Turk J Gastroenterol 2003; 14 (1): 44-49

Predictors of vitamin B-12 deficiency: Age and helicobacter pylori load of antral mucosa

Vitamin B12 eksikliğinin prediktörleri: yaş ve antral mukozanın helicobacter pylori yükü

Yüksel GÜMÜRDÜLÜ[1], Ender SERİN[1], Birol ÖZER[1], Fazilet KAYASELÇUK[2], Kemal KUL[1], Cengiz PATA[1], Mustafa GÜÇLÜ[1], Gürden GÜR[3], Sedat BOYACIOĞLU[3]

Başkent University Faculty of Medicine Departments of Gastroenterology[1] and Pathology[2], Adana Teaching and Medical Research Center and Division of Gastroenterology[2] Ankara Hospital

Background/aims: *The aim of this study was to assess the frequency of vitamin B12 deficiency in patients with non atrophic gastric mucosa and any relationship between the presence of vitamin B12 deficiency and demographic, hematologic, and histopathotogic parameters.* ***Methods:*** *Three hundred and ten patients with no gastric mucosal atrophy on histologic evaluation were included in the study. Chronic inflammation, neutrophil activity and H. pylori load were scored using the Sydney classification system. Variables that might influence or predict the presence of vitamin B12 deficiency (age, gender, hemoglobin, mean corpuscular volume, serum folate level, scores of histologic parameters) were evaluated by univariate I multivariate analysis.* ***Results:*** *The percentages of patients with vitamin B12 concentrations of < 250 pglmL, < 200 pglmL, and <100 pglmL were 67.4%, 46.8% and 6.5% respectively. Patient age and all three histologic were inversely related to vitamin B12 deficiency (p<0.05). By multivariate analysis, factors independently associated with serum vitamin B12 deficiency were age and antral H. pylori load (p<0.05).* ***Conclusions:*** *The higher frequency of vitamin B12 deficiency in this study compared with a western study maybe a reflection of the effect of H. pylori infection on serum vitamin BJQ level. In addition, age was shown to be an independent risk factor for vitamin B12 deficiency irrespective of gastric atrophy. It is already known that the presence of H. pylori on gastric mucosa influence serum vitamin B12 levels. Hematologic parameters are not useful in predicting the deficiency of this vitamin.*

Key words: *H. pylori,* vitamin B_{12}, age, gender, hemoglobin, mean corpuscular volume

Amaç: *Non atrofik gastrik mukozası olanlar hastalarda vitamin B12 eksikliğinin sıklığını ve vitamin* B12 *eksikliği ile demografik, hematolojik ve histolojik parametrelerin ilişkisini değerlendirmeyi amaçladık.* ***Yöntem:*** *Histolojik değerlendirmede gastrik mükozal atrofisi olmayan 310 hasta çalışmaya dahil edildi. Kronik inflamasyon, nötrofil aktivasyonu ve H. pylori yükü Sydney sınıflaması kullanılarak skorlandı.vitamin B12 eksikliğini etkileyebilecek veya predikte edebilecek yaş, cinsiyet, hemoglobin, MCV, serum folat seviyesi ve histolojik parametrelerin skoru gibi değişiklikler univaryant / multivaryant analizlerle değerlendirildi.* ***Bulgular:*** *Hastaların %67,4'ünde vitamin B_{12}< 250 pglmL, 46.8%'inde < 200 pglmL ve 6.5%'inde <100 pg/mL idi. Hastaların yaşı ve histolojik özelliklerin tümü ile vitamin B12 eksikliği arasında negatif korelasyon vardı (p<0.05). Multivaryant analizde yaş ve antral H. pylori yükü vitamin B12 eksikliği ile bağımsız olarak ilişkiliydi (p<0.05). Sonuç: Batıda yapılan çalışmaya göre yüksek sıklıkta vitamin B12 eksikliğinin olması H. pylori enfeksiyonunun serum vitamin B12 enfeksiyonuna etkisini yansıtıyor olabilir. Bu gastrik mükozal atrofi bulunmayan hasta grubu, yaşın gastrik atrofi ile ilişkisiz olarak vitamin B12 eksikliğinin bağımsız risk faktörü olduğunu ve her halükarda gastrik mukozada H. pylori bulunmasının vitamin B12 seviyesini etkilediğini göstermiştir. Bu vitaminin eksikliğini predikte etmekte hematolojik parametreler kullanışlı değildir.*

