pathway that produces pro-inflammatory cytokines such as IL-1 β , TNF- α , IL-6, MCP-1 dan IFN- β . These cytokines will have further interaction with inflammatory mediators such as neutrophils or lymphocytes and then will activate immune response. Gastritis, which is caused by *H. pylori* infection, is characterized by increased amounts of acute and chronic inflammatory cells that secrete cytokines and will contribute to greater local inflammation.^{16,17}

Determining the Dominant Factors of Functional Dyspepsia in Patients with Psychological Stress

In our study, we attempted to find out the dominant determinant factors in functional dyspepsia and we obtained results that the determinant factors included afternoon plasma cortisol levels, morning plasma cortisol levels, IL-6 expression, *H. pylori* activity and IL-8 expression. Then, we found that in individuals with functional dyspepsia and psychological stress, the dominant factor was plasma cortisol levels, especially afternoon plasma cortisol levels.

A study in Thailand showed that *H. pylori* infection, anxiety and depression were frequently found in patients with functional dyspepsia. The highest prevalence of those symptoms was found in individuals with post-prandial distress syndrome (PDS). *H. pylori* infection, anxiety and depressive are important factors that may lead to functional dyspepsia especially in patients with PDS. However, the study does not suggest which factor is more dominant. Appropriate management of *H. pylori* eradication and also treating anxiety and depression is the best treatment regimen. In addition, it can play a role in preventing gastric cancer.

Increased morning plasma cortisol levels in patients with psychological stress, particularly those with depression, had been observed in previous studies. It may also occur in individuals with chronic stress, as it may lead to negative feedback failure which happens repeatedly, thus maintain higher levels of cortisol and affect certain organs.

The afternoon plasma cortisol levels also affects the development of dyspepsia syndrome.

Our study has demonstrated increased afternoon plasma cortisol levels, which was within the normal limit. The increased cortisol levels may be caused by inflammatory response characterized by increasing IL-6 expression and when we observed the correlation between IL-8 expression and cortisol levels, we found a significant difference.

Therefore, morning cortisol and afternoon cortisol levels are expected to be the objective parameters to confirm whether an individual has psychological stress problem or not. Appropriate stress management could reactivate the negative feedback mechanism, therefore the progression to gastric mucosa damage can be prevented.

The inflammatory response in gastric mucosa occurs due to various factors and one of them is psychological stress. In our study, we found that IL-6 expression is more dominant than IL-8. The evidence indicates that stress can lead to increased IL-6 and IL-8 expression, although, no significant difference was found.

IL-8 is more frequently associated with *H. pylori* activity. Our study demonstrated that there were colonies of *H. pylori* in both stress and non-stress group. Our result indicated that there was no significant difference in *H. pylori* activity; Nevertheless, we found greater activity of *H. pylori* in the stress group than the non-stress group. When causing tissue or mucosal damage, *H. pylori* will interact with IL-8.

Psychological stress will activate the HPA axis and alter the immune system or inflammatory system, which is characterized by activation of IL-1, IL-6 and IL-8. Moreover, it will also modulate stress response through some neurotransmitters such as serotonin and cortisol. The feedback mechanism of microbiota and *H. pylori* can also affect inflammation and activate HPA axis to increase cortisol levels in the circulation. When feedback mechanism of cortisol is still good, accordingly the circuit will be stopped and the inflammatory response can be repressed. Therefore, it may have affected our study result in which IL-6 and IL-8 levels were found to be lower in the stress group compared to non-stress group.

LIMITATIONS OF STUDY

Our study did not consider the role of neuronal and neurotransmitter that can affect the inflammatory process in gastric mucosa. Our study only observed the role hormonal pathway, which was measured through the increased cortisol levels, which were firstly assumed to have a correlation with increased IL-6 and IL-8 expressions. However, our study showed different result as we found that IL-6 and IL-8 expressions were higher in the non-stress group than the stress group.

Our study did not assess IL-6 and IL-8 expression in individuals with negative *H. pylori* findings. Immune response may occur due to *H. pylori* activity, but it does prove that the psychological stress can affect the *H. pylori* activity.

CONCLUSION

Psychological stress is associated with increased cortisol serum levels, lower IL-6 and IL-8 expression and increased *H. pylori* and the dominant factor is afternoon plasma cortisol levels.

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