CLINICAL STUDY

Plasma ghrelin concentrations in type 1 diabetic patients with autoimmune atrophic gastritis

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Abstract

Objective: Type 1 diabetes mellitus patients (DM1) show increased prevalence of pernicious anaemia, the histological substrate of which is type A chronic atrophic gastritis (CAG) in the stomach corpus, the main source of ghrelin. We aimed to compare plasma ghrelin concentrations in DM1 patients with type A CAG (DM1-CAG), DM1 patients without type A CAG and healthy controls and in DM1-CAG group, to ascertain a possible relationship between ghrelin and biochemical markers of gastric mucosa atrophy and/or neuroendocrine (NE) cell hyperplasia and histological gastric biopsy findings.

Design and methods: Fifteen DM1-CAG patients were matched for age, sex and body mass index with 15 DM1 patients without type A CAG and 15 controls. Pepsinogen I, pepsinogen II, gastrin, parietal cell antibodies, chromogranin A (CgA) and ghrelin were determined in all subjects. In DM1-CAG patients, immunohistochemical analysis of gastric biopsies using antibodies to CgA and ghrelin was performed.

Results: Ghrelin concentrations differed among groups; however, paired comparisons between groups were not significant. In DM1-CAG, no correlation was found between ghrelin and gastric body atrophy markers, pepsinogen I and the pepsinogen I/II ratio. Immunohistochemical studies of DM1-CAG patients showed CgA staining in 12 and ghrelin staining in 6, which was confined to the foci of NE cell hyperplasia. Those patients who stained positive for ghrelin had higher ghrelin concentrations when compared with the negative patients.

Conclusions: Ghrelin concentrations are not decreased in DM1-CAG patients; thus, our data suggest that ghrelin is not a good marker of gastric mucosa atrophy in these patients, given the possible ghrelin synthesis in hyperplastic gastric endocrine/enterochromaffin-like cells.

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Introduction

Ghrelin is a 28-amino acid peptide produced in a variety of human tissues; however, the major source of circulating ghrelin is the stomach. The oxyntic mucosa of the stomach is rich in endocrine cells, the most predominant of which are enterochromaffin-like cells (ECL; 60–70%), other endocrine cells including X/A-like cells (20%), the likely source of ghrelin (1–3), somatostatin cells (D cells; 2–5%) and enterochromaffin cells (0–2%) (2).

Most studies on chronic atrophic gastritis (CAG) observed a low plasma ghrelin concentration due to *Helicobacter pylori* (*H. pylori*) infection (4–6) with a positive association between this concentration and biochemical markers of gastric mucosa atrophy such as pepsinogen I and pepsinogen I/II ratio (4, 5, 7). Severity of histologic changes and gastritis topography has been reported to affect circulating ghrelin levels which are markedly decreased in cases of extensive atrophic

gastritis involving the corpus (4). A possible explanation for this low ghrelin concentration is the loss of ghrelin-producing cells. In fact, attenuation of ghrelin mRNA expression and reduction in ghrelin-positive cell number in gastric mucosa have been described in patients with *H. pylori* infection (5). Atrophic autoimmune gastritis, or type A CAG, is characterised by chronic inflammation of oxyntic mucosa predominantly affecting the gastric corpus which results in achlorhydria and atrophy of oxyntic glands and progressive disappearance of parietal and chief cells. Pernicious anaemia (PA) is considered to be the end stage of type A CAG. Immunohistochemical studies have revealed ghrelin expression in gastric neuroendocrine (NE) cell hyperplasia lesions of type A CAG patients (8, 9) and gastric carcinoid tumours (10), although scarce data exist about plasma ghrelin concentrations in these patients. We recently reported that DM1 patients present an increased prevalence of latent pernicious

anaemia when low plasma pepsinogen I concentrations are considered the main marker of early gastric mucosa atrophy (11). To our knowledge, no data exist regarding plasma ghrelin concentrations in DM1 patients with type A CAG (DM1-CAG). Thus, the question remains as to whether plasma ghrelin concentrations are useful for the diagnosis of gastric mucosa atrophy or NE cell hyperplasia in DM1-CAG patients.

The aim of this study, therefore, was to evaluate plasma ghrelin concentrations in a group of DM1-CAG patients and compare them with a group of DM1 patients without evidence of type A CAG, and a healthy control group. In addition, in DM1-CAG patients, we sought to ascertain whether a possible relationship exists between ghrelin concentrations, biochemical markers of gastric mucosa atrophy, inflammatory markers and histopathologic gastric biopsy findings.