Anahtar kelimeler: : *H. pylori,* vitamin B_{12}, yaş, cinsiyet, ortalama eritrosit hacmi

INTRODUCTION

Pernicious anemia with its classical feature of atrophic gastric mucosa has been considered leading cause of cobalamin deficiency by earlier studies. Growing evidence of the relationship between H. pylori infection and food-cobalamin malabsorption (1,2) has led to the suggestion that chronic H. pylori gastritis may be the most frequent cause of cobalamin deficiency, especially in developing countries. This suggestion was supported by two different studies evaluating the effect of eradication treatment on the improvement of vitamin B12 deficiency in patient groups either with atrophic (3) or non-atrophic gastric mucosa (4). This probable link between cobalamin deficiency and H. pylori infection even in patients with non-atrophic gastric mucosa, led to present study, evaluating the frequency of this deficiency in subjects with non-atrophic gastric mucosa. In addi-

Address for correspondence: Dr. Ender SERİN
Başkent Üniversitesi Tıp Fakültesi Adana Uygulama ve Araştırma Merkezi Dadaloğlu Mahallesi, 39 Sokak, No: 6 01250 Adana, Turkey
Telefon: +90-322-3272727-Fax: +90-322-3271273
E-mail: eserin67@yahoo.com - yukselgumurdulu@hotmail.com

Manuscript received: 25.2.2003 **Accepted:** 20.3.2003

tion, any relationship between the presence of cobalamin deficiency and demographic, hematologic, and histopathologic parameters was evaluated.

MATERIALS AND METHODS

Three hundred and ten patients who were referred for upper gastrointestinal endoscopic examination to evaluate dyspeptic complaints and who fulfilled the criteria as follows were included in the study: no previous of history of gastrointestinal tract surgery, organic and metabolic disease (diabetes mellitus, chronic liver diseases, renal failure), bile lake (which is a feature of duodenogastric reflux) at endoscopy no blood transfusion in the previous-six months; minimal or no atrophy of gastric mucosa in histopathologic examination and no intake of antibiotics, proton pump inhibitors, H2 receptor blockers or non-steroidal anti inflammatory drugs for two months prior to the study. The protocol of this study was approved by the Institutional Review Board and Local Ethics Committee.

Two biopsy specimens from the gastric antrum and two from the corpus were examined in each patient. The tissues were prepared with hematoxylin and eosin and Giemsa staining. Findings for chronic inflammation (based on lymphocyte and plasma cell infiltration of the lamina propria), neutrophil activity, atrophy, and *H. pylori* density were scored using a semi-quantitative scale, as follows: 0=none, l=mild, 2=moderate, and 3=marked. Histologic gastritis was defined using the Sydney classification (5). Biopsies showing at least a grade *I* of antrum mucosa and grade O of corpus mucosa for chronic inflammation were diagnosed as histologic antral gastritis, and grade 1 in both locations as histologic pangastritis. The same pathologist, who was blinded to the patient's clinical condition, performed all histological examinations.

Serum cobalamin and folate levels were determined by chemiluminescent enzyme immunoassay in an IMMULITE automated analyzer (DPC, Los Angeles, CA, USA), and hemoglobin level and mean corpuscular volume (MCV) were measured by standard methods.

Statistical Analysis: Demographic, hematologic, and histopathologic variables that might influence or predict the presence of cobalamin deficiency were analyzed. Variables included in univariate analysis were age, gender, hemoglobin, mean corpuscular volume, serum folate level, scores of histologic parameters. The variables for which the p value was less than 0.1 by univariate analysis were analyzed by multivariate analysis.

The Student t-test or chi-square test was used to evaluate the variables that can influence serum vitamin B 12 level or predict the presence of deficiency of this vitamin. The mean values of serum vitamin B12 in patients with different grades of histologic features were compared using one-way ANOVA. Spearman correlation analysis was performed to evaluate the relationship between histologic parameters. Logistic regression analysis was performed to identify independent factors associated with vitamin B 12 deficiency, the presence of which was accepted if the serum level was less than 200 pg/rnL. This level is commonly used clinical cutoff for a vitamin B12 deficiency status (6). Quantitative data were summarized as mean ± SD and compared with Mann-Whitney U test or Student-t test as appropriate. A p value of < 0.05 was considered statistically significant.

