

Effects of *Helicobacter pylori* infection and long-term proton pump inhibitor use on enterochromaffin-like cells

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Abstract

Background Excessive release of gastrin leads to hypertrophy and hyperplasia of enterochromaffin-like cells (ECL) and prolonged stimulation of these cells causes functional impairment. The purpose of this study was to investigate the effect of *Helicobacter pylori* (*H. pylori*) infection and long-term proton pump inhibitors (PPI) use on ECL cells.

Methods Fifteen patients who underwent endoscopy because of dyspeptic symptoms were enrolled in the present study. Biopsies were taken from corpus and antrum and existence of *H. pylori* was investigated with culture, cytology and CLOtest. The patients were divided into 3 groups. Group-A: *H. pylori*-negative, never treated previously with PPI; Group-B: *H. pylori*-positive, never treated previously with PPI; and group-C: *H. pylori*-negative and continuously treated with PPI for more than 6 months before the subject recruitment period. The features of ECL cell in oxyntic glands were examined with electron microscopy on biopsy specimens.

Results ECL cells were completely normal in Group A. In group B, moderate hyperplasia and vacuolization was seen in ECL cells. In group C, ECL cell hyperplasia was observed and vacuoles with greater amounts of granules in enlarged vesicles were found more intensely in cytoplasm.

Conclusion The use of PPI for a long period of time and presence of *H. pylori* infection are risk factors for ECL hyperplasia.

Keywords Enterochromaffin-like cells, proton pump inhibitors, *Helicobacter pylori* infection
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Introduction

The epithelium of the mammalian stomach consists of parietal cells, chief cells, mucous cells, and endocrine cells. So-called enterochromaffin-like cells (ECL) are predominant among the endocrine cells [1]. They respond to gastrin and play an important role in the regulation of gastric acid secretion by mobilizing histamine to stimulate the parietal cells via H₂-receptor activation [2].

The ECL cells display a characteristic ultrastructure with

numerous, fairly large secretory vesicles, and a few electron-dense granules and small clear microvesicles.

Mobilization of histamine is associated with ultrastructural changes in the ECL cells, most notably the loss of secretory vesicles, which represents the presumed storage site of histamine [1,3,4]. In response to sustained hypergastrinemia, the ECL cells increase in size and number and start to develop vacuoles and lipofuscin bodies in their cytoplasm [5]. It has been suggested that both vacuoles and lipofuscin bodies are products of the process of crinophagy (autodigestion of secretory organelles), which allows overstimulated ECL cells to cope with excess secretory material [3-5].

Hypergastrinemia associated with long-term proton pump inhibitor (PPI) therapy may lead to ECL cell hyperplasia [6-9]. However, atrophic gastritis of corpus is also known to be associated with ECL cell hyperplasia and severity of atrophic gastritis is correlated with the grade of hyperplasia [10]. Chronic *Helicobacter pylori* (*H. pylori*) infection can also affect ECL cells via mucosal pro-inflammatory cytokines such as tumor necrosis factor (TNF)- α or interleukin (IL)-1 β elevated by *H. pylori*, and causes inhibition of histamine synthesis and secretion which

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Conflict of Interest: None

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can predispose to hypochlorhydria and hypergastrinemia. Additionally, continuous long-term PPI therapy has been found to be associated with progression of chronic atrophic gastritis and ECL cell hyperplasia in patients infected by *H. pylori* [8,9,11,12].

The aim of the present study was to examine structural changes in ECL cells with electron microscopy in the biopsy samples of gastric corpus mucosa in patients who were *H. pylori* negative, treated with PPI for a long time because of reflux disease and *H. pylori*-positive, not treated with PPI.

Patients and Methods

Fifteen patients undergoing endoscopic examination because of dyspeptic symptoms were enrolled in the study. The presence of *H. pylori* was sought on biopsies by CLOtest, culture and cytology. Nine patients were male and 6 were female. Mean age was 36.2 (20-56) years. The patients were divided into 3 groups. Group A: Five *H. pylori*-negative patients, who never previously received PPI therapy; Group B: Five *H. pylori*-positive patients, who never received PPI treatment; Group C consisted of 5 patients who were using long-term (> 6 months) PPI due to gastroesophageal reflux disease and were *H. pylori*-negative. The patients underwent repeat endoscopy and 4 biopsies were obtained from corpus for electron microscopy examination.