Subjects and methods

Patients

Fifteen DM1-CAG patients (nine women and six men, mean age: 36.87 ± 13.5 years) were matched with 15 DM1 patients without evidence of CAG for age, sex, body mass index (BMI), diabetes duration and metabolic control and with a healthy control group for sex, age and BMI. DM1 patients were recruited from the outpatient clinic of the Department of Endocrinology-Diabetology of the University Hospital Germans Trias i Pujol Hospital (Badalona, Spain). All DM1 patients were under intensified insulin treatment (mean daily insulin dose of 0.70 ± 0.17 IU/kg in DM1-CAG and 0.75 ± 0.23 in DM1 without CAG) with stable metabolic control in the last year (average of three HbA1c determinations; Table 1).

DM1-CAG patients were selected from a cohort of a previously published study (11), carried out in our DM1 patients for early detection of chronic autoimmune gastritis. Specifically, the diagnosis of type A CAG was based on the presence of a low serum pepsinogen I concentration and hypergastrinaemia (12), a pepsinogen I/II ratio under 3 (13), positive parietal cell antibodies (PCAs) and histological confirmation. Since *H. pylori* infection has been found in some studies to lower ghrelin concentrations, we decided that DM1 patients without CAG and controls had to have a negative urea breath test to rule out the infection (14). However, *H. pylori* infection was not considered as an exclusion criterion of DM1-CAG patients given the low prevalence of CAG among DM1. In fact, four patients had *H. pylori* infection.

Patients with cachexia, other autoimmune diseases, liver disease, infection, renal dysfunction (serum creatinine ≥ 1.5 mg/dl) or a history of eradication therapy for *H. pylori* infection were excluded. None were using antibiotics, anti-inflammatories, prokinetics or proton pump inhibitors.

The protocol was approved by the Hospital Ethics Committee and all patients gave their written informed consent in accordance with the Declaration of Helsinki.

Laboratory assays

Blood was drawn between 0800 and 0900 h after an overnight fast of at least 12 h. For ghrelin determination, blood was transferred into chilled tubes containing EDTA and aprotinin, and stored on ice until centrifuged. Plasma and serum samples were kept frozen at -80°C until assayed. Glycosylated haemoglobin (HbA1c) was measured in blood samples with EDTA by high-pressure liquid chromatography using a fully automated Menarini HI-AUTO A1c 8140 analyser manufactured by Arkay

Table 1 Clinical characteristics and biochemical results of type 1 diabetes mellitus patients with and without type A chronic atrophic gastritis and the control group.

	DM1-CAG	DM1	Control	P value
N (male/female)	15 (6/9)	15 (6/9)	15 (6/9)	NS
Age (years)	36.87 ± 13.5	37.4 ± 13	35.5 ± 13	NS
BMI (kg/m^2)	24.1 ± 2.8	23.8 ± 2.7	23.0 ± 2.5	NS
HbA1c (%)	7.7 ± 0.7	7.7 ± 1.1	—	NS
Insulin (mIU/l)	7.0 (2.6–12.5)	7.4 (4.1–9.8)*	3.8 (2.0–5.2)	0.015
Duration (years)	17.9 ± 6.3	18.7 ± 6.8	—	NS
PCA(+) (%)	100	0	0	<0.001
Pepsinogen I ($\mu\text{g/l}$)	$18.5 \pm 15^\dagger$	113.7 ± 33	125.5 ± 32.6	<0.001
Pepsinogen II ($\mu\text{g/l}$)	13 ± 5	11.4 ± 4.2	14 ± 8.2	NS
Pepsinogen I-II ratio	$1.4 \pm 1.0^\dagger$	11 ± 5.3	10.2 ± 4.5	<0.001
Gastrin (ng/l)	469 (225–708) [†]	26 (22–30)	21 (17.2–30)	<0.001
Chromogranin A (ng/ml)	191 (115–282) [†]	68 (59–77)	45.6 (1.5–57)	<0.001
Ghrelin (pg/ml)	651 ± 282	515 ± 148	671 ± 224	0.038
TNF- α (pg/ml)	5.49 ± 1.08	5.28 ± 1.16	4.52 ± 0.67	0.033
CRP (mg/l)	1.04 (0.52–2.95)	1.2 (1.13–3.02)	0.96 (0.36–1.53)	NS

DM1-CAG, type 1 diabetes mellitus with type A chronic atrophic gastritis; DM1, type 1 diabetes mellitus; BMI, body mass index; PCA, parietal cell antibodies; NS., non-significant. Data are expressed as mean $\pm$ s.d. median (interquartile range) or percentage. Comparisons among groups were tested by the Kruskal–Wallis test. Comparisons between two groups were tested by the Mann–Whitney *U* test followed by Bonferroni correction. *Significantly different from control group ($P=0.004$); [†]significantly different from DM 1 ($P<0.001$) and control group ($P<0.001$).

