

Mucus Thickness of the Gastric Mucosa and *Helicobacter pylori* Infection in Dyspeptic Patients with or without Diabetic Symptom

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ABSTRACT

Background: Chronic *Helicobacter pylori* (*H. pylori*) infection affects the mechanisms of gastric mucosal protection. In patients with diabetes mellitus, data on the prevalence of *H. pylori* infection are scanty and contradictory. We have examined, using histological fixation technique, the thickness of the adherent mucus gel layer in the gastric mucosal and *H. pylori* infection in dyspeptic patients with or without diabetes.

Method: A cross-sectional study was conducted in 86 dyspeptic patients consisted of 43 diabetics and 43 non-diabetics patients. In all cases, upper gastrointestinal endoscopy were performed, measurement of the gastric corpus and antral mucus thickness was carried out at the corpus and antral biopsy specimens were snap frozen and cryostat sections were stained using a hematoxyline eosin. One biopsy within 2 cm of the pylorus was examined for detection of *H. pylori* status by using polymerase chain reaction (PCR).

Results: In all sections the mucus layer was continuous. At the gastric corpus and antrum, the mucus thickness of diabetic patients was thinner ($35.2 \pm 2.2 \mu\text{m}$ and $43.9 \pm 3.8 \mu\text{m}$) than non diabetic patients ($45.2 \pm 2.5 \mu\text{m}$ and $51.2 \pm 2.2 \mu\text{m}$). The results were significantly different ($p = 0.001$). The difference of *H. pylori* prevalence between diabetics (52.5%) and nondiabetics patients (47.5%) was not significant ($p = 0.67$).

Conclusion: This study shows a significant thinning of the adherent mucus gel layer both in diabetic patients and *H. pylori*-positive individuals. No difference has been found between patients with *H. pylori* infection and diabetes mellitus.

Keywords: mucus thickness, *Helicobacter pylori* infection, diabetic patient

INTRODUCTION

Complaint related to upper gastrointestinal tract (dyspepsia syndrome) is often experienced by diabetic patients. The results of previous studies showed that the prevalence of dyspepsia in diabetes ranges from

13-20%.¹ Dyspepsia syndrome is often associated with delayed gastric emptying (gastro paresis). The prevalence of gastroparesis in type I diabetes ranges from 27–58%; and it ranges from 30-60% in type 2 diabetes.^{2,3} The main cause of complex diabetic gastroparesis is autonomic neuropathy, others are genetic factor, inflammation, and fibrosis in gastric smooth muscles.³ Consequently, gastroparesis may increase the risk of *Helicobacter pylori* (*H. pylori*) infection and defensive factors disturbance.⁴⁻⁹ It has been estimated that defensive factors in diabetes mellitus have been disturbed causing increased risk of

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aberration in gastric and duodenal mucosa.^{10,11} Suzuki et al reported that their study in diabetic rats showed disturbance on the gastric mucosal defensive factors.¹²

The defensive factors are originated in mucosa microcirculation, which in normal condition they have a role to maintain mucus, bicarbonate secretion, and epithel restitution process. The microcirculation is affected by blood pressure, vasoconstrictors, such as thromboxane A₂, adrenalin and noradrenalin, also by vasodilators, such as prostaglandin type E₂ (PGE₂) and nitric oxide (NO₂) that secreted by capillary endothel.^{9,13,14} One of chronic complications in diabetes is microangiopathy that will cause gastric mucosal blood flow disturbance. A study conducted by Suzuki in diabetic rats showed that there was a reduction in gastric mucosal blood flow.¹² A study by Shengyuan et al utilizing the laser Doppler flowmeter on 14 points of gastric mucosa in diabetic patients showed a significant reduction on gastric mucosal blood flow compared with control.¹⁵ There was a correlation between mucus thickness and intragastric acid production in order to maintain the gastric balance.⁹ Hassler et al who studied the intragastric pH testing in diabetic patients, found that intragastric pH in diabetic patients was higher than non-diabetics.¹¹ Allen et al reported that minimal mucus thickness in human was 50 µm. While Phillipson et al reported that minimal mucus thickness in rats *in vivo* was 80 µm, which act as a protection from gastric acid, pepsin, drugs, and irritants that may damage gastric mucosal layer.^{16,17,18} So far, studies about mucus thickness in diabetic patients have never been performed.