RESULTS

The patient population consisted of 102 men and 208 women with a mean age of 43 ± 12 (rangelG to 75) years. The mean serum vitamin B-12 concentration for 310 patients was 216 pg/mL. The percentages of patients with concentrations < 250 pg/mL, < 200 pg/mL and <100 pg/mL were 67.4%, 46.8%, and 6.5% respectively. Patients with <200 pg/mL of serum vitamin B12 concentration were older than those with >200 pg/mL ($p<0.01$). Sex and other hematologic parameters of patients with or without vitamin B12 deficiency did not show any significant difference by univariate analysis (Table 1).

Table 1. Demographic and hematologic data of patients with or without vitamin B -12 deficiency.

Variable	Patients with B_{12}<200 pg/mL (N=145)	B_{12}>200 pg/mL (N=165)	p value
Age	46 ± 12	42 ± 12	0.007
Sex (male/female)	52/93	50/115	0.299
Hemoglobin (g/dL)	13.8 ± 1.4	13.6 ± 1.4	0.153
MCV (fL)	88 ±7	87 ±6	0.338
Serum folate (ng/mL)	10.8 ± 4.6	11.4 + 5.0	0.329

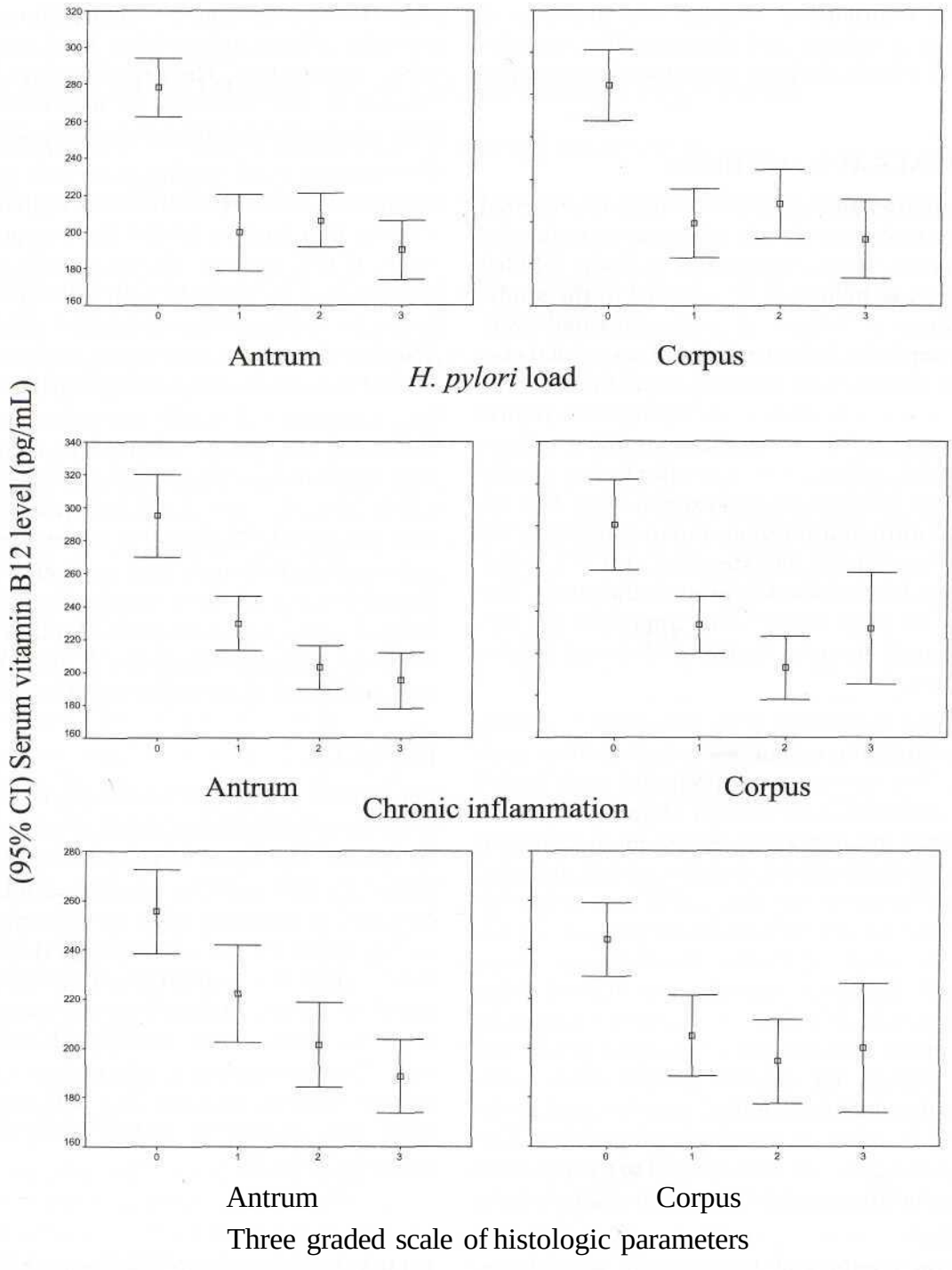

Figure 1. The effects of *H. pylori* load, chronic inflammation, and neutrophil activity in antrum and corpus on serum vitamin B12 level. Analysis was performed by one-way ANOVA and p value for each graphic was <0.001.

H. pylori load in both the antrum and corpus was well correlated with inflammation and activity scores (Table 2). Sixty-eight patients were found to be negative for H. pylori by histopathologic examination. Serum vitamin B-*12* level was inversely related with H. pylori load, chronic inflammation, and neutrophil activity in the antrum and corpus (p<0.001) (Figure 1). The mean value of serum vitamin B12 was higher in patients without H. pylori compared to those with H. pylori (280±64 pg/mL and *199+75* pg/mL respectively, p<0.001), but the mean value of serum folate did

Table 2. Relationships between *PI. pylori* load and other histologic findings in gastric mucosa.

		Correlation coefficient	p value
Antrum	*H. pylori* load-inflammation	0.653	<0.001
	H. pylori load-activity	0.700	<0.001
Corpus	*H. pylori* load -inflammation	0.590	<0.001
	H. pylori load-activity	0.660	<0.001

not show difference between these two patient groups (11.3+5.2 ng/mL and 11.1±4.8 ng/mL, respectively, p>0.05).