(Kyoto, Japan) with an inter-assay coefficient of variation (CV) of 3.0 and 1.8% at HbA1c levels of 4.8 and 9.0% respectively (reference range 3.7–5.1%). Immunoreactive insulin concentrations were measured using an automated enzyme chemiluminescent assay (Immulite 2000, DPC, Los Angeles, CA, USA). Assay sensitivity was 2 mIU/l. Inter-assay CV values were <7.3%. Serum pepsinogen I was measured in duplicate by an ELISA (Biohit Diagnostics, Helsinki, Finland); intra-assay CV values were below 5.9% and inter-assay CV values below 4.9%. Sensitivity of the assay was 1.9 µg/l (reference range 33.9–168.7 µg/l). Serum pepsinogen II was measured in duplicate, by an ELISA (Biohit Diagnostics); intra-assay CV values were below 5.6% and inter-assay CV values below 8.3%. Sensitivity of the assay was 1.0 µg/l. Gastrin was measured by RIA (DPC, Los Angeles, CA, USA) as previously described (11). Total plasma ghrelin concentrations were measured by a commercial double antibody/PEG RIA (Linco Research Inc., St Louis, MO, USA). The intra-assay CV was below 10% and inter-assay CV below 16%. Assay sensitivity was 93 pg/ml. Serum chromogranin A (CgA) concentrations were measured by a commercial solid-phase two-site immunoradiometric assay (CGA-RIACT, CIS Bio International, Schering S.A., Sur-Yvette, Cedex, France). The intra-assay CV was below 6.0% and inter-assay CV below 8.5%. Assay sensitivity was 1.5 ng/ml. Tumour necrosis factor α (TNF- α) concentrations were measured by an enzyme chemiluminescent immunometric assay (Immuline ONE, DPC). Assay sensitivity was 1.7 pg/ml. Inter-assay CV values were <6.5%. The high sensitivity C-reactive protein (CRP) concentrations were determined by an ultrasensitive CRP test (N High Sensitivity CRP; Dade Behring Marburg GmbH, Marburg, Germany) with an inter-assay CV <3.9%. The detection limit of the assay was 0.175 mg/l performed using a sample dilution of 1:20. PCAs were determined by indirect immunofluorescence on rat gastric mucosa as substrate following the standard procedures (Menarini Diagnostics, Barcelona, Spain). A titre higher than 1:40 was regarded as positive. Antibodies to intrinsic factor were measured by Enzyme Immunoassay (D-Tek, SA, Mons, Belgium; normality: binding index <1.0).

H. pylori infection was assessed by a urea breath test (^{13}C -UBT) (TAU-KIT, Isomed, SL, Madrid, Spain) as proposed in the standardised European protocol (14). Thus, those (^{13}C -DOB, δ over baseline) values higher than 5 were considered positive.

Histological examination

Oesophagogastrroduodenoscopy (GIF-Q145 endoscope; Olympus Optical Co. Ltd, Japan) was performed in all DM1-CAG patients. Each patient underwent three biopsies taken from the gastric antrum and three biopsies from the midbody along the greater curve using Paul Drach Jumbo biopsy forceps. The biopsies were examined by a blinded experienced histopathologist. Serial 5 µm thick sections were stained with haematoxylin–eosin for

conventional histopathologic examination. Giemsa staining for *H. pylori* was also performed. The degree of gastritis was assessed according to the Updated Sydney System (15) and defined as mild, moderate or severe oxyntic gland loss and/or replacement by metaplastic pyloric or intestinal glands. Endocrine cells were evaluated after immunostaining with a monoclonal antibody against CgA (1:200 dilution; Dako, Glostrup, Denmark) followed by the streptavidin–biotin complex procedure. For visualisation, diaminobenzidine tetrahydrochloride was used as a chromogen substrate and nuclear counterstaining with haematoxylin was performed. The stages of NE cell growth were classified in a sequence with presumed increasing oncologic potential, useful for prognostic evaluation of patients at risk of carcinoid development such as those with CAG, according to Solcia *et al.* (16). Ghrelin immunostaining was performed on formalin-fixed and paraffin-embedded slides. The primary antibody was a polyclonal serum antihuman ghrelin (amino acids 13–28; Phoenix Pharmaceuticals Inc., Belmont, CA, USA), diluted 1:300 and incubated for 1 h at room temperature. A standard immunoperoxidase procedure with streptavidin peroxidase and diaminobenzidine as the final reaction product was used. Control experiments included the immunoperoxidase reaction in serial sections by omitting the primary antibody. Hypothalamus, pituitary gland and normal oxyntic gastric mucosa slices served as positive controls for ghrelin immunostaining.