The correlation between *H. pylori* infection and dyspepsia in diabetic patients are still controversial.^{1,5,8} Papamichael et al summarized several studies in type 1 and type 2 diabetes mellitus.⁸ Controversial findings had been reported, especially about *H. pylori* infection and duration of illness, blood fasting glucose, age, sex, body mass index (BMI), the severity of dyspepsia complaint, hypertension, and cardiovascular neuropathy. Such findings may be caused due to different identification methods for *H. pylori*.

METHOD

The present study was a cross-sectional study design which was performed in 2 groups of study samples. The first group was 43 diabetic patients with dyspepsia complaints and the second group was 43 non-diabetic dyspeptic patients. All patients

underwent upper gastrointestinal endoscopy to look for the abnormalities/lesion and they also had gastric mucosa biopsy.

The sampling was performed consecutively at the outpatient clinic and endoscopic procedure room at the hospitals: Cipto Mangunkusumo, Pantiwilasa, and Gatot Soebroto between April 2010 and June 2010. The inclusion criteria in this study were: (1) Patients 18–60 years of age; (2) Dyspeptic patients with or without diabetes. The exclusion criteria were: (1) Patients with acute or chronic liver disease; (2) Patients with chronic kidney disease; (3) Patients with gall stone; (4) Patients with history of abdominal/upper gastric surgery; (5) Patients with heart failure who were at New York Heart Association (NYHA) class III or IV; (6) Patients with history of stroke; (7) Patients who took aspirin and non-steroidal anti-inflammation drugs; (8) Patients or patients' family who refused to undergo endoscopy; (9) Patients who had other gastric mucosa abnormality during the endoscopic examination, for example patients who have been suspected for malignancy; (10) Uncooperative patients.

Of the *H. pylori* DNA testing in biopsy specimens, 86 samples from the gastric antrum of dyspeptic patients were analyzed by polymerase chain reaction (PCR) procedure to detect the presence of *H. pylori* by using urease C/ureC gene (*glmM*, 294 bp). For the gastric mucosal mucus examination, the biopsy specimen should be confirmed that it was adequately large and intact to be adhered with the embedding medium for frozen preparation (Cryo matrix) and be frozen. The tissues were cut at 12 µ by cryo cut microtome set at -20°C, and then it was fixated and stained with hematoxylin eosin stain. The preparation was analyzed under the light microscope to identify the thickness of gastric mucosal mucus as being measured by ocular micrometer scale under magnification of 40 x 10.

In order to differentiate the mean of mucus thickness in dyspeptic patients who also had type 2 diabetes mellitus and the non-diabetic patients, 2 mean difference for independent t-test was used. For categorical data, the non-parametric test, Chi-square was used and when the data has fulfilled the requirements, the Fisher and Kolmogorov Smirnov tests were utilized. Afterward, the data was analyzed by using SPSS 15 for Windows. Study protocol had been submitted to and approved by Ethical Committee at Faculty of Medicine, University of Indonesia.