Corpus biopsy samples were fixed in buffered 10% formaldehyde and they were submitted to the same pathologist. After fixing for at least 24 h, the samples were dehydrated in alcohol and carefully oriented before embedding in paraffin. Sections from each corpus biopsy sample were stained by the Grimelius silver impregnation method to identify argyrophil cells. Biopsy samples were cut into 1 mm³ blocks and fixed in 2.5% glutaraldehyde for 2 h at 4 °C then post fixed in 2% osmium tetroxide for 2 h at 4 °C. The sections were then hydrated with ethanol and embedded in epoxy resin. Ultra-thin sections were prepared and double stained with uranium acetate and lead citrate, the sections were observed through transmission electron microscope (Leo 906 E transmission electron microscope, 80 kV Oberkochen- Germany).

ECL cell properties of oxyntic glands were investigated by transmission Jeol 100 electron microscopy. The ECL cells were identified by their characteristic ultrastructure [4-13]. Granules were defined as cytoplasmic membrane-enclosed organelles (with a diameter of 25–200 nm), displaying an electron-dense core and a thin, electron-lucent halo between the membrane and the dense core, the diameter of the dense core representing more than 50% of the diameter of the entire organelles.

Vesicles were membrane-enclosed, electron-lucent organelles, sometimes possessing a small, often eccentrically located dense core, which was less than 50% of the diameter of the vesicle. The lipofuscin bodies were identified by their high electron density and irregular shape. The ECL

cells contained few lipofuscin bodies, numerous vesicles and vacuoles (Fig. 1). The cell numbers and cytoplasmic changes in all three groups were evaluated qualitatively. Fasting serum gastrin concentrations (normal range; 5.6-29.3 pg/mL) were measured in 5 mm³ blood samples drawn via venous route.

Results

Regarding the electron microscopic examination in Group A, hyperplasia was not observed in ECL cells. While small numbers of vacuoles were detected in the cytoplasm, no histamine storage was observed. In Group B, moderate increase in the number of ECL cells was observed. There was also increase in the number of cytoplasmic vacuoles. In Group C, patients who were *H. pylori*-negative with a history of long-term PPI usage, ECL cell hyperplasia was more prominent compared to Group B and wide-large vacuoles and lipofuscin pigments were detected in the cytoplasm (Fig. 2).

Mean serum gastrin levels in group A and group B were detected as 18.42 pg/mL (10-28) and 27.62 pg/mL (14-36) respectively. Among patients in Group C who received PPI treatment for more than 6 months, serum gastrin level was detected as 125.67 pg/mL (68-267).

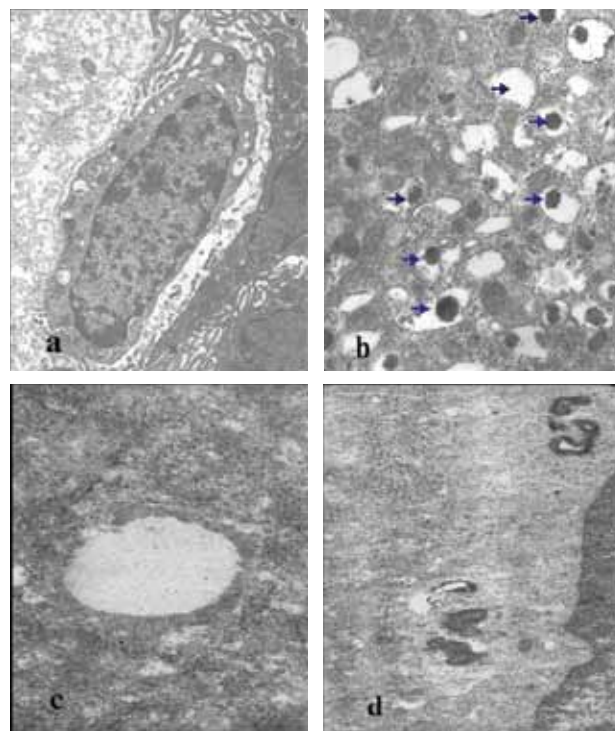


Figure 1 Enterochromaffin-like (ECL) cell and cytoplasmic organelles. (a) normal ECL cell, (b) granules, (c) vacuoles, (d) lipofuscin