Statistical analysis

Descriptive results are expressed as mean $\pm$ s.d. or median (interquartile range). Differences among groups were examined by the Kruskal–Wallis test and when the test was significant at the 0.05 level, pairwise comparisons were based on the Mann–Whitney *U* test followed by Bonferroni correction. Differences in proportions were analysed using χ^2 or Fisher's exact tests. Departure from normality was assessed using the Kolmogorov–Smirnov distribution test. Relationships among variables were tested using Spearman's correlation coefficient. A *P* value <0.05 was considered statistically significant. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS/Windows version 12.0; SPSS Inc., Chicago, IL, USA).

Results

Anthropometric and clinical data

The clinical characteristics of DM1 patients with and without CAG and the control group are shown in Table 1. No differences were observed for age, BMI, metabolic control and diabetes duration among groups.

Biochemical parameters

Serum pepsinogen I concentrations and pepsinogen I/II ratio were significantly decreased in the DM1-CAG group when compared with DM1 patients without CAG and control group ($P < 0.001$). No differences were found in pepsinogen II concentrations among groups. Serum gastrin and CgA concentrations were significantly higher in DM1-CAG patients when compared with DM1 patients without CAG ($P < 0.001$) and controls ($P < 0.001$). Plasma ghrelin concentrations differed among groups. However, pairwise comparisons between groups were not significant. No differences in TNF- α and CRP concentrations were observed in DM1 patients, regardless of type A CAG association. With regard to immunological analysis, all DM1-CAG patients had positive PCA at high titres (3: 1/160, 1: 1/320, 6: 1/640 and 5: 1/1280). Only three of the 15 had positive intrinsic factor antibodies.

A significant inverse correlation was found between ghrelin and serum insulin concentrations ($r = -0.315$, $P = 0.045$) when all the patients studied were considered.

In DM1-CAG patients, plasma ghrelin concentrations did not correlate with any of the biochemical markers of gastric body atrophy, serum pepsinogen I concentration or pepsinogen I/II ratio. In these patients, serum CgA concentrations were positively associated with serum gastrin concentrations ($n = 15$, $P < 0.002$) and negatively associated with serum pepsinogen I concentrations ($n = 15$, $P < 0.05$) and pepsinogen I/II ratio ($n = 15$, $P < 0.02$). No correlation was found between CgA and plasma ghrelin concentrations. No correlation was found between plasma ghrelin concentrations and serum TNF- α concentrations or CRP concentrations.

Histological findings

Gastric body mucosa atrophy and NE cell hyperplasia Histological examination of DM1-CAG patients confirmed different glandular atrophy degrees in all: mild in five out of 15, moderate in five out of 15 and severe in five out of 15. Intestinal metaplasia was present in nine of the 15. Immunostaining for CgA for the evaluation of NE cell hyperplasia was positive in 12 of the 15 biopsied patients. With regard to Solcia's pattern, four of the 12 had linear hyperplasia, six had both micronodular and linear hyperplasia, whereas only two had micronodular hyperplasia. None showed adenomatoid or dysplastic lesions (Table 2).

H. pylori *H. pylori* infection was present in four of the DM1-CAG patients, all of them with a positive UBT.

Ghrelin expression in gastric NE cell hyperplasia

Immunohistochemical staining for ghrelin was positive in six of the 15 cases and was almost exclusively confined to the foci of NE cell hyperplasia (linear or micronodular) and coincided with positive CgA immunostaining. However, the number of ghrelin-positive cells was not as high as that in CgA-reactive cells (Fig. 1). Of the 15 patients, six showed negative ghrelin but positive CgA immunostaining. Finally, three patients showed negative immunostaining for both CgA and ghrelin.