RESULTS

Table 1. Characteristic of dyspepsia patients with and without diabetes

Variable	Dyspepsia		p
	Diabetic	Non diabetic	
Number of subject	43	43	
Sex			
Male	22	21	
Female	21	22	
Age (year)	49.2 ± 7.6	46.5 ± 9,6	0.158
Fasting blood glucose (mg/dL)	162 ± 29.64	91.97 ± 7,03	
Postprandial blood glucose (mg/dL)	241.51 ± 39.73	121.72 ± 13.66	
HbA1C	6.9 ± 0.7		
Body mass index	24.2 ± 1.0	23.9 ± 1.1	0.333
Duration of diabetes (year)	5.2 ± 3.8		
Antrum			
Hyperemic	43 (51.8)	28 (48.2)	
Erosive	30 (69.8)	12 (30.2)	
Ulcus	10 (76.9)	3 (23.1)	
Corpus			
Hyperemic	40 (57.1)	30 (42.9)	
Erosive	12 (54.8)	9 (45.2)	
Ulcus	0	0	
Mucus thickness			
Antrum	35.9 ± 2.2	44.1 ± 3.8	< 0.001
Corpus	45.2 ± 2.5	51.0 ± 2.7	< 0.001
<i>Helicobacter pylori</i> infection			
Positive polymerase chain reaction	21 (52.5%)	19 (47.5%)	< 0.001
Positive histopathology	2 (50%)	2 (50%)	< 0.001

Table 2. The gastric mucosal mucus thickness in dyspepsia patients with *Helicobacter pylori* infection

Mucus thickness	Dyspepsia (n = 86)		p
	Positive <i>H. pylori</i>	Negative <i>H. pylori</i>	
Antrum (µm)	38.0 ± 4.3	41.8 ± 5.2	< 0.001
Corpus (µm)	46.7 ± 3.3	49.3 ± 3.9	< 0.001

Table 3. The gastric mucosal mucus thickness in diabetic patients with *Helicobacter pylori* infection

Mucus thickness	Dyspepsia with diabetic (n = 43)		p
	Positive <i>H. pylori</i>	Negative <i>H. pylori</i>	
Antrum (µm)	34.6 ± 1.7	37.1 ± 2.0	< 0.001
Corpus (µm)	42.7 ± 2.6	48.9 ± 2.3	< 0.001

Table 4. The gastric mucosal mucus thickness in non-diabetic patients with *Helicobacter pylori* infection

Mucus thickness	Dyspepsia non-diabetic (n = 43)		p
	Positive <i>H. pylori</i>	Negative <i>H. pylori</i>	
Antrum (µm)	41.7 ± 3.2	46.0 ± 3.2	< 0.001
Corpus (µm)	49.0 ± 2.5	52.6 ± 1.7	< 0.001

Table 5. Histopathological gastropathy in diabetic and non-diabetic patients

Gastropathy–histopathology	Dyspepsia (n = 86)		p
	Diabetic (%)	Non diabetic (%)	
Polymorphonuclear cell			
Mild	1 (20.0)	4 (80.0)	
Moderate-severe	1 (50.0)	1 (50.0)	
Mononuclear cell			
Mild	10 (26.3)	28 (73.7)	< 0.001
Moderate-severe	31 (75.6)	10 (24.4)	< 0.001
Gland atrophy			
No atrophy	20 (47.6)	22 (52.4)	1.0
Mild	20 (52.6)	18 (47.4)	1.0
Moderate-severe	3 (50.0)	3 (50.0)	

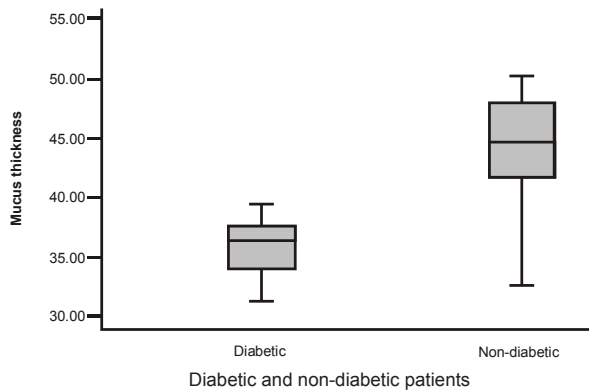


Figure 1. Boxplot of the antral gastric mucosal mucus thickness in diabetic and non-diabetic patients