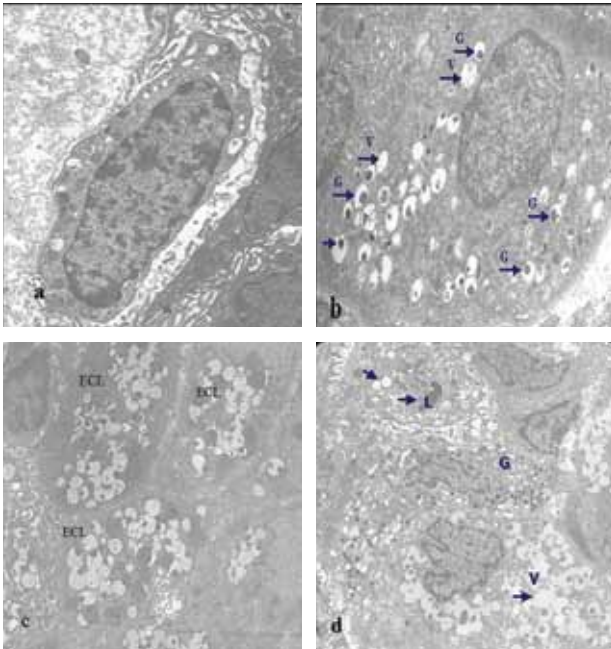


Figure 2 (a) *Helicobacter pylori* (*H. pylori*) and proton pump inhibitor (PPI)-negative group. Enterochromaffin-like (ECL) hyperplasia was not observed; only a small number of vacuoles were seen in the cytoplasm; no histamine storage was observed; (b) The *H. pylori*-positive and PPI-negative group. Histamine-containing granule accumulation within vesicles and vacuoles. (c-d) PPI-positive group. Hyperplasia, vacuoles, lipofuscin and granules in ECL cells

Discussion

ECL cells can be visualized by light microscopy, immunohistochemistry, and the electron microscopy (morphometry) technique [4,14]. Zhao et al identified characteristic ultrastructure of ECL cells with the electron microscopy technique [4,13]. The ECL cells display a characteristic ultrastructure with numerous, fairly large secretory vesicles, and a few electron-dense granules and small clear microvesicles [4]. The granules and vesicles in the ECL cells have been classified in electron-dense granules and electron-lucent secretory vesicles and microvesicles [15]. The granules are defined as membrane-enclosed, dense-cored organelles with a diameter of 25–200 nm, displaying a thin, electron-lucent halo between the membrane and the dense core, the diameter of the dense core being more than 50% of the diameter of the entire organelles. Vesicles are membrane-enclosed, electron-lucent organelles without a dense core or with a small, often eccentrically located dense core, the diameter of the dense core being less than 50% of the diameter of the organelle. Based on their profile size, vesicles belong to one of the three populations: 1. secretory vesicles with a diameter of 125–500 nm (with or without visible dense core); 2. vacuoles (seen in ECL cells of omeprazole-treated rats only) with a diameter of at least 500 nm (with one or more dense cores); and 3. clear, electron-lucent microvesicles (without dense core) with a diameter of 25–125 nm [15]. In the present

study, ECL cells were examined by electron microscopy and a qualitative method was used during the examination. This is a limitation for our study. In the group with *H. pylori*-negative patients who had never received PPI treatment previously, the number of ECL cells was normal and hyperplasia was not observed in ECL cells.

In patients treated with omeprazole, serum gastrin levels may increase up to 4-fold; this is accompanied by development of diffuse, linear, and/or micronodular ECL cell hyperplasia. Nishi et al administered high-dose omeprazole to patients with Barrett’s esophagus for 2 years, and investigated changes in gastric ECL cells using endoscopic biopsy specimens to clarify the etiology of hyperplasia of the parietal cells [16]. In another study, Rindi et al determined the effects of 5-year treatment with rabeprazole or omeprazole on the gastric mucosa. Two hundred and forty-three patients received rabeprazole (20 mg or 10 mg) or omeprazole (20 mg) once daily for up to 5 years, for gastro-esophageal reflux disease and 51% of the patients completed the whole 5 year period. ECL cell hyperplasia occurred in a minority of patients, and was associated with serum gastrin concentrations. No ECL cell dysplasia or tumors were observed [8]. In the present study, in *H. pylori*-negative patients who received PPI treatment for more than six months, there was a distinct hyperplasia in ECL cells and the numbers of granules, vesicles, vacuoles and inclusions were significantly greater with electron microscopy.

