The Relations Among Serum Ghrelin, Motilin and Gastric Emptying and Autonomic Function in Autoimmune Gastritis

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ABSTRACT

Background: Gastric emptying (GE) of solids is delayed and autonomic dysfunction is detected in autoimmune gastritis (AIG). The goals of this study were to: (1) compare serum levels of ghrelin and motilin in subjects with delayed and normal GE and (2) investigate whether circulating antimyenteric antibodies (CAA), serum ghrelin levels and motilin levels have any effect on autonomic function.

Materials and Methods: Noninvasive cardiovascular reflex tests were used in order to evaluate the autonomic function. GE was evaluated by a standard 2-hour scintigraphic test. Serum ghrelin and motilin levels were tested by enzyme-linked immunosorbent assay and CAA were tested by immunofluorescence.

Results: The serum ghrelin and motilin levels in the patients with delayed GE ($n = 22$) were significantly decreased compared to the normal GE patients ($n = 19$), (67.55 ± 8.81 versus 126.79 ± 25.81 pg/mL, $P < 0.001$ and 279.59 ± 111.12 versus 500.42 ± 155.95 pg/mL, respectively, $P < 0.001$). Whereas, the serum ghrelin and motilin levels in the patients with deranged autonomic function ($n = 26$) were significantly decreased compared to the patients with normal autonomic function ($n = 15$), (80.73 ± 28.46 versus 127.79 ± 28.06 pg/mL, $P < 0.001$ and 316.92 ± 160.47 versus 490.20 ± 141.02 pg/mL, $P < 0.001$, respectively). None of the patients were positive for CAA.

Conclusions: Ghrelin and motilin levels in AIG subjects with delayed GE and deranged autonomic function were significantly decreased. The decrease in serum ghrelin and plasma motilin levels in AIG suggest their potential role in the delayed GE observed in these subjects.

Key Indexing Terms: Autoimmune gastritis; Autonomic nerve function; Gastric emptying; Ghrelin; Motilin. **[Am J Med Sci 2018;355(5):428–433.]**

INTRODUCTION

Autoimmune gastritis (AIG) is an organ-specific autoimmune disease of the stomach characterized by autoantibodies directed against hydrogen potassium ATPase and intrinsic factor. In this disorder, the loss of parietal cells and the destruction of oxyntic glands of the corpus cause atrophy of the corpus mucosa, which in turn leads to hypo-achlorhydria, hypergastrinemia and antral G-cell hyperplasia.[1] In advanced stages of the disease, vitamin B_{12} metabolism is impaired and may lead to iron deficiency anemia due to the loss of gastric parietal cells. The clinical presentation may be variable, ranging from silent disease in the early phase to upper gastrointestinal (GI) symptoms such as bloating, epigastric pain, nausea and, in certain cases, vitamin B_{12} or iron deficiency anemia.[2] It has been documented that gastric emptying (GE) of solids is delayed in most of the patients with AIG and may be a cause of symptoms. This disorder is also an important etiology of the delayed GE.[3] It has also been reported that some patients with AIG have autonomic nerve dysfunction and there is a close relationship between autonomic nerve dysfunction and delayed GE.[4]

In humans, ghrelin is secreted from the endocrine cells in the oxyntic glands of the corpus and fundus, and it enhances GE by affecting the vagus nerve. Motilin is an endogenous peptide released from duodenal endocrine cells, which increases gastric motility by increasing myenteric cholinergic activity within the myenteric plexus of the stomach.[5] The higher prevalence of circulating antimyenteric antibodies (CAA) in achalasia supports an autoimmune etiology in this disorder.[6] It is also possible that same antibodies exist in AIG due to an autoimmune etiology. Besides this finding, there is a lack of information regarding CAA, ghrelin and motilin levels in AIG patients. Considering the facts that ghrelin and motilin are putative regulators of GE and some AIG patients may have delayed GE leading to upper GI

symptoms, we hypothesized that the presence of CAA, ghrelin and motilin may be related to delayed GE and autonomic nerve dysfunction. Thus the goals of this study were to: (1) define and compare the serum levels of ghrelin and motilin in the patients with delayed GE and normal GE and (2) investigate whether CAA and serum levels of ghrelin and motilin have any effect on autonomic nerve function.