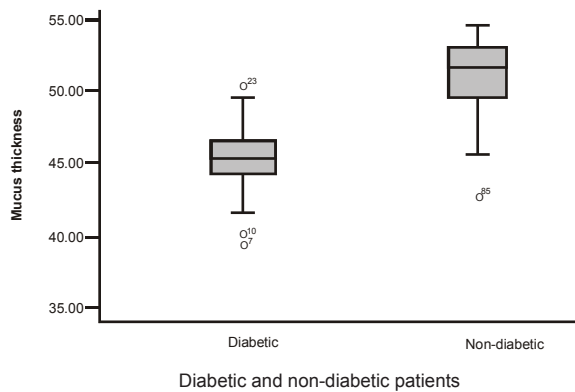


Figure 2. Boxplot the corpus gastric mucosal mucus thickness in diabetic and non-diabetic patients

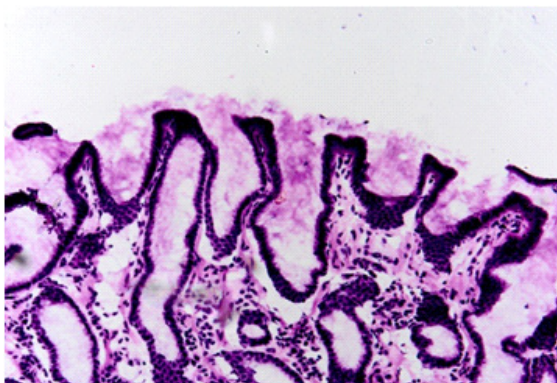


Figure 3. Antral mucus with hematoxylin eosin staining

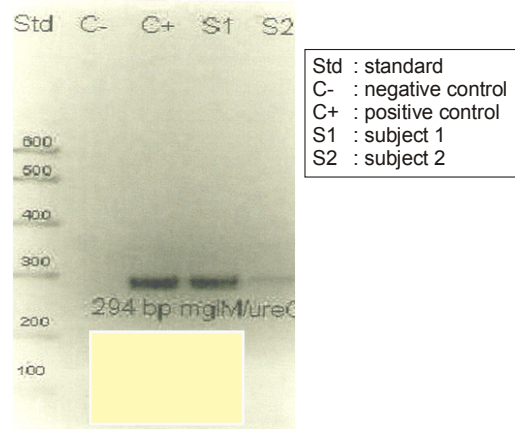


Figure 4. Specific band of *Helicobacter pylori* based on PCR method using ureC/glmM primer

DISCUSSION

The study about gastric mucosal mucus thickness was previously reported in animals. After Jordan et al had found a method visualizing mucus layer in human, then studies on mucus thickness had been started.¹⁹ Atuma et al evaluated mucus thickness of rats *in vivo* under anesthesia by using micropipette for gastric acid sampling. Such technique was considered to be more representatives to evaluate the overall gastric mucus thickness.²⁰ Theoretically, the mucus layer is an initial barrier for upper gastrointestinal tract from any substance entering the intestinal lumen. It protects cells from the digestive process or chemical contacts.^{21, 22, 23}

In the present study, the antral gastric mucosal mucus thickness in diabetic patients was $35.9 \pm 2.2 \mu\text{m}$; while in non-diabetic patients was $45.9 \pm 3.9 \mu\text{m}$. Statistically, such difference was significant ($p = 0.000$). Moreover, the mucus thickness of gastric corpus in diabetic patients was $45.2 \pm 2.5 \mu\text{m}$ and $51.2 \pm 2.2 \mu\text{m}$ in non-diabetics. It was statistically showed significant difference ($p = 0.000$). Antono found that the mucus thickness of NSAIDs users was $37.4 \pm 9.2 \mu\text{m}$ at the antrum area and $43.3 \pm$

13.1 μm at the corpus.²⁴ Atuma et al, who performed in vivo technique in rats, reported that the mucus layer thickness at antrum was $154 \pm 16 \mu\text{m}$ and at corpus was $80 \pm 5 \mu\text{m}$.²⁰ Various constraints or causes may become the reason for those differences, such as fixation process with organic materials and embedded paraffin causing gel dehydration leading to mucus shrinkage or thinner adherent mucus.²⁰

