Plasma Chromogranin A in Patients with Inflammatory Bowel Disease

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Background: Circulating chromogranin A (CgA) levels, a marker for neuroendocrine tumors including carcinoids, have recently been found elevated in some patients with inflammatory bowel disease (IBD), although their significance is unclear. Therefore, we aimed to evaluate CgA levels and their possible relationship with clinical and biochemical disease activity indexes in 119 IBD patients.

Methods: The study groups comprised 75 patients with ulcerative colitis, 44 with Crohn's disease, in both active and quiescent phases, and 85 controls.

Results: Mean CgA levels were significantly higher in IBD patients than in controls (20.4 ± 14.0 [SD] versus 11.3 ± 4.3 U/L, $P < 0.001$), without any statistical significant difference among the IBD subgroups. However, CgA levels were above the normal range (20 U/L) in 25/45 patients with active IBD (55%; 95% confidence interval [CI]: 40%–70%) and in 18/74 patients with remission IBD (24%; 95% CI: 15%–36%) ($P < 0.001$, Fisher's test). Among biochemical parameters, CgA correlated with serum TNF-α levels ($r_s = 0.398$, $P < 0.001$).

Conclusions: High CgA levels can occur in IBD. The disease activity and TNF-α levels seem to influence the CgA pattern, which could reflect the neuroendocrine system activation in response to inflammation. From a clinical point of view, the possibility of high CgA levels in IBD should be taken into consideration when a carcinoid is suspected in such patients, since this event seems to be more frequent than previously considered. Indeed, revision of our 83 patients with gastrointestinal carcinoids, studied between 1997 and 2006, showed that 4 patients had IBD, with a prevalence of 4.8%, which is markedly higher than that of the general population.

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Key Words: Crohn's disease, chromogranin A, ulcerative colitis

Circulating levels of chromogranin A (CgA), a 49-kDa acidic glycoprotein, are a sensitive marker for neuroendocrine tumors, including gastrointestinal (GI) carcinoids.[1–7] However, high CgA values are also common in patients with renal failure, atrophic body gastritis, and ongoing proton-pump inhibitors (PPI) therapy, as a result of reduced metabolic CgA clearance or gastric enterochromaffin-like cell activation, respectively. In other conditions, including heart failure, chronic obstructive pulmonary disease, rheumatological diseases, and nonendocrine malignancies, the finding of increased CgA levels has been related to a possible involvement of the neuroendocrine system.[8–18] Interestingly, slightly elevated circulating CgA levels have also been recently reported in some patients with inflammatory bowel disease (IBD), although the CgA pattern in this setting has not been fully elucidated.[2,13] This finding should be taken into account if a neuroendocrine tumor is suspected in a patient with IBD. Actually, even if this association has been considered to be quite rare,[19,20] recent studies have reported an increased risk of carcinoid tumors in IBD patients, as a possible consequence of the chronic inflammatory stimulus.[21]

The aim of the present study was to evaluate plasma CgA levels in a series of consecutive patients with either ulcerative colitis (UC) or Crohn's disease (CD), in both acute phase and remission, and to assess the possible relationship between CgA plasma levels and both the clinical stage of the disease and biochemical parameters related to the disease, including serum tumor necrosis factor (TNF)-α levels.[15,22] A possible relationship between CgA plasma levels and the number of CgA-positive colonic mucosal cells was also investigated in a subset of UC patients, on the basis of the recently reported gut endocrine cell hyperplasia in inflamed gut of patients with IBD.[23–28]

Moreover, a 10-year series of patients with GI carcinoids, diagnosed and followed up at our institution, was reviewed in order to evaluate the actual prevalence of IBD

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among them, as current data regarding this association are scanty and conflicting.[19–21]