MATERIALS AND METHODS

Patients

The Institutional Review Board of Ankara University, Faculty of Medicine approved this study. All subjects gave informed consent. A total of 41 patients with AIG were included in this study. AIG was diagnosed depending upon histopathologic findings in gastric biopsy specimens. Histopathologically, AIG is characterized by a significant reduction or complete absence of oxyntic glandular units, which are replaced by fibrosis of the lamina propria and pseudopyloric and intestinalized metaplastic glands.[7] The patients with accompanying conditions that might affect autonomic nerve function were eliminated from the study, as depicted by Stojanovich et al.[8] Exclusion criteria were conditions such as diabetes mellitus, pregnancy, renal, liver, cardiac or respiratory decompensation, and any known autoimmune disorders, thyroid and adrenal diseases, or a diagnosis of previous or present cancer. Patients receiving drugs such as anticholinergic agents, β-blockers, calcium channel blockers, angiotensin-converting enzyme inhibitors, angiotensin-2 receptor blockers or pilocarpine that might affect autonomic nervous function and GE were also excluded from the study.[9] In the same manner, patients with disorders that might affect GE were also excluded from the study.[4]

Autonomic Nerve Function Tests

Autonomic nervous system function was evaluated using cardiovascular reflex tests, as described by Ewing et al.[10] Valsalva maneuver, postural index, and heart rate response to deep breathing were used to assess parasympathetic function. Blood pressure response to standing and handgrip test were used to assess sympathetic function. All tests were performed appropriately, as described elsewhere.[10]

Each test was depicted as normal (0), borderline (1) or abnormal (2), as demonstrated in reference values by Ewing et al.[10] In this computation, maximal possible cumulative score is 10 (i.e., if all 5 tests were considered to be abnormal). A cumulative score of 0 or 1 was interpreted as normal, a score of 2 or 3 was accepted as mild, a score between 4 and 6 was accepted as moderate dysfunction and a score of 7 or higher were accepted as severe autonomic dysfunction.[8] Some data included in this study were used in previous studies.[4]

GE Study

GE time was evaluated using a 2-hour scintigraphic test, as described elsewhere.[3] Briefly, an isotope-labeled scrambled egg white meal of 300 kcal was given as a scintigraphic GE protocol. This test meal was prepared by mixing 55 MBq Tc-99 m macroaggregated albumin with a scrambled egg. A GE half-time (GET ½) of longer than 110 minutes was determined to be delayed GE in this study.[11]

Determination of Serum Total Ghrelin, Motilin and CAA

Blood samples were obtained between 08:00 and 10:00 AM, after an overnight fast. The serum was immediately separated by centrifugation and stored in 0.5 mL aliquots at −80°C until tested. The serum total ghrelin and motilin levels were determined by enzyme-linked immunosorbent assay (Cloud Clone Corp., Houston, TX) according to the manufacturer's instructions. CAA were tested by immunofluorescence (IFA) (Euroimmun, Luebeck, Germany) by using a commercially available Neurology Mosaic 1 kit following the manufacturer's instructions. This kit includes monkey cerebellum, monkey nerves and monkey intestinal tissue. For determining CAA positivity, these tissues were stained and myenteric plexus neurons were considered.

Statistics

All statistical analyses were performed using SPSS 16.0 (SPSS, Chicago, IL) for Windows. The Shapiro-Wilk test was used to determine the normality of the distribution of data, and depending on the results, parametric or nonparametric tests were preferred. If the distribution was normal, the values were expressed as the mean (standard deviation), and nominal variables were expressed as *n* and percentage (%). The significance of the differences in mean values between the groups was compared using Student's *t* test, and the significance of the differences in median values was investigated by the Mann-Whitney *U*-test. Nominal variables were evaluated by Pearson chi-square χ-test or Fisher's exact test. The relationship between 2 continuous variables was evaluated by Pearson correlation test when the distribution was normal, otherwise, Spearman correlation test was employed. In the case of more than 2 groups, the significance of the difference of the mean values between the groups was analyzed using analysis of variance, and the significance of the difference of median values was analyzed using the Kruskal-Wallis test. $P < 0.05$ was considered statistically significant.

RESULTS

Overall, 41 patients (27 women), mean age 56.61 ± 11.79 years with AIG were included. Of them, 22 (53.6%) patients showed delayed GE and 19 patients showed normal GE (GET ½: 241.19 ± 199 versus 90 ±