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If the *Helicobacter pylori* infection may affect ghrelin secretion in children

Czy zakażenie *Helicobacter pylori* u dzieci ma wpływ na wydzielanie ghreliny

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Summary

Introduction. *Helicobacter pylori* is considered as an etiologic agent of the gastric mucosa diseases. Therefore, it may affect ghrelin secretion, known as "hunger hormone", by neuroendocrine cells of gastric mucosa.

Aim. The aim of our study is to determine serum ghrelin concentrations in *H. pylori* infected and noninfected children.

Materials and methods. The study involved 84 children, 6-18 years old, living in urban area (Bytom). All patients underwent endoscopy. Biopsy speciments were taken in antrum of the stomach. On the base of the results of urease test and/or positive histological examination (hematoxylin-eosin stained) *H. pylori* infection was recognized in 33 children (Group I) and in 51 children (Group II) was excluded. Histological lesions of gastric mucosa were diagnosed as: chronic gastritis non active of mild grade or active of mild grade. Serum ghrelin concentration was measured by RIA (Human Radioimmunoassay kit, total ghreline; Millipore).

Results. There was no significant difference between serum ghrelin concentration in children from Groups I and II. Moreover, in both groups evaluated seperately as well as in the whole group, there was no relationship between the ghrelin levels and the histological estimation of gastric mucosa lesions.

Conclusions. Our results suggests that *H. pylori* infection with mild grade of gastric mucosa inflammation does not affect serum ghrelin level in children.

Key words: ghrelin, *Helicobacter pylori*, children

Streszczenie

Wstęp. *Helicobacter pylori* jest uznanym czynnikiem patogenetycznym chorób błony śluzowej żołądka. Może zatem wpływać na wydzielanie ghreliny określanej mianem „hormonu głodu".

Cel. Celem pracy jest ocena stężeń ghreliny w surowicy dzieci zakażonych i niezakażonych *H. pylori*.

Materiał i metody. Badaniami objęto 84 dzieci z bólami brzucha, w wieku 6-18 lat, mieszkających w środowisku wielkomiejskim (Bytom). Na podstawie wyników testu ureazowego i/lub obecności *H. pylori* w badaniu histopatologicznym bioptatów błony śluzowej pobranych w czasie gastroskopii z *antrum* żołądka, u 33 rozpoznano (grupa I), a u 51 dzieci wykluczono (grupa II) zakażenie *H. pylori*. Stwierdzane zmiany zapalne miały charakter *gastritis chronica non activa minimalis* lub *activa minimalis*. Stężenie ghreliny oznaczono w surowicy badanych metodą radioimmunologiczną (Human Radioimmunoassay kit, total ghreline; Millipore).

Wyniki. Nie stwierdzono statystycznie znamiennej różnicy stężeń ghreliny w surowicy dzieci z grup I i II, ani związku tych stężeń z obrazem histologicznym błony śluzowej badanych w obu grupach oddzielnie, ani też u wszystkich dzieci rozpatrywanych łącznie.

Wnioski. Uzyskane wyniki sugerują, że zakażenie *H. pylori* powodujące zmiany zapalne błony śluzowej żołądka o niewielkim nasileniu nie powoduje u dzieci zmian stężenia ghreliny w surowicy.

Słowa kluczowe: ghrelina, *Helicobacter pylori*, dzieci

INTRODUCTION

In addition to the central nervous system, the gastrointestinal tract plays a significant role in appetite regulation and medical conditions of the individual organs of this multi-organ system are of fundamental importance for nutritional behaviours and the nutritional condition of persons. An inflammatory condition of the gastric mucosa accompanied by a *Helicobacter pylori* (*H. pylori*) infection may also affect the appetite of sick children. The *H. pylori* influence on the secretion of ghrelin, called the "hunger hormone", is one of the possible mechanisms here. It is synthesised by Gr cells located mostly in the gastric mucosa of the body of the stomach (1). It is a 28-amino-acid peptide encoded by the preproghrelin gene. Ghrelin is a strong stimulator of growth hormone secretion, it stimulates the appetite, resulting in an increase in the body weight (2). It influences pre-adipocyte differentiation, accumulation of fat in the fatty tissue as well as the energy balance of the human body (3), both in the short-term and long-term regulation (4). It also plays an important role in the regulation of stomach motility and hydrochloric acid secretion (5).