Biochemical results according to histopathologic findings in the DM1-CAG group

H. pylori infection Within the DM1-CAG group, those with *H. pylori* infection ($n = 4$) showed significantly

Table 2 Pathologic and immunohistochemical features of type 1 diabetes mellitus patients with type A chronic atrophic gastritis.

Patient no.	Sex/age (yr)	Corpus atrophy	NE hyperplasia (CgA)	Ghrelin IHC	HP	Serum Ghrelin (pg/ml)	Serum CgA (ng/ml)	Serum Gastrin (ng/l)
1	M/28	Moderate	—	—	—	467	53	222
2	M/33	Mild	—	—	+	535	1.5	32
3	M/51	Mild	—	—	—	691	118	225
4	F/31	Severe	+	+	—	645	184	314
5	F/37	Mild	+	—	+	600	161	708
6	F/72	Moderate	+	—	—	578	309	490
7	F/28	Moderate	+	+	—	1057	87	514
8	F/29	Mild	+	—	—	535	270	469
9	F/34	Severe	+	+	—	672	262	1649
10	F/23	Moderate	+	—	—	513	115	199
11	M/20	Moderate	+	+	+	280	151	446
12	F/48	Severe	+	—	—	801	355	436
13	M/30	Mild	+	—	—	618	499	683
14	F/51	Severe	+	+	—	1437	238	717
15	M/38	Severe	+	+	+	370	282	1085

M, male; F, female; NE hyperplasia, neuroendocrine cell hyperplasia; HP, *Helicobacter pylori*; IHC, immunohistochemistry; CgA, chromogranin A. For IHC, (+) indicates positive staining and (—) indicates negative staining.

Figure 1 Chromogranin A and ghrelin immunostaining in gastric mucosa of a DM1-CAG patient (case 11). (a) Marked neuroendocrine cell hyperplasia, mostly linear, with occasional nodules depicted by chromogranin A ($\times 100$). (b) Ghrelin expression in the same area shows only slight hyperplastic changes ($\times 100$).

lower plasma ghrelin concentrations when compared with *H. pylori*-negative patients (438.10 ± 141.38 vs 728.67 ± 285.18 pg/ml, $P=0.026$). In fact, when DM1-CAG *H. pylori*-infected patients were not considered for the statistical analysis, plasma ghrelin concentrations were significantly higher in DM1-CAG patients when compared with DM1 without CAG (728.67 ± 285.18 vs 515.10 ± 148.20 pg/ml, $P=0.024$) and did not differ from those of the control group (Fig. 2).

NE cell hyperplasia Patients with NE cell hyperplasia showed higher CgA and serum gastrin concentrations than those without (CgA: 250 (167.5 – 295.5) ng/ml versus 53 (27.2 – 85.5) ng/ml, $P=0.018$; gastrin: 502 (441 – 712) ng/l versus 222 (127 – 223) ng/l, $P=0.018$). No differences were found in plasma ghrelin concentrations in relation to the presence or not of NE cell hyperplasia.

Gastric mucosa atrophy Patients with severe gastric mucosa atrophy showed higher plasma ghrelin, gastrin and CgA concentrations when compared with those with mild or moderate atrophy, although the

Figure 2 Plasma ghrelin concentrations in the different groups evaluated. DM1: type 1 diabetic patients without chronic autoimmune gastritis. All DM1-CAG+: type 1 diabetic patients with chronic autoimmune gastritis. CAG+HP-: type 1 diabetic patients with chronic autoimmune gastritis without *H. pylori* infection. CAG+HP+: type 1 diabetic patients with chronic autoimmune gastritis and *H. pylori* infection.

difference did not reach statistical significance (data not shown).

Discussion

The present study is the first to describe plasma ghrelin concentrations in patients with DM1 associated with type A CAG. Plasma ghrelin concentrations in these patients are not decreased, in contrast to results reported in DM1 patients and in CAG due to *H. pylori* infection (4, 5).