MATERIALS AND METHODS

From September 2006 to December 2006, 119 patients with IBD (71 males and 48 females, age 22–83 years, mean 48 years), met the inclusion criteria and were consecutively enrolled in the study. Among these, 75 had UC, 32 active (a-UC) and 43 in remission (r-UC), and 44 had CD, 13 active (a-CD) and 31 in remission (r-CD). Disease activity was assessed using the disease activity indexes DAI[29] and CDAI[30] for UC and CD patients, respectively. Remission for CD or UC patients was defined by a CDAI level $\leq$150 and a DAI $\leq$1, respectively. Among UC patients, 33 (44%) had proctitis or proctosigmoiditis, 17 (23%) left-sided colitis, and 25 (33%) extensive colitis; among CD patients, 14 (32%) had ileitis, 7 (16%) colitis, and 23 (52%) ileocolitis. Routine blood parameters and serum TNF-α levels were assayed in all patients. The number of CgA-positive cells was evaluated in multiple colonic mucosal biopsies of 22 UC patients, 11 with active and 11 with quiescent disease. Exclusion criteria were concomitant PPI therapy, atrophic body gastritis, renal failure, endocrine diseases, heart failure, chronic obstructive pulmonary disease, rheumatological diseases, and malignancies.

The control group consisted of 85 healthy volunteers, 45 males and 40 females, age 18–79 years, mean 46 years, recruited among medical staff, blood donors, and acquaintances of patients. None of them was taking any medication and physical examination and routine blood tests were normal. Serum TNF-α levels were assayed in 30 subjects. Thirteen subjects had to do a screening colonoscopy and accepted undergoing multiple rectal and colonic mucosal biopsies that were assayed for CgA-positive cells.

To assess the prevalence of IBD in GI carcinoids, hospital files of 83 patients with a carcinoid, referred to our institution between January 1997 and December 2006 for clinical investigation, treatment, and follow-up, were reviewed. This group included 46 men and 37 women, age 13–78 years, mean 55 years. Twenty-five patients had a foregut, 36 a midgut, and 22 a hindgut carcinoid. Lymph node and/or liver metastases were present in 31 patients.

All subjects gave their informed consent to the study, which was approved by the local ethics committee.

Venous blood samples were drawn into tubes with and without EDTA, between 8 and 10 AM, after an overnight fast. The samples were centrifuged at 4°C and plasma and serum were separated and stored at –30°C until assayed. As previously described,[31] plasma CgA levels were measured with an enzyme-linked immunosorbent assay purchased from DAKO A/S (Glostrup, Denmark) and the results expressed as U/L. The 95% confidence interval (CI) was 2.1 U/L and the intra- and interassay coefficients of variation (CV) were 4.1% and 6.8%, respectively. Serum TNF-α levels were determined as previously described[32] by means of a solid-phase enzyme immunometric assay (Quantikine HS; R&D Systems, Minneapolis, MN) and the results given as pg/mL. The 95% CI of detection was 0.12 pg/mL and the intra- and interassay CVs were 6.7% and 11.6%, respectively. Routine blood determinations were performed using standardized laboratory techniques.

Endoscopic biopsies were formalin-fixed, paraffin-embedded, and stained with hematoxylin and eosin for routine evaluation. Immunohistochemical tests for chromogranin A (mouse monoclonal clone LK2H10, dilution 1:300, Biogenex, San Ramon, CA) were performed using the streptavidin-biotin-peroxidase complex method, as previously described.[33] Negative controls were obtained by substituting the immunoglobulin fraction of nonimmune mouse serum for the primary antibody. The number of CgA immunoreactive cells was evaluated with gridded eyepiece, at $\times$630 magnification; immunoreactive cells were counted in at least 50 glands.

STATISTICAL ANALYSIS

Results were given as mean $\pm$ SD. All data were tested for normality of distribution by the Kolmogoroff–Smirnoff test. Differences between groups were evaluated by the Mann–Whitney test and the Kruskal–Wallis test followed by Dunn's multiple comparison test, when appropriate. Relationships between variables were assessed by Spearman coefficient. Differences between percentages were evaluated by Fisher's exact test. A P-value < 0.05 was considered statistically significant.