H. pylori infections occur all over the world and they affect over 50% of the adult population (5). In the population of children, the frequency of infections increases with the age (6). In view of little possibility of spontaneous eradication, this infection lasts for many years – from childhood to adulthood. Taking into consideration the frequent occurrence of the lack of appetite in children and its changeability in children infected with *H. pylori*, one can assume that altered ghrelin secretion can be one of the reasons for this. The results of serum/plasma ghrelin concentrations in persons infected with *H. pylori* are divergent.

AIM

The aim of this study is to assess serum ghrelin concentrations in children infected with *H. pylori*.

MATERIALS AND METHODS

84 children aged 6-18, living in urban area (Bytom), took part in the study. The children were patients at the Chair and Department of Paediatrics in Bytom of the Medical University of Silesia in Katowice. In connection with reported chronic or recurrent abdominal pains located in the epigastric or middle abdominal regions, these children had diagnostic tests performed. There were no patients after *H. pylori* eradication in the group. Diagnostic tests included: blood tests (ESR, the concentrations of aminotransferases, urea and creatinine), urine and stool tests (tests for parasites, assessment of the presence of human haemoglobin in faeces) and an ultrasound examination of the abdomen.

On the basis of a fiberoscopic examination (Olympus GIF-XP 20) combined with a urease test (National Food and Nutrition Institute, Warsaw) and an histopathology assessment of gastric mucosa specimens collected from the prepyloric region, an *H. pylori* infection was diagnosed in 33 persons (23 girls and 10 boys – Group I), and it was ruled out in 51 children (23 girls and 28 boys – Group II). The gastric mucosa preparations were dyed with haematoxylin and eosin. A positive result of the urease test and/or confirmation of the presence of this bacteria obtained in the histopathologic examination were the basis for the diagnosis. Together with blood samples collected after night fast for tests connected with the reason of hospitalisation, 2 ml of blood was collected to determine the ghrelin concentration. After centrifuging, serum samples were frozen and stored until measurements at -30°C. The ghrelin concentration was determined by means of a radioimmunological method (Human Radioimmunoassay kit, total ghrelin; Millipore). The results are presented in pg/ml.

On the basis of the histopathologic assessment of gastric mucosa specimens, an *H. pylori* infection was ruled in or out and the normal picture of the gastric mucosa was found in 2 Group I patients and 20 Group II patients, *gastritis chronica non activa minoris gradus* in 25 Group I patients and 27 Group II patients, *gastritis chronica activa minoris gradus* in 8 persons from Group I and 2 persons from Group II.

An approval for the research was obtained from the Bioethics Committee of the Medical University of Silesia in Katowice (KNW/0022/KB1/208/11/12).

The Mann-Whitney U-test was used to compare ghrelin concentrations in Groups I and II and single classification analysis of variance (confirmed by Tukey's test) was performed to examine the relationship between ghrelin concentrations and the type of histopathological lesions of the gastric mucosa in Group I, Group II and in both groups combined. Double classification analysis of variance was used to check the simultaneous influence of the patients' gender and the presence of *H. pylori* infection on the levels of ghrelin concentrations in the serum and a simultaneous influence of the intensity of histopathological lesion and the presence of *H. pylori* on the serum ghrelin concentrations.

Low absolute values of skewness and kurtosis (< 4) made it possible to use parametric tests.

RESULTS

No statistically significant difference was observed between the ghrelin concentrations in the serum of children infected and not infected with *H. pylori* (tab. 1).

The hypothesis of the existence of the influence of the patient's gender and *H. pylori* infection on the serum ghrelin concentrations was verified by double classification analysis of variance. It was found that the influence of the gender is not significant in this respect and the influence of *H. pylori* was on the borderline of significance ($p = 0.054$). The interaction between the two variables was not significant ($p = 0.72$). No statistical relationship was between ghrelin concentrations and the intensity of histopathological lesions of the gastric mucosa in Group I and Group II and in both groups considered together.