In the presence of *H. pylori* infection, acid suppression results in more marked gastrin increase and a higher risk of developing ECL cell hyperplasia than in non-infected subjects [12,17-20]. Infection with *H. pylori* may stimulate the production of IL-1 and nitric oxide, which are recognized inhibitors of gastric acid secretion. *H. pylori*-positive subjects under acid suppression also have elevated gastrin levels, which are 30-50% greater than in *H. pylori*-negative subjects. The reason seems to be that gastrin stimulates both ECL cell histamine secretion and proliferation [21]. Gastric carcinoids are rare neoplasms, and usually are seen only in the stomach of patients with severe atrophic gastritis, pernicious anemia or Zollinger Ellison syndrome [22-24]. However, other than a single case report of carcinoid in the apparent absence of atrophic gastritis or *H. pylori* infection, rigorous studies in adults have not shown carcinoids in patients treated with acid-blocking agents [22,25,26]. These studies showed that ECL cell hyperplasia in the context of patients treated with long-term PPIs is a benign change. It occurs with greater prevalence in PPI-treated patients who are *H. pylori*-positive, but it also occurs in individuals with chronic *H. pylori* antral gastritis who were never treated with acid-blocking agents [10,22,26,27].

There has been no evidence of the relationship between *H. pylori* infection and gastric carcinoids in humans. However, gastric cancer and gastric carcinoids have been reported in *H. pylori*-infected Mongolian gerbils [28,29]. Honda et al showed that gastric cancers appeared in 2 of 5 animals infected with *H. pylori* 18 months after *H. pylori* inoculation. Gastric cancers were similarly observed in 5 of 10 infected animals, 24 months after infection [30]. In another study,

Kawaga *et al* evaluated serum gastrin and histopathological examination of the stomach at 6, 12, 18, and 24 months after *H. pylori* inoculation in Mongolian gerbils. They observed gastric carcinoids in 5 of 10 infected animals at 24 months after infection [29].

In the present study, the ECL cell hyperplasia was moderately increased in the *H. pylori*-positive group compared to the *H. pylori*-negative group, which received no PPI treatment. The serum gastrin levels were found higher than in *H. pylori*-negative patients. However, the increase both in ECL cell hyperplasia and serum gastrin levels was not as high in the group using long-term PPI.

During long-term acid suppression, gastric fundic ECL cell hyperplasia may develop, especially in the presence of *H. pylori* infection. Acid-suppressive therapy alters the *H. pylori* gastritis, transforming the antrum-predominant pattern into a body-predominant pattern. Eissele *et al* investigated 42 patients with reflux esophagitis or peptic ulcer disease, who were treated with lansoprazole for up to 5 years. They

found an increased prevalence of linear/micronodular ECL cell hyperplasia from 6.7% to 54.5% in the *H. pylori*-positive patients, compared with an increase from 0% to 7.7% in the *H. pylori*-negative subjects [31]. In the present study, ECL cells were not evaluated in patients with *H. pylori*-positive and long-term acid suppressive therapy groups. This is a limitation of our study.

Chromogranin A (CgA) is a well-recognized marker of neuroendocrine neoplasia and is released into the circulation from the ECL cell population of the stomach [32–36]. The release of CgA increases during profound gastric acid inhibition, possibly reflecting the trophic effect of gastrin on the ECL cells. Sanduleanu *et al* evaluated serum CgA levels in 230 dyspeptic patients. The CgA levels were found to correlate with hypergastrinemia, duration of acid inhibition, *H. pylori* infection, body gland atrophy and ECL cell hyperplasia. They showed that in subjects on long-term acid inhibition, serum CgA was equally sensitive but more specific than serum gastrin for the detection of ECL cell hyperplasia [37]. In the present study, serum CgA levels were not measured in all study groups.

To conclude, in the present study an increased density of ECL cells was observed in the *H. pylori*-positive group and patients treated with long-term acid suppressive therapy. *H. pylori* may be an important factor for the progression of fundic gastritis and the development of argyrophil cell hyperplasia during long-term treatment with PPI. For this reason, if PPI therapy is needed for a long time, *H. pylori* infection must be eradicated.

Summary Box

What is already known:

- The use of proton pump inhibitors for a long period and presence of *H. pylori* infection are risk factors for enterochromaffin-like cell hyperplasia
- Long-term PPI therapy has been associated with progression of chronic atrophic gastritis and ECL cell hyperplasia in patients infected by *Helicobacter pylori*
- Hypergastrinemia which is caused by long-term proton pump inhibitor therapy may lead to ECL cell hyperplasia
- Chronic *Helicobacter pylori* infection can also affect ECL cells via mucosal pro-inflammatory cytokines such as tumor necrosis factor- α or interleukin- 1β .
- These cytokines cause inhibition of histamine synthesis and secretion which can predispose to hypochlorhydria and hypergastrinemia

What the new findings are:

