The Effect of Psychological Stress on Mucosal IL-6 and *Helicobacter pylori* Activity in Functional Dyspepsia

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ABSTRAK

Latar belakang: *patofisiologi dispepsia fungsional masih belum bisa dipahami sepenuhnya. Terdapat banyak faktor yang mempengaruhi, diantaranya gangguan motilitas gaster, hipersensitifitas viseral, infeksi* Helicobacter pylori *(Hp), stres psikologis, dan sekresi asam lambung yang berlebihan. Penelitian ini bertujuan menentukan aktifitas Helicobacter pylori, ekspreksi mukosa IL-6, dan hubungannya dengan stres psikologis.* ***Metode:*** *studi potong lintang dilakukan pada 40 pasien rawat jalan di Rumah Sakit Umum M. Djamil dan 2 Puskesmas di Padang. Mereka dibagi dalam dua kelompok, dengan atau tanpa stres psikologi, yang diidentifikasi dengan menggunakan DASS 42. Sampel biopsi gaster dan darah perifer diambil saat esofagoduodenoskopi. Metode imunohistokimia digunakan untuk menentukan ekspresi IL-6 dan Hp di mukosa gaster. Hubungan masing-masing variabel dalam kelompok yang mengalami tekanan psikologis dan non-stres dianalisis dengan uji Chi-kuadrat.* ***Hasil:*** *penelitian ini dilakukan pada 40 penderita dispepsia fungsional dengan rerata umur 37,58±11,82 tahun. Didapatkan nilai kortisol plasma berbeda bermakna di antara kedua kelompok (non-stress vs stress), bahkan kortisol pagi pada kelompok stress melebihi nilai normal. Ekspresi IL-6 sebagai bukti terdapat aktifitas inflamasi terlihat lebih banyak pada kelompok non stress dibandingkan dengan kelompok stress (8,25 % vs 7,25%). Aktifitas* Helicobacter pylori *terlihat meningkat pada kelompok stress, ditandai dengan terlihatnya jumlah yang menginvasi ke submukosa lebih banyak dibandingkan kelompok non-stress (11 vs. 7).* ***Kesimpulan:*** *stres psikologis terlihat tidak berhubungan dengan IL-6 pada dispepsia fungsional mukosa gaster namun terdapat bukti adanya peningkatan aktifitas* Helicobacter pylori.

Kata kunci: *dispepsia fungsional,* Helicobacter pylori*, stres psikologis, interleukin 6 (IL-6).*

ABSTRACT

Background: *pathophysiology of functional dyspepsia remains poorly understood. Many factors such as gastric motility disorder, visceral hypersensitivity, Helicobacter pylori (Hp) infection, psychological stress and excessive gastric acid secretion play roles in this symptom. Psychological stress may promote peptic ulcer and has an effect on ulcers-associated Hp. This study aimed to determine Helicobacter pylori activity and expression of mucosal IL-6 and their association with psychological stress.* ***Methods:*** *a cross-sectional study was done among 40 outpatients with dyspeptic syndromes in M. Djamil General Hospital and two-community health centers in Padang. The subjects were divided into two groups, with and without psychological stress, which were identified using DASS 42. Gastric biopsy specimens and peripheral blood samples were taken while performing esophagogastroduodenoscopy. Immunohistochemistry methods was used to determine the expression of IL-6*

and Hp in gastric mucosa. The correlation of each variable in the group experiencing psychological stress and non-stress was analyzed using Chi-square test. ***Results:*** *there were 40 patients with functional dyspepsia with average age of 37.58 (SD 11.82) years old. The cortisol levels were significantly different between both groups (non-stress vs. stress groups); moreover, morning cortisol level in psychological stress group was higher beyond normal limit. Inter-Leukin-6 expression, as the evidence of inflammatory activity, seemed higher in non-stress group than the group with psychological stress (8.25% vs. 7.25%). Helicobacter pylori activity was seemed to be increased in the stress group as characterized by higher numbers of invasion to the sub mucosa epithelium compared to the non-stress group (11 vs. 7 subjects).* ***Conclusion:*** *psychological stress seems to have no correlation with IL-6 in gastric mucous of patients with functional dyspepsia; however, there is an evidence of increasing activity of Helicobacter pylori.*

Key words: *functional dyspepsia, Helicobacter pylori, psychological stress, interleukin 6 (IL-6).*