Table 1. Comparison of serum ghrelin concentrations in *H. pylori* infected and not infected children.

Patients	Serum ghrelin concentration (pg/ml)				
	$\bar{x}$	SD	min	max	p
Boys and girls Hp(+) (group I) n = 33	1042.8	445.3	284.0	2167.0	0.09*
Boys and girls Hp(-) (group II) n = 51	874.7	411.6	208.0	1844.0	
Girls Hp(+) n = 23	1012.9	418.5	284.0	1722.0	0.25**
Girls Hp(-) n = 10	780.4	372.1	208.0	1546.0	
Boys Hp(+) n = 23	1111.7	519.1	350.0	2167.0	0.83**
Boys Hp(-) n = 28	952.2	432.5	308.0	1844.0	

*Mann-Whitney U-test
**Tukey's test

DISCUSSION

H. pylori plays the key role in the aetiopathogenesis of gastritis, peptic ulcer disease in the stomach and duodenum as well as in stomach tumours (cancer, MALT lymphoma) (5). It was proven that in persons infected with this bacteria, the secretion of hydrogen ions and leptin is changed under the influence of a meal (7). Gao et al. found that ghrelin concentrations in persons infected with *H. pylori* (8) were lower, and Furuta et al. (9) described the eradication of these bacteria and an improvement of appetite. Thjodleifsson et al., on the other hand, suggest on the basis of multicentre research, that such infections may be a risk factor in the development of overweight (10). Konturek et al. think that *H. pylori* seems to be a factor affecting the appetite of infected adults (7). Thus, it could be expected that an inflammatory condition of the gastric mucosa caused by *H. pylori* infection will be a factor modifying ghrelin secretion in children.

In the described group of paediatric patients living in urban area, we did not find a significant relationship between ghrelin concentrations in the serum and *H. pylori* infection. This fact could confirm the results of our previously published research, in which we did not find a relationship between *H. pylori* infection and the appetite of the children who participated in the study, which was reported by parents as either very good or bad (11). Similarly, no difference between ghrelin concentrations in the serum of prepubertal children with a diagnosed or ruled-out *H. pylori* infection was found by Pacifico et al. (4). As in the group of patients presented in this study, the afore-mentioned authors did not find any features of gastric mucosa atrophy in the group infected with *H. pylori* or in the uninfected group. The differentiation in the plasma ghrelin concentration in *H. pylori* – infected children is confirmed by studies devoted to the influence of bacteria eradication on this concentration. Deng et al. (12) found an increase in ghrelin concentrations in the blood plasma and in the gastric mucosa after an effective eradication of *H. pylori* in children with functional dyspepsia diagnosed according to the Rome III criteria. Similar results were obtained by Konturek et al. (13) after treating *H. pylori* – infected children from shepherd families living in the Tatra Mountains. The results obtained by Pacifico et al. (4) were different – after the eradication of the bacteria, the ghrelin concentration in the children's plasma decreased. In the serum of the children participating in our study, ghrelin concentrations were insignificantly lower in children uninfected with *H. pylori*. Isomoto et al. (14) suggest that the influence of *H. pylori* infection on ghrelin concentrations may depend on bacterial virulence – bacteria producing cagA and vacA have a greater influence on the reduction of ghrelin concentrations. Deng et al. (12), on the other hand, indicate differences between *H. pylori* strains occurring in developed and developing countries, which may be connected with socio-economic conditions. They may also be connected with differences in the results of research obtained in various countries, according to the authors quoted above. Such a suggestion shows that it is justified to verify what *H. pylori* strains occur in the environment under analysis. By relating the results of ghrelin determination to the clinical symptoms, such as appetite, one should take into account the fact that, apart from ghrelin, leptin and obestatin are also hormones synthesised in the gastric mucosa, which have an influence on the appetite regulation (2). Płonka et al. (15) compared the results of their own research conducted on children with research results on adults and suggested that *H. pylori* infection could have a different influence on leptin secretion by gastric mucosal cells at different ages. Therefore, this issue requires further research. Taking into account the influence of *H. pylori* infection on the appetite, it would be especially important to verify the ghrelin and obestatin levels at the same time. These peptides, which have opposite effects in the complex process of appetite regulation (obesta-

tin has anorectic effects), are derived from a shared precursor – preproghrelin (2). These complex relationships, which can be modified by *H. pylori* infection, require further research.