By multivariate analysis, factors independently associated with serum vitamin B^2 deficiency were age and antral H. pylori load. Patients with a high score of neutrophil activity in antrum tended to have vitamin B-^ deficiency (p=0.059) (Table 3).

Table 3. Independent factors associated by multivariate analysis with vitamin B12 deficiency (< 200pg/mL).

Variables	OR	95% CI	p value
Age	1.0357	1.0135 - 1.0584	0.0015
H. pylori load (antrum)	1.4828	1.0023 - 2.1937	0.0487
Chronic inflammation (antrum)	1.2130	0.7426 - 1.9814	0.4405
Neutrophil activation (antrum)	1.4409	0.9859 - 2,.1058	0.0592
H. pylori load (corpus)	1.2188	0.8328 - 1.7837	0.3085
Chronic inflammation (corpus)	1.0811	0.6834 - 1.7103	0.7388
Neutrophil activation (corpus)	0.8737	0.5865 - 1.3015	0.5066

DISCUSSION

One of the factors accounting for a possible etiologic relationship between H. pylori infection and coronary heart disease has been suggested to be hyperhomocysteinemia (7). Based on the fact that vitamin B12 > B6, and folate act as-cofactors in homocysteine metabolism, the effect of H. pylori infection on the malabsorption of these vitamins and the expected outcome of hyperhomocyteinemia has become the subject of some recent studies. Helicobacter pylori has been determined as a an etiologic factor in vitamin B 12 deficiency, either by using the specific absorption test (1,2) or evaluating the effect of eradication treatment on serum vitamin B12 level (3,4). Occult vitamin B-*12* deficiency can be the cause of severe neurologic diseases (8,9). In populations with a high prevalence of H. pylori infection, the frequency of vitamin B12 deficiency and its clinical consequences can be expected to be high. In this study the most commonly accepted cutoff value for low vitamin B12 status (<200 pg/mL) was used in patients undergoing upper endoscopic evaluation, and a markedly high frequency of vitamin B12 deficiency was found (46.8%). This figure is significantly higher than that of the Framingham Offspring Study (consisting of 2999 subjects) which was under taken to evoluate the association between vitamin B12 status and intake (10). This can be explained by different types of dietary intake and behavior among subjects from various countries and/or may be a reflection of the effect of H. pylori infection on serum vitamin B-12 level, due to a higher prevalence of this infection in countries such as Turkey compared with western countries.