RESULTS

Plasma CgA levels in both controls and IBD patients are detailed in Figure 1. The upper reference limit for CgA levels, defined as 2 SD above the mean obtained in our healthy subjects, was 20 U/L. Plasma CgA levels were significantly higher in IBD patients than in healthy subjects (20.4 $\pm$ 14.00; range 6.3–88.4; versus 11.3 $\pm$ 4.33; range 2.6–20.4; U/L [correction made here after initial online publication], $P < 0.001$, Mann–Whitney test). In the IBD group there were no significant differences in mean CgA levels among patients with UC and CD, in both active and remission phase (Fig. 1). However, CgA values were above the normal range in 25/45 patients with active IBD (55%; 95% CI, 40–70) and in 18/74 with quiescent IBD (24%; 95% CI, 15–36) ($P < 0.001$, Fisher's exact test). In the IBD group, plasma CgA levels correlated positively with serum TNF-α levels ($r_s = 0.398$, $P < 0.001$), although TNF-α values were significantly higher in active than in quiescent IBD and in controls, with mean $\pm$ SD of 1.85 $\pm$ 1.206, 1.24 $\pm$ 0.526, and 1.00 $\pm$ 0.543 pg/mL, respectively ($P < 0.001$, Kruskal–Wallis and Dunn's test). No significant relationship was observed between CgA levels and routine biochemical param-

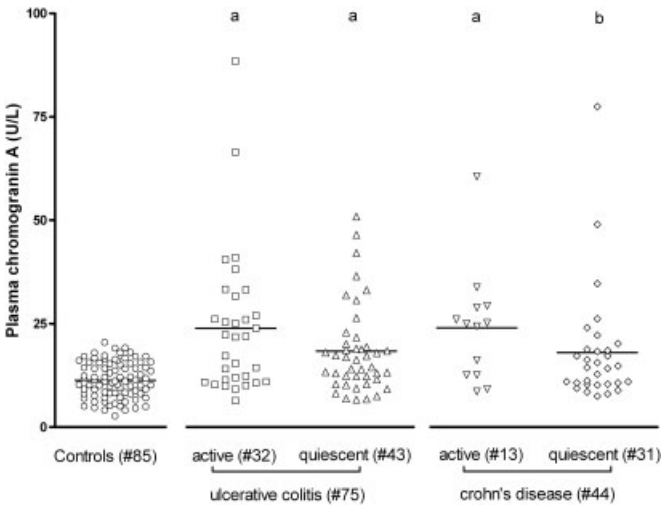

FIGURE 1. Plasma chromogranin A levels in normal subjects (controls), in patients with active ulcerative colitis, quiescent ulcerative colitis, active CD, and quiescent CD. Each individual patient or control is shown as a symbol; bold lines are the mean values. (a) $P < 0.001$ and (b) $P < 0.05$ versus controls (Kruskal–Wallis and Dunn's test).

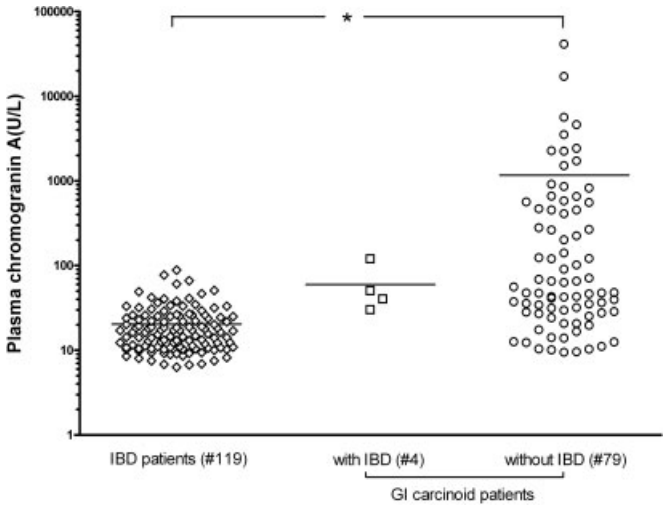

FIGURE 2. Plasma chromogranin A levels in 119 patients with IBD and 83 GI carcinoid patients, 4 of which with concomitant IBD and 79 without IBD. Chromogranin A levels are plotted logarithmically to accommodate extreme values. Each individual patient or control is shown as a symbol; bold lines are the mean values. *$P < 0.001$ (Kruskal–Wallis and Dunn's test).