Most gastroscopic lesions in diabetic patients, about 100%, were found at hyperemic antrum; while 93% lesions were found at the corpus. In non-diabetic patients, most lesions were also found at the antrum; with 93% hyperemic, and at the corpus 69.7%. Anastasios et al also found that gastroscopic lesions were located mostly at the antrum.²⁵ One of the reasons was because anatomically antrum is in the lowest part.²⁵ Foods and drugs that taken will directly move down to antrum and stop for a while, before entering into duodenum. Other reason may be because of the longest contact duration of drugs and foods to the gastric mucus are at the antrum. Longer duration will increase topical irritation, and cause more mucosal damage.²⁶

The mucus thickness at the antrum tended to be thinner compared to the other parts, in accordance with the visible gastroscopic images. Such finding is consistent with the histological theory about gastric mucosal at the corpus, where the epithelial cells are thicker and more mucus cells found there compared with the antrum.⁹ Theoretically, in my opinion, the mucus layer at the corpus was thicker than antrum (table 1). So, it can be said that mucus protection at the corpus was better than the antrum and this also explained why the most gastroscopic lesions were found at the antrum.

A study by Allen et al reported that the minimal mucus thickness in human was $50 \mu\text{m}$; while Phillipson et al reported that study in rats demonstrated that the minimal gastric mucosal mucus thickness was $80 \mu\text{m}$ as a protection against gastric acid, pepsin, drugs and irritant agents that can damage the gastric mucosal mucus layer.^{16,17} The results of Fixa et al study reported that the mucus thickness may also be affected by sub-mucosal inflammation process. Our study found that there was more histopathologic gastropathy in diabetic patients, either moderate or severe mononuclear inflammation, compared to the non-diabetic patients and statistically, the difference was significant (table 5).²⁷

The gastric mucosal mucus layer thickness may act as protection against irritant substances, including NSAIDs, which had been proven by in vitro study of prostaglandin E_1 analog effect (misoprostol) on the protection of gastric mucosa in rats. In the study, the mucus thickness was increased two-fold in insoluble gel/firmly adherent mucus layer (the mucus layer that firmly attached with the mucosa) and increased

three-fold in soluble mucus/loosely adherent mucus (the dynamic and mobile mucus, a lubricating layer).²⁸

It has been proven that prostaglandin E_1 analog may significantly decrease gastroscopic lesion compared to cimetidine and sucralfate. A study by Taye et al reported that in diabetic rats using pyoglitazone the mucus thickness also increased.²⁹ Similar results were also reported by Iijima et al by using the technique of endoscopy gastrin test. They showed that rebamipide may also increase mucus thickness.³⁰ We believe that the more thickness in gastric mucosal mucus, the easier to prevent gastric epithel cells damage. In addition to gastric mucosal mucus thickness, the quality of mucus glycoprotein components (serine, threonine, proline, and oligosaccharides chains) may also have role to maintain its integrity.⁹ Moreover, a lot of other factors that may have role, such as prostaglandin, secretin, gastrin and cholecystokinin that stimulate the mucus secretion.⁹ Those factors have not been investigated yet regarding its correlation or effects on the mucus thickness and the study may be difficult. Therefore, whether thicker mucus will be comparable with the quality of gastric mucosa is still unknown. Based on the data, we believe that the gastric mucosal mucus layer thickness was a reflection or final result of an overall pre-epithelial protection function.

In the present study, there were 86 biopsy samples collected from dyspeptic patients aged 28–60 years, all the patients experienced gastritis as determined by pathological anatomy test. This study also detected *H. pylori* in gastric biopsy of the patients through PCR technique using ureC primary gene/glmM that has closely correlated to colonization and bacteria pathogenesis activity in gastrointestinal tract. Of the 86 tested biopsy samples, 4 (5.4%) samples showed positive result by histopathological examination; while by ureC/glmM PCR methods, 38 (46.3%) patients showed positive results. Zsikla et al reported that PCR methods may increase the detection rate of *H. pylori* about 20% compared to the histopathological examination.³¹