INTRODUCTION

Functional dyspepsia is a disease characterized by recurrent gastrointestinal complaints and causes the patients to seek frequent treatments. The prevalence of dyspepsia in the world ranges from 5-40% of the population and approximately 60% of them have functional dyspepsia (FD).[1] A study by Mahadeva and Goh[2] showed that there was a relatively large prevalence of functional dyspepsia in the world ranging from 10-30% and it is expected to reach 60% in the primary health care units. Functional dyspepsia (FD) is a highly prevalent and heterogeneous disorder. Most patients with FD complain symptoms that are related to meals intake. However, the pathophysiology of FD remains poorly understood. Functional dyspepsia involves many pathogenic factors, such as gastric motility disorders, visceral hypersensitivity, psychological factors, *Helicobacter pylori* (Hp) infection, and excessive gastric acid secretion. The role of factors causing functional dyspepsia remains controversial and there are many challenges to prove the role of each factor.[3,4]

Psychological stress may be a factor associated with FD or it could also be a precipitating factor. During stress, corticotrophin releasing factor (CRF) from the hypothalamus stimulates secretion of adrenocorti-cotrophic hormone (ACTH) from the pituitary, which in turn releases glucocorticoids (cortisol) from the adrenal gland.[5] Cortisol hormone that is secreted due to psychological stress exposure will trigger gastric acid secretion (aggressive factor) and will inhibit prostaglandin (defensive factor), which has protective effect on the stomach. Decrease in prostaglandin will then facilitate the damage of gastric mucous. A study by Bohmelt[6] demonstrated that there was a significant increase in cortisol level among patients with DF compared to control group. Murni[7] also shows that there is a significant increase of morning corticol level in patients with DF and depression.

The role of H. pylori infection on the pathophysiology of FD is still being debated. Activated immune response will increase inflammatory process and will then triggers the release of various mediators and chemotactic factors such as IL-6, IL 8, Il1β, TNFα IL-10 and others. The release of these factors will further cause inflammatory reaction in gastric mucous and lead to mucosal microscopic or macroscopic damage.[8] *Helicobacter pylori* is a Gram-negative spiral bacterium that colonizes the gastric mucous of human, causing chronic gastritis, peptic ulcers, gastric adenocarcinoma, and mucous-associated lymphoma. Despite the development of strong immune responses against Hp infection in human, the bacteria are rarely eliminated from the stomach and infection is usually lifelong.[9,10] Infection of *Helicobacter pylori* induces strong local immune responses in the gastric mucous of infected host. It is characterized by the recruitment of neutrophils, T and B lymphocytes, plasma cells, macrophages and dendritic cells (DCs), together with epithelial cell damage.[11] Innate immune system is the first-line defense against invading Hp. Toll-like reseptor-2 (TLR2) is a major innate receptor for the recognition of Hp infection and may

cause inflammation. Activation of these innate receptors led to activation of NF-κB, caspase, and interferon pathways that result in production of pro-inflammatory cytokines such as IL-1β, TNF-α, IL-6, MCP-1 and IFN-β. These cytokines attract acute inflammatory mediators such as neutrophils as well as lymphocytes leading to activation of the adaptive immune response.[12,13]

We conducted a study to strengthen the role of psychosomatic medical science on the treatment of functional disorders in everyday practice as well as providing evidence on the involvement of psychological stress factors in the pathogenesis of functional dyspepsia, so that the management of patients can be done comprehensively. The aim of our study is to provide evidence on the correlation between psychological stress and IL-6 as well as the activity of *Helicobacter pylori* in patients with gastric mucous functional dyspepsia and to determine a non-invasive and convenient diagnostic procedure that can be used in primary health care units.

METHODS

The study was a cross-sectional study using a comparative analytical observation. It was conducted in March 2016 and the participants were patients with dyspepsia syndrome at the Andalas Health Center and Padang Pasir Health Center as well as outpatients of M. Djamil General Hospital. The study had been approved by the Ethical Committee on Health Research, Faculty of Medicine, Andalas University on May 25th, 2015 with a reference number of 081/KEP/FK/2015.

Patient Selection

Forty patients enrolled in this study were 18-65 years of age who had suffered dyspepsia syndrome for more than two months. There was no subjects with sign of bleeding (alarm symptoms), history of chronic disease, pregnancy, and under medication. All patients were tested for psychological stress using Depression Anxiety and Stress Scale (DASS 42). Esophagogastroduodenoscopy (EGD) was done to determine the underlying causes of the symptoms, and mucosal tissues were taken during EGD.