The present study confirmed the weak correlation between vitamin B-12 deficiency and MCV that has been noted in the literature and that subjects with low serum vitamin B-12 level are not absolutely anemic (8,9,11). In spite of the suggestion that H. pylori-induced chronic atrophic gastritis decreases plasma folate level (12), we did not observe a difference between serum folate levels of patients with or without nonatrophic H. pylori, gastritis. These different observations may depend on the presence of gastric mucosal atrophy in patients included in other studies since decreased gastric acid secretion may cause reduced dietary folate absorption (13) or be due to a higher dietary intake of folate in Turkey (such as tea which is a very commonly consumed drink) (14). This may be clinically important because high or normal serum folate levels can mask hematologic abnormalities seen in cobalamin deficiency, and furthermore some severe and irreversible neurologic disorders may occur (15).

The possible etiologic factors behind the high prevalence of vitamin B-12 deficiency in older persons are dietary deficiency and malabsorption from atrophic gastritis. This study indicated the older age is an independent factor for the presence of vitamin B-12 deficiency and disproved the possibility of malabsorption, because the study included only patients without gastric mucosal atrophy. Studies showing that some elderly patients have inadequate dietary vitamin intake (16) and that most elderly patients with low vitamin B12 concentrations have normal Schilling test results (17) confirm the likelihood that dietary deficiency is the etiologic factor in a low vitamin B12 status of elderly subjects.

Three of the histologic parameters of Sydney classification (neutrophil activitiy, chronic inflammation and *H. pylori* load) in both antrum and corpus showed a strong correlation with serum vitamin B-12 concentration in the univariate analysis. Multivariate analysis indicated that low vitamin B12 status was significantly related to antral H. pylori load (p=0.048), and to antral neutrophil activity (p=0.059). Recent studies have suggested a strong relationship between *H. pylori* infection and vitamin B 12 deficiency. The mucosal atrophy of the corpus mucosa in pernicious anemia is thought to be the reason for malabsorption of vitamin B12 i[n] *H. pylori* infection. However, this hypothesis has been refuted in studies showing either improvement of vitamin B 12 deficiency after eradication treatment of patients with non-atrophic *H. pylori* gastritis (4) or food-cobalamin malabsorption during *H. pylori* gastritis (1,2). The observation of *H. pylori* load as an independent factor in vitamin B12 deficiency only in the antrum is difficult to explain. Distribution of food in the stomach is a complex process. Although the extent to which *H. pylori* consumes vitamin B12 for its metabolism has not been precisely established, it may hav6 more time to take away vitamin B12 from food in the antrum compared with that in proximal stomach.

Histological activity has been recently shown to have some impact on gastric physiology, with suppression in somatostatin activity and hypergastrinemia during *H. pylori* infection being well-established findings (18-21). One of the suggested underlying mechanisms behind these hormonal changes is the observation of interaction between some cytokines and neuropeptides (22,23). Neutrophils may affect somatostatin levels through stimulating interleukin-8 secretion from gastric epithelium (24). Somatostatin is known to inhibit gastric acid secretion and intrinsic factor release (25,26). Reduced somatostatin in antral epithelium, probably due to the effect of neutrophils, does not support the negative relationship between histological activity and low vitamin B12 status in terms of vitamin B12 malabsorption due to low gastric acid and intrinsic factor. Thus, the trend for negative correlation between antral activity and vitamin B12 deficiency in the present study is probably a reflection of the fact that the severity of activity depends on *H. pylori* load.

In conclusion, this patient cohort without gastric mucosal atrophy showed that hematologic parameters are not useful in the prediction of vitamin B12 deficiency. Age is an independent factor for vitamin B12 deficiency, irrespective of gastric atrophy. The presence of *H. pylori* on gastric mucosa influences serum vitamin B12 level by anyway and thus vitamin B12 deficiency is a strong indication that diagnostic evaluation for *H. pylori* infection should be undertaken. The prevalence of vitamin B12 deficiency can be expected to be high in countries with a high incidence of *H. pylori* infection and precautions against the clinical consequences of vitamin B12 deficiency, some of which can be subtle, should be taken.