H. pylori may enter human body during childhood and may survive until adulthood. Moreover, it is not easy to be cultured or need a very specific condition for culture. Therefore, one of the techniques performed for fast detection is the specific PCR test since it can trace a very specific *H. pylori* genes and very sensitive as it can double its DNA to million times, even when originated only from 1 or 2 bacteria.³² In this study, detection of *H. pylori* was based on deoxyribonucleate acid which have potency to be utilized for diagnosis by using markers that was a general marker for bacteria structure and pathogenesis factors such as species specific ureC/glmM genes.^{31,32} The facts that PCR may quantitatively yield product and genotype with

sensitivity and specificity nearly 100% compared to the conventional methods. However, sometimes due to something or another, such sensitivity was not obtained or the concentration of DNA product was very low.^{32,33,34}

In the present study, 21 (52.5%) dyspeptic patients with diabetes and 19 (47.5%) dyspeptic patients without diabetes showed positive results of the *H. pylori* presence on specific band; however, the difference was not statistically significant ($p = 0.67$). Also, *H. pylori* were detected in 1 sample histopathologically despite its negative result on PCR. Various constraints or causes may be the reasons, such as: (1) Biopsy sampling that was only performed once and may be less precise site where *H. pylori* were suspected.⁵ It could be managed or at least be minimized by biopsy in more than 2 sites, the antrum and corpus; however, such action might endanger the patients because it could injure their gastric wall; (2) Biopsy contained only a very little or some *H. pylori* bacteria, then it disappeared during a quite long DNA extraction process where the DNA concentration became lower because it was diluted by tissue DNA leading to a concentration below the detection threshold.³⁴ It can be managed by performing nested PCR with inner primer and DNA resulting from the first amplification product as a template which has sensitivity nearly 100 times.^{33,34} Another way is using reverse transcriptase PCR (RT-PCR) which had already been performed by some researchers;³² (3) The condition of PCR amplification such as less specific primer, which means that *H. pylori* bacteria did not contain analyzed genes or was not expressing the related genes. Utilization of more primers may help to overcome this.^{32,33,34} The 16S RNA gene was proposed as the most suitable gene to be used in PCR testing because its number of template molecules are most commonly found in *H. pylori*;³³ (4) Bacteria may contain target genes, but the annealing temperature of PCR was less fit to produce the desired DNA. It should be considered that many factors may affect the success of PCR, including the possibility of contaminated DNA; (5) DNA might be amplified by PCR, but sometimes problems arose in electrophoresis and visualization that actually caused the loss of or undetectable DNA bands. Other sampling such as feces can also be used for supporting or completing the PCR test results.³⁴

The prevalence study of *H. pylori* infection showed variation among countries and ranged between 25–80% in developing countries.^{18,35} The study demonstrated that the prevalence of *H. pylori* in dyspeptic patients was 46.3%; while in dyspeptic patients with diabetes was 52.5% and without diabetes was 47.5%. The existing data about the correlation between *H. pylori* infection and diabetes is still controversial.⁸ Some studies

showed higher prevalence of *H. pylori* in diabetic patients compared to non-diabetic.^{36,37,38,39,40,41,42} It was also higher in diabetic patients with micro complication and macroangiopathy.⁴⁰⁻⁴⁴ Other studies showed that there was no difference regarding prevalence between diabetic and non-diabetic patients, i.e. 37.3% vs. 35.2%; 50.8% vs. 56.4%, 28.1% vs. 29.25%, 33% vs. 32%.^{44,45,46,47} Dore et al stated that the absence of such difference may be due to infection that occur before manifestation of diabetes, i.e. during the childhood.⁴⁸

In Indonesia, the prevalence of *H. pylori* was 10.2% and 2.9% in dyspeptic patients, respectively as it has been reported by Syam et al by histopathological method and rapid urease test (RUT) pronto dry,⁴⁹ and Saragih et al by histopathological method.⁵⁰ Moreover, Tokudome et al reported that in region with low prevalence of gastric cancer, the used of serology method and urea breath test (UBT) produced the results of 5% and 3%.⁵¹ Such prevalence is different from the collaboration study conducted by Murdani et al, which used histopathological methods, cultures, and RUT with a result of 68% in Indonesia;⁵² while the result in Japan was 59.5%, a quite similar result compared to our study using the PCR methods.⁵²