Blood Cortisol

Serum samples were taken from patients and examined using Elecysys Cortisol Reagent Kit and Electrochemiluminescence Immunoassay system (ECLIA) on Roche Elecsys 1010/2010 device with modular analitycs E 170. The normal morning serum value: 4. 30 – 22.40 µg/dL (Adult, Age: 18 year) and the normal evening serum value: 3. 09 – 16.66 µg/dL (Adult, Age: 18 year).

Histopathology Assessment using Immunohistochemical Methods

Immunohistochemistry of gastric tissue was taken from two sites, i.e. the antrum and fundus. Once the tissue had been harvested, fixation was done using paraffin block. IHC staining process was performed to examine the expression of IL-6 and *H. pylori*.

Procedure of IHC for Immunohistochemistry

Antigen detection in tissues and cells was performed in a multi-step immunohistochemical process. The initial step was to bind the primary antibody to its specific epitope. A secondary antibody was applied to bind with the primary antibody, which was followed by an enzyme-labeled polymer; or the polymer may be applied directly to bind with the primary antibody. The bound primary antibody was detected by an enzyme-mediate colorimetric reaction.

Statistical Analysis

Univariat analysis was performed to observe the distribution of each variable using a computer system, which was then presented in charts and graphs. Statistical analysis of the correlation between each variable in the group experiencing psychological stress and non-stress group was done using Chi-square test.

RESULTS

The study was conducted for approximately 4 (four) months with 40 eligible subjects out of 47 patients participated. There were 20 patients who suffered dyspepsia with psychological stress and 20 patients without psychological stress. From the **Table 1**, we can observe that the average age of patients with dyspepsia syndrome who were included in this study is of 37.58 (SD 11.82) years with a greater total number of female

Table 1. Subjects' characteristics (N=40)

Variables	value
Age (years), mean (SD)	37.58 (11.82)
Gender, n (%)	
- Male	14 (35.00)
- Female	26 (65.00)
Psychology, n (%)	
- Non-stress	20 (50.00)
- Stress	20 (50.00)
Morning cortisol, mean (SD), µg/dL	
- Non-stress	23.100 (11.041)
- Stress	29.015 (10.395)
Evening cortisol, mean (SD), µg/dL	
- Non-stress	8.360 (6.519)
- Stress	12.944 (1499.9)
IL-6, mean (SD)	
- Non-stress	8.25 (3.1)
- Stress	7. 25 (3.2)

patients than the male. We found that the number of patients who experienced psychological stress and non-stress were 20 subjects for each group. The level of cortisol in every sample was tested twice, i.e. in the morning and evening. There was a significant difference of cortisol level in the morning and evening obtained from both groups. The morning and evening cortisol levels were significantly different between non-stress and psychological stress group. The morning cortisol level was also higher in psychological stress group than the normal value (4.30 – 22.40µg/dL); however, the cortisol level was still in normal range in the evening (3.09 – 16.66 µg/dL).

IL-6 expressions were observed by counting the number of cells marked with IL-6 marker which could be seen as brown-colored cells. Positive cells were counted from 100 cells that existed. From the result, we found that the group without stress had greater IL-6 expression than the group with psychological stress (8.25% vs. 7.25 %, p=0.00)

The study also showed *Helicobacter Pylori* activity in both groups, either with psychological stress or non-psychological stress.

From the **Table 2**, we can observe that *Helicobacter pylori* activity in non-stress group was lower compared to group with psychological stress, as demonstrated by higher number of specimens showing invasion to submucous membrane in the psychological group (11 subjects vs. 7 subjects).

Table 2. Difference of *Helicobacter pylori* activity in patients with gastrointestinal mucous functional dyspepsia patients that suffered psychological stress and non-psychological stress.

Helicobacter pylori Activity	Non-stress (n)	Stress (n)
No activity	4	3
Activity limited to epithelial	9	6
Activity up to sub mucous	7	11

DISCUSSION

Functional dyspepsia is a disease that is commonly found in health care practices, especially in primary care. The pathophysiology is still unclear, which causes the treatment to be less optimal. The role of psychological stress and *Helicobacter pylori* infection is referred to as one of the many other factors that cause complaints in functional dyspepsia. The aim of our study was to observe the IL-6 expression and *Helicobacter pylori* activity in patients with functional dyspepsia that suffers from psychological stress, which assumed to differ from patients without psychological stress.