REFERENCES

1. Carmel R, Perez-Perez GI, Blaser MJ. Helicobacter pylori infection and food-cobalamin malabsorption. Dig Dis Sci 1994; 39: 309-14.
2. Carmel R, Aurangzeb I, Qian D. Associations of food-cobalamin malabsorption with ethnic origin, age, Helicobacter pylori infection, and serum markers of gastritis. Am J Gastroenterol 2001; 96: 63-70.
3. Kaptan K, Beyan C, Ural AU et al. Helicobacter pylori-Is it a novel causative agent in vitamin B12 deficiency? Arch Intern Med 2000; 16,0: 1349-53.
4. Serin E, Gümürdülü Y, Özer B et al. Impact of Helicobacter pylori on the development of vitamin B12 deficiency in the absence of gastric atrophy. Helicobacter 2002; 7:337-41.
5. Dixon MF, Genta RM, Yardley JH et al. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. Am J Surg Pathol 1996;20:1161-81.
6. World Health Organization. Nutritional anemias. Report of a Scientific Group. World Health Organ Tech Rep Ser 1968;405.
7. Welch GN, Loscalzo J. Homocysteine and atherothrombosis. N Engl J Med 1998;338:1042-50.
8. Shanoudy H, Salem AG. Neuropsychiatric manifestations of vitamin B12 deficiency in the absence of anemia or macrocytosis: a case report. S D J Med 1992; 45:129-31.
9. Lindenbaum J, Healton EB, Savage DG et al. Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. N Engl J Med 1988; 318: 1720-28.
10. Tucker KL, Rich S, Rosenberg I et al. Plasma vitamin B-12 concentrations relate to intake source in the Framingham offspring studyl-3. Am J Clin Nutr 2000;71:514-522.
11. Carmel R. Pernicious anemia, the expected findings of very low serum cobalamin levels, anemia, and macrocytosis are often lacking. Arch Intern Med 1988;318:1720-8.
12. Tamura A, Fujioka T, Nasu M. Relation of Helicobacter pylori infection to plasma vitamin B12, folic acid, and homocysteine levels in patients who underwent diagnostic coronary arteriography. Am J Gastroenterol. 2002;97:861-6.

13. Lucock MD, Priestnall M, Daskalakis I et al. Nonenzymatic degradation and salvage of dietary folate: Physicochemical factors likely to influence bioavailability. Biochem Meloc Med 1995;55:43-55.
14. Chen TS, Lui CK, Smith CH. Folacin content of tea. J Am Diet Assoc 1983; 82: 627-32.
15. Reynolds EH. Benefits and risk of folic acid to the nervous system. J Neurol Neurosurg Psychiatry. 2002;72:567-71.
16. Van Asselt DZB, de Groot CPGM, van Staveren WA et al. Role of cobalamin intake and atrophic gastritis in mild cobalamin deficiency in older Dutch subjects. Am J Clin Nutr 1998;68:328-34.
17. Blundell EL, Matthews JH, Allen SM et al. Clin Pathol 1985;38:1179-84.
18. Moss SF, Legon S, Bishop AE et al. Effect of *H. pylori* on gastric somatostatin in duodenal ulcer disease. Lancet 1992;340:930-2.
19. 0dum L, Peterson HD, Anderson IB et al. Gastrin and somatostatin in Helicobacter pylori infected antral mucosa. Gut 1994;35:615-8.
20. Gür G, . Boyacıoğlu S, Gül C et al. Impact of Helicobacter pylori infection on serum gastrin in hemodialysis patients. Nephrol Dial Transplant 1999;14:2688-91.
21. Maconi G, Lazzoroni M, Sangaletti O et al. Effect of Helicobacter pylori eradication on gastric histology, serum gastrin and pepsinogen I levels, and gastric emptying in patients with gastric ulcer. Am J Gastroenterol 1997;92:1844-8.
22. Lehmann FS, Golodner EH, Wang J et al. Mononuclear cells and cytokines stimulate gastrin release from canine antral cells in primary culture. Am J Physiol 1996;270:G783-8.
23. Beales I, Blaser MJ, Srinivasan S et al. Effect of Helicobacter pylori products and recombinant cytokines on gastrin release from cultures canine G cells. Gastroenterology 1997;113:465-71.
24. Yamamato S, Kaneko H, Konagaya T et al. Interactions among gastric somatostatin, interleukin-8 and mucosal inflammation in Helicobacter pylori-positive peptic ulcer patients. Helicobacter 2001;6:136-145.
25. Komasaka M, Horie S, Watanabe K et al. Antisecretory effect of somatostatin on gastric acid via inhibition of histamine release in isolated mouse stomach. Eur J Pharmacol 2002;4:235-43.
26. Oddsdottir M, Ballantyne GH, Adrian TE et al. Somatostatin inhibition of intrinsic factor secretion from isolated guinea pig gastric glands. Scand J Gastroenterol 1987;22:233-8.