The correlation between *H. pylori* infection and diabetic complication is still a debate and need further study. Demir et al reported that neuropathy complication was often found in diabetic patient with *H. pylori* infection.⁴ A study by Gucelik et al showed that there was a correlation between the virulence of *H. pylori* (CagA) and macroangiopathy complication.³⁹ Similar results were also reported by Quadri et al and Gasbarrini et al in cerebral thrombosis.^{53,54} Benner et al reported that more *H. pylori* infection was commonly found in diabetic patients with obesity.⁵⁵

Several studies also showed that the effectiveness of *H. pylori* eradication in diabetic patients is lower compared to the non-diabetic patients; therefore, they needed a longer time for antibiotics treatment.⁴⁶ Likewise, the reinfection rate in a year is higher in diabetic patients compared with non-diabetics.^{56,57,58} This may be caused by micro vascular changes in the stomach of diabetic patients leading to the reduced antibiotics absorption or may be caused by the frequent use of antibiotics thus possibly develop antibiotics resistance against *H. pylori* strain.^{52,53} The development of *H. pylori* vaccine may provide hope of effective treatment for diabetic patients.^{56,57}

The mucus thickness in dyspeptic patients who had been infected by *H. pylori* with antrum $38.0 \pm 4.3 \mu\text{m}$; corpus $46.7 \pm 3.3 \mu\text{m}$, was thinner than non-infected patients with antrum $41.8 \pm 5.2 \mu\text{m}$; corpus: $49.3 \pm 3.9 \mu\text{m}$. Such difference was statistically significant ($p = 0.001$). Similar results were also found in diabetic patients with antrum $34.6 \pm 1.7 \mu\text{m}$; corpus

42.7 ± 2.6 µm vs. antrum 37.1 ± 2.0 µm; corpus: 48.4 ± 2.3 µm. The difference was also statistically significant. A study by Newton et al reported that there was thinning in the mucus layer of *H. pylori*-infected patients.⁵⁸ Beil et al reported that the thinning was caused by *H. pylori* cytotoxic process in ribosome stage from mucus-producing cells.⁵⁹ Furthermore, Celli et al reported that there was rheological changes of mucus due to increased pH by urease activities so that the mucus layer became thinner.⁶⁰ Hassler et al reported that the increase of intragastric pH was caused by *H. pylori* infection which induced glands atrophy; while in our study, most glands atrophy was mild atrophy and statistically there was no significant differences between diabetic and non-diabetics patients.¹¹

The limitations of our study were: (1) The evaluation of mucus thickness was conducted in situ; (2) Detection of *H. pylori* was performed by biopsy at the antrum and using the PCR methods. At this moment, the golden standard for detecting the presence of *H. pylori* was by culture or by using two evaluation methods which have high sensitivity and specificity; (3) The measurement of blood glucose level in non-diabetic dyspeptic patients was based on the fasting blood glucose and 2-hours postprandial blood glucose; it should be better by the oral glucose tolerance test (OGTT).

In this study, there were 4 non-diabetic subjects according to the measurement only by random blood glucose level and the fasting blood glucose and 2-hours postprandial blood glucose testing were not performed, but they were still included in the non-diabetic group. However, statistically it showed no significant difference since the percentage was less than 10% of all non-diabetic dyspeptic subjects. Moreover, the mean value of random blood glucose in those subjects were still in confidence interval of fasting blood glucose level and 2-hours postprandial glucose level; therefore, the 4 subjects were probably had normal blood glucose level indeed.

CONCLUSION

The gastric mucus thickness in diabetic patients is thinner than non-diabetics. The prevalence of *H. pylori* infection in diabetic patients shows no difference with non-diabetics. In addition, the mucus thickness is thinner in diabetic dyspeptic patients with *H. pylori* infection compared to patients without *H. pylori* infection.

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