The fact that plasma ghrelin concentrations were not low in our DM1-CAG patients could be explained by the results obtained on histologic studies. Given the close proximity of NE and parietal cell compartments in gastric corpus mucosa, ghrelin biosynthesis could be affected by atrophic events associated with an autoimmune reaction. On the other hand, endocrine hyperplasia of ECL due to an increase in gastrin levels secondary to achlorhydria is a frequent finding in gastric mucosa of CAG patients (17). Although ghrelin is known to be synthesised by X/A-like cells, immunohistochemical studies of ECL hyperplastic lesions in the setting of type A CAG, as well as gastric carcinoids, have shown that these lesions are strongly positive for ghrelin and do express ghrelin mRNA, which demonstrates that this cell population also synthesise the protein (5). For this reason, it has been hypothesised that ECL acquire the capability to synthesise ghrelin during proliferative states or that ghrelin is the product of a peculiar neoplastic cell population, increased by gastrin stimulation. However, in the study of Corbetta *et al.* (10)

plasma ghrelin concentrations were not different from healthy subjects in a group of patients with gastroenteropancreatic NE tumours, six of which were gastric carcinoids. In our DM1-CAG patients, immunohistochemical analyses of gastric mucosa using CgA as a marker of NE cell hyperplasia showed ghrelin expression to be enhanced, almost exclusively, in NE cell hyperplastic lesions when compared with normal gastric mucosa. However, plasma ghrelin did not differ between patients with or without NE cell hyperplastic lesions probably due to the fact that only half of the patients with NE cell hyperplasia stained positive for ghrelin. As reported by De Block *et al.* (18) serum gastrin and CgA concentrations were higher in DM1-CAG patients with NE cell hyperplasia than those without.

On the other hand, it has been suggested that ghrelin could play a role in modulating immune responses and inflammatory processes (19). In fact, ghrelin concentrations have been reported to be high in some inflammatory diseases and to correlate positively with inflammatory cytokines such as TNF- α (20, 21). In our study, TNF- α levels and CRP did not differ between DM1 patients with or without type A CAG; thus, differences in ghrelin concentrations between these groups could hardly be explained by the inflammatory process.

Several studies found low plasma ghrelin concentrations correlating with biochemical markers such as pepsinogen I and pepsinogen I/II ratio and the degree of gastric atrophy when *H. pylori* was present (5, 7). In contrast to *H. pylori* studies, ghrelin concentrations in our DM1-CAG patients did not correlate with biochemical markers of gastric mucosa atrophy. A possible explanation for these differences between both types of CAG could be the hyperplastic ECL cells in type A CAG, which could synthesise ghrelin, while in *H. pylori* atrophy these ECL cells would be destroyed. However, the results of our study concur with *H. pylori* infection-induced chronic gastritis, since the few DM1-CAG *H. pylori*-infected patients had lower ghrelin concentrations than those without the infection.

Few studies have reported ghrelin concentrations in DM1 patients. Most reports in DM1 children, showed total (22–24) and acylated (22) ghrelin concentrations to be reduced in these patients when compared with healthy subjects. However, other studies found total ghrelin concentrations to be similar to controls (25) and acylated ghrelin concentrations only decreased at DM1 diagnosis (23). Our study concurs with the former, since our DM1 patients had lower total ghrelin concentrations, once type A CAG had been ruled out. The described determinants of ghrelin secretion in non-diabetic subjects include insulinaemia (26–28) and blood glucose levels (29–31), both of which are parameters with a negative relationship. Furthermore, in DM1 patients, an inverse correlation between serum insulin and plasma ghrelin was found and insulinaemia shown to be a decisive signal for ensuring postprandial plasma ghrelin suppression (28). An inverse correlation between plasma ghrelin and serum insulin

was also found in the present study when all the patients studied were considered.

Our study has some limitations, the major one being the small number of patients included. This could be explained by the low prevalence of type A CAG in DM1 patients and by the strict inclusion criteria for atrophic autoimmune gastritis diagnosis. A second limitation is that oesophagogastroduodenoscopy was not performed in DM1 patients without CAG or in controls to rule out gastric corpus atrophy. However, a low serum pepsinogen I concentration and a low pepsinogen I/II ratio are widely accepted as good biochemical markers of gastric corpus atrophy. In the present study, all DM1 patients without CAG and controls had normal pepsinogen I, pepsinogen I/II ratio, gastrin concentrations and a negative urea breath test, which permitted gastric corpus atrophy to be ruled out and obviated fibrogastroscopy on ethical grounds.

In conclusion, plasma ghrelin concentration is not a good biochemical marker of gastric mucosa atrophy in DM1-CAG patients, given the possible ghrelin synthesis in NE cell hyperplasia lesions of gastric mucosa. Moreover, considering the results of the present study, we believe plasma ghrelin concentrations to be inferior to gastrin and CgA concentrations in predicting NE cell hyperplasia in DM1-CAG patients. Future studies aiming to assess ghrelin concentrations in DM1 patients should rule out the presence of CAG, since concentrations may be altered by this entity.

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