Dyspepsia is frequently experienced by patients of productive age, in this case at average age of 37.58 (SD 11.82) years old. The average age found in our study is younger than the results of previous study obtained by Murni[7], which is 41.6 (SD 14.66) years old. Age does play a role in one's ability to deal with stressors in life. Depression is more commonly found at a young age due to behavioral and environmental factors, which affect the ability to adapt to stressors. Females are more likely to suffer from functional dyspepsia compared to male patients in our study. This is because females are assumed to have less ability to withstand stressors and this should be proven in a subsequent study.

In our study, increased cortisol levels, both morning and evening levels were found in patients with functional dyspepsia who experienced psychological stress compared to those that were not experiencing psychological

stress. Stressful life events can induce persistent changes in the ability of the HPA axis, that play an important role in the pathogenesis of depressive disorders. Considerable evidence suggests that this vulnerability for developing psychiatric disorders is associated with changes in neurobiological systems related to stress regulation.

Excessive stress will cause HPA axis to be hyperactive or hypersensitive, which causes biological vulnerability towards stressors. Chronic stress, which results in increased production of catecholamines such as norepinephrine and epinephrine from the adrenal medulla and sympathetic neurons, has long been believed to adversely influence health. Stress mediators are known to promote tumor development as well as progression and are associated with increased risk of heart disease and infection.[14,15]

The process occurs in the hippocampal that regulate HPA outflow, which may enable cortisol to partially escape negative feedback inhibition and lead to the relative increase in diurnal cortisol output. A similar dynamic operating in cells of the immune system, particularly monocytes, could diminish glucocorticoid receptors capacity to inhibit NF-κB, AP-1, and other proinflammatory transcriptional-control pathways, which in turn could result in the heightened IL-6 responses to TLR stimulation.[16]

Increased production of peripheral cytokines and other pro-inflammatory markers have been linked to psychiatric disorders such as major depressive disorder and post-traumatic stress disorder. Another study found that inflammation may be an important developmental mediator linking adverse experiences in early life to poor adult physical and mental health.[17]

There are some significant positive correlation between indices of IL-6 and age; however, no significant correlations have been found emerged for any of the IL-6 measures and sex, depression symptoms, quality of life score, state anxiety, trait anxiety, or perceived stress level. Cytokines such as IL-6 are integral parts of the innate inflammatory response to a physical stressor (e.g. infection, inflammation). The mechanisms by which psychosocial stress initiates cytokine responses, as well as the clinical consequences of an exaggerated cytokine response to stress, remains to be determined.

Our study found that percentage of IL-6 in gastric mucous of FD patients with psychological stress was lower than patients without psychological stress. IL-6 may play an important role in the gastric mucosal response to *H. pylori* infection and in the development of clinical *H. pylori*-related disease. the signaling pathways regulating IL-6 gene expression in *H. pylori* infection remains largely unstudied. Although many studies have shown that *H. pylori* infection is associated with increased IL-6 production within the gastric mucosa the mechanisms involved are largely unresolved.[17,18]

H. pylori preferentially stimulates IL-12 secretion over that of IL-6 and IL-10 from human dendritic cells. The relatively poor ability of *H. pylori* to induce IL-6 and IL-10 may be due to the low endotoxin activity of its LPS. This explanation implies that IL-12 secretion is due to a different bacterial stimulus than that causing secretion of IL-6 or IL-10 in the case of *H. pylori*.[19]

In our study, activity of *H. pylori* was shown to be higher in gastric mucous in FD patients with psychological stress. Stress can impact the developmental trajectory of the intestinal barrier and has been associated with an increase in gut permeability. The effects of stress on intestinal permeability are complex and likely involve both the gut and the brain. Corticotrophin releasing factor (CRF) and its receptors play a key role in stress-induced gut permeability dysfunction. In response to an acute stressor, colonic paracellular permeability increases and has been associated with the development of visceral hypersensitivity and elevated central corticotropin releasing hormone (CRH) expression occurred concomitantly with changes in the gut microbiota.[20]

The results of our study is in line with research done by Murni[7] that shows that histopathological description of gastric mucous are more severe in patients with functional dyspepsia that suffers depression compared to the non-depressed groups.

CONCLUSION

Psychological stress is associated with expression of pro-inflammatory cytokine (IL-6) in patients with gastric mucous functional dyspepsia and also involved the increase in activity of *Helicobacter pylori*.

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