- The changing of enterochromaffin like cells in long-term users of proton pump inhibitors and *Helicobacter pylori* in patients can be shown with electron microscopic examination
- *Helicobacter pylori* infection is an important factor for the progression of fundic gastritis and the development of argyrophil cell hyperplasia during long-term PPI therapy
- For this reason, if PPI therapy is needed for a long time, *Helicobacter pylori* infection must be eradicated

References

1. Håkanson R, Chen D, Sundler F. The ECL cells. In: Johnson LR (editor): Physiology of the gastrointestinal tract. Raven: New York; 1994, pp.1171-1184.
2. Andersson K, Chen D, Mattsson H, Sundler F, Håkanson R. Physiological significance of ECL-cell histamine. *Yale J Biol Med* 1998;71:183-193.
3. Chen D, Zhao CM, Lindström E, Håkanson R. Rat stomach ECL cells: up-date of biology and physiology. *Gen Pharmacol* 1999;32:413-422.
4. Zhao CM, Chen D, Lintunen M, Panula P, Håkanson R. Secretory organelles in rat stomach ECL cells. An immunohistochemical and electron-microscopic study. *Cell Tissue Res* 1999;298:457-470.
5. Zhao CM, Chen D, Andersson K, Håkanson R. ECL cells of the rat stomach: development of lipofuscin in response to sustained gastrin stimulation. *Cell Tissue Res* 1998;291:315-323.
6. Andersson K, Chen D, Håkanson R, Mattsson H, Sundler F. Enterochromaffin-like cells in the rat stomach: effect of alpha-fluoromethylhistidine-evoked histamine depletion. A chemical, histochemical and electron microscopic study. *Cell Tissue Res* 1992;270:7-13.
7. Genta RM, Rindi G, Fiocca R, Magner DJ, D'Amico D, Levine DS. Effects of 6-12 months of esomeprazole treatment on the gastric mucosa. *Am J Gastroenterol* 2003;98:1257-1265.
8. Rindi G, Fiocca R, Morocutti A, Jacobs A, Miller N, Thjodleifsson B. Effects of 5 years of treatment with rabeprazole or omeprazole on the gastric mucosa. *Eur J Gastroenterol Hepatol* 2005;17:559-566.
9. Solcia E, Fiocca R, Havu N, Dalvåg A, Carlsson R. Gastric endocrine cells and gastritis in patients receiving long-term