CONCLUSIONS

Our results suggests that *H. pylori* infection with mild grade of gastric mucosa inflammation does not affect serum ghrelin level in children.

BIBLIOGRAPHY

1. Konturek SJ, Brzozowski T, Konturek PC et al.: Brain-gut and appetite regulating hormones in the control of gastrin secretion and musosal protection. J Physiol Pharmacol 2008; 59(2): 7-31.
2. Bossowski A, Urban M: Znaczenie ghreliny i obestatyny w molekularnym aspekcie rozwoju otyłości. Potencjalna rola osi podwzgórze-przysadka-tarczyca. Pediatr Endokrinol 2006; 5(4): 47-53.
3. Muccioli G, Tschop M, Papotti M et al.: Neuroendocrine and peripheral activities of ghrelin: implications in metabolism and obesity. Eur J Pharmacol 2002; 440(2-3): 235-254.
4. Pacifico L, Anania C, Osborn JF et al.: Long-term effects of *Helicobacter pylori* eradication on circulating ghrelin and leptin concentrations and body composition in prepubertal children. European J Endocrinol 2008; 158: 323-332.
5. Osawa H: Ghrelin and *Helicobacter pylori* infection. World J Gastroenterol 2008; 14(41): 6327-6333.
6. Iwańczak F, Iwańczak B, Gościniak G, Pytrus T: Ocena serologiczna występowania zakażenia *Helicobacter pylori* u dzieci do lat 5. Pediatr Pol 2001; 76: 263-268.
7. Konturek JW, Konturek SJ, Kwiecień N et al.: Leptin in the control of gastric secretion and gut hormones in humans infected with *Helicobacter pylori*. Scand Gastroenterol 2001; 36(11): 1148-1154.
8. Gao X-Y, Kuang H-Y, Liu X-M at al.: Circulating ghrelin/obestation ratio in subjects with *Helicobacter pylori* infection. Nutrition 2009; 25: 506-511.
9. Furuta T, Shirari N, Takashima M, Hanai H: Effect of *Helicobacter pylori* infection and its eradication on nutrition. Aliment Pharmacol Ther 2002; 16: 799-806.
10. Thjodleifsson B, Olafsson I, Gislason D: Infections and obesity: a multinational epidemiological study. Second J Infect Dis 2008; 40: 381-386.
11. Obuchowicz A, Zeckei J, Kniażewska M et al.: Zakażenie *Helicobacter pylori* u dzieci a ich łaknienie według oceny rodziców. Pediatr Współcz Gastroenterol Hepatol i Żywienie Dziecka 2011; 13, 1L: 48-51.
12. Deng Z-H, Chu B, Xu Y-Z et al.: Influence of *Helicobacter pylori* infection on ghrelin levels in children. World J Gastroenterol 2012; 18(36): 5096-5100.
13. Konturek PC, Cześnikiewicz-Guzik M, Bielanski W, Konturek SJ: Involvement of *Helicobacter pylori* infection in neuro-hormonal control of food intake. J Physiol Pharmacol 2006; 57 (Suppl. 5): 67-81.
14. Isomoto H, Nakazato M, Ueno H et al.: Low plasma ghrelin levels in patients with *Helicobacter pylori* infection. Helicobacter 2009; 14: 54-60.
15. Płonka M, Bielański W, Konturek SJ et al.: *Helicobacter pylori* infection and serum gastrin, ghrelin and leptin in children of Polisch shepherds. Digestive and Liver Disease 2006; 38: 91-97.

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