- omeprazole treatment. *Digestion* 1992;**51**:82-92.
10. Havu N, Maarooos HI, Sipponen P. Argypophil cell hyperplasia associated with chronic corpus gastritis in gastric ulcer disease. *Scand J Gastroenterol* 1991;**186**:90-94.
 11. Klinkenberg-Knol EC, Festen HP, Jansen JB, et al. Long-term treatment with omeprazole for refractory reflux esophagitis: efficacy and safety. *Ann Intern Med* 1994;**121**:161-167.
 12. Kuipers EJ, Lundell L, Klinkenberg-Knol EC, et al. Atrophic gastritis and Helicobacter pylori infection in patients with reflux esophagitis treated with omeprazole or fundoplication. *N Engl J Med* 1996;**314**:1018-1022.
 13. Zhao CM, Chen D, Lintunen M, Panula P, Håkanson R. Effects of reserpine on ECL-cell ultrastructure and histamine compartmentalization in the rat stomach. *Cell Tissue Res* 1999;**295**:131-140.
 14. Solcia E, Rindi G, Havu N, Elm G. Qualitative studies of gastric endocrine cells in patients treated long-term with omeprazole. *Scand J Gastroenterol* 1989;**24**:129-137.
 15. Chen D, Zhao C-M, Andersson K, Sundler F, Håkanson R. Ultrastructure of enterochromaffin-like cells in rat stomach: effects of alpha-fluoromethylhistidine-evoked histamine depletion and hypergastrinemia. *Cell Tissue Res* 1996;**283**:469-478.
 16. Nishi T, Makuuchi H, Weinstein WM. Changes in gastric ECL cells and parietal cells after long-term administration of high-dose omeprazole to patients with Barrett's esophagus. *Tokai J Exp Clin Med* 2005;**30**:117-121.
 17. Katelaris PH, Seow F, Lin BP, Napoli J, Ngu MC, Jones DB. Effect of age, Helicobacter pylori infection, and gastritis with atrophy on serum gastrin and gastric acid secretion in healthy men. *Gut* 1993;**34**:1032-1037.
 18. Mossi S, Meyer-Wyss B, Renner EL, Merki HS, Gamboni G, Berlinger C. Influence of Helicobacter pylori, sex, and age on serum gastrin and pepsinogen concentrations in subjects without symptoms and patients with duodenal ulcers. *Gut* 1993;**34**:752-756.
 19. El-Omar EM, Penman ID, Ardill JE, Chittajallu RS, Howie C, McColl KE. Helicobacter pylori infection and abnormalities of acid secretion in patients with duodenal ulcer disease. *Gastroenterology* 1995;**109**:681-691.
 20. Kuipers EJ, Uytendaele AM, Peña AS, et al. Increase of Helicobacter pylori associated corpus gastritis during acid suppressive therapy: implications for long-term safety. *Am J Gastroenterology* 1995;**90**:1402-1406.
 21. Modlin IM, Tang LH. The gastric enterochromaffin-like cell: an enigmatic cellular link. *Gastroenterology* 1996;**111**:783-810.
 22. Kuipers EJ. Proton pump inhibitors and gastric neoplasia. *Gut* 2006;**55**:1217-1221.
 23. Solcia E, Bordi C, Creutzfeldt W, et al. Histopathological classification of nonantral gastric endocrine growths in man. *Digestion* 1988;**41**:185-200.
 24. Jensen RT. Consequences of long-term proton pump blockade: insights from studies of patients with gastrinomas. *Basic Clin Pharmacol Toxicol* 2006;**98**:4-19.
 25. Haga Y, Nakatsura T, Shibata Y, et al. Human gastric carcinoid detected during long-term antiulcer therapy of H2 receptor antagonist and proton pump inhibitor. *Dig Dis Sci* 1998;**43**:253-257.
 26. Klinkenberg-Knol EC, Nelis F, Dent J, et al. Long-term omeprazole treatment in resistant gastroesophageal reflux disease: efficacy, safety, and influence on gastric mucosa. *Gastroenterology* 2000;**118**:661-669.
 27. Lamberts R, Creutzfeldt W, Struber HG, Brunner G, Solcia E. Long-term omeprazole therapy in peptic ulcer disease: gastrin, endocrine cell growth, and gastritis. *Gastroenterology* 1993;**104**:1356-1370.
 28. Watanabe T, Tada M, Nagai H, Sasaki S, Nakao M. Helicobacter pylori infection induces gastric cancer in Mongolian gerbils. *Gastroenterology* 1998;**115**:642-628.
 29. Kagawa J, Honda S, Kodama M, Sato R, Murakami K, Fujioka T. Enterocromaffin-like cell tumor induced by Helicobacter pylori infection in Mongolian gerbils. *Helicobacter* 2002;**7**:390-397.
 30. Honda S, Fujioka T, Tokieda M, Satoh R, Nishizono A, Nasu M. Development of Helicobacter pylori-induced gastric carcinoma in Mongolian gerbils. *Cancer Res* 1998;**58**:4255-4259.
 31. Eissele R, Brunner G, Simon B, Solcia E, Arnold R. Gastric mucosa during treatment with lansoprazole: Helicobacter pylori is a risk factor for argyrophil cell hyperplasia. *Gastroenterology* 1997;**112**:707-717.
 32. O'Connor DT, Deftos LS. Secretion of chromogranin A by peptide producing endocrine cells. *N Engl J Med* 1986;**314**:1145-1151.
 33. Stridsberg M, Oberg K, Li Q, Engstrom U, Lundqvist G. Measurement of chromogranin A, chromogranin B (secretogranin I), chromogranin C (secretogranin II) and pancreastatin in plasma and urine of patients with carcinoid tumours. *J Endocrinol* 1995;**144**:49-59.
 34. Håkanson R, Ding XQ, Norlén P, Chen D. Circulating pancreastatin is a marker for the enterochromaffin-like cells of the rat stomach. *Gastroenterology* 1995;**108**:1445-1452.
 35. Stabile BE, Howard TJ, Pasaro E, O'Connor DT. Source of plasma chromogranin A elevation in gastrinoma patients. *Arch Surg* 1990;**125**:451-453.
 36. Borch K, Stridsberg M, Burman P, Rehfeld JF. Basal chromogranin A and gastrin concentrations in circulation correlate to endocrine cell proliferation in type-A gastritis. *Scand J Gastroenterol* 1997;**32**:198-202.
 37. Sanduleanu S, De Bruïne A, Stridsberg M, et al. Serum chromogranin A as a screening test for gastric enterochromaffin-like cell hyperplasia during acid-suppressive therapy. *Eur J Clin Invest* 2001;**31**:802-811.