eters, including CRP. Again, in UC and CD patients plasma CgA levels did not correlate with the disease activity indexes (DAI and CDAI, respectively), either with endoscopic indexes or with disease site. However, there was a trend toward higher CgA levels in extensive as compared with localized disease (UC: proctitis versus left-sided colitis and pancolitis 17.2 ± 7.64 versus 23.6 ± 17.05 U/L, $P < 0.05$, Mann–Whitney test; CD: colitis and ileitis versus ileocolitis 15.0 ± 7.95 versus 24.2 ± 16.78 U/L, $P < 0.03$, Mann–Whitney test). Moreover, no influence of treatment on CgA levels was found.

In UC patients no association was found between plasma CgA levels and the number of mucosal CgA-positive cells. Further, there were no significant differences in mean CgA-positive cells among the mucosal biopsies of active and quiescent UC and controls (35 ± 20.7, 43 ± 20.1, and 59 ± 32.8 cells/50 glands, respectively).

From the revision of our series of 83 patients with GI carcinoids we found that 4 of them had also IBD, with a prevalence of 4.8%. They were 4 male patients, aged 47–68 years, mean 59 years; 2 had UC and 2 had CD. Carcinoid site was rectum in the 2 UC patients, appendix in 1 CD patient, and ileum in the other CD patient. Appendiceal and rectal tumors were well differentiated, whereas the ileum tumor was a malignant carcinoid with liver and lymph node metastases. All carcinoids were diagnosed in inactive disease sites. No patient had carcinoid syndrome (Table 1).

As shown in Figure 2, patients with carcinoids and IBD had intermediate plasma CgA levels and not significantly different from those of patients with IBD alone and those of patients with carcinoids alone, while the difference in CgA values between these 2 groups was statistically significant ($P < 0.001$). CgA values above 100 U/L occurred in all carcinoid patients with metastases, including the 1 with CD.

DISCUSSION

Consistent with preliminary data from Granberg et al[2] and Spadaro et al,[13] the present results demonstrate that

TABLE 1. Characteristics of 4 Patients with Concomitant GI Carcinoid and IBD

No.	Sex, Age (yr)	IBD Type	Carcinoid		Metastases
			Localization	Histology	
1	M, 55	UC	Rectum	Well differentiated	Absent
2	M, 47	UC	Rectum	Well differentiated	Absent
3	M, 68	CD	Appendix	Well differentiated	Absent
4	M, 65	CD	Ileum	Malignant carcinoid	Present

plasma CgA levels are significantly higher in IBD patients than in healthy subjects. The highest CgA levels have been detected in patients with extensive disease, whereas no significant difference has been found in the mean CgA plasma values between UC or CD patients, in both active and quiescent phase of the disease. Interestingly, the finding that a significantly higher proportion of patients with CgA plasma levels above the normal range was found in those with active than quiescent IBD clearly indicates an influence of the disease activity on the CgA pattern. This suggestion is further supported by the finding in the present series of a significant relationship between CgA and TNF-α circulating levels. Indeed, the proinflammatory role of TNF-α in IBD is well known.[34] Recently, increased CgA plasma levels often related with serum TNF-α and/or soluble TNF-α receptor levels have been found in patients with different inflammatory diseases, such as rheumatoid arthritis, systemic lupus erythematosus, chronic heart failure, and chronic obstructive pulmonary disease,[10,14,15,18] even if the actual pathophysiological meaning of these data remains to be elucidated. Taking into consideration the wide expression of CgA in the secretory granules of neuroendocrine cells and its corelease with hormones and neurotransmitters, it has been suggested that in chronic inflammatory disorders circulating CgA could indicate the neuroendocrine system activation in response to the noxious stimulus.[7] The role of neuropeptides and their interplay with cytokines in the inflammatory response has recently been reviewed.[34,35] Moreover, CgA itself and CgA-derived peptides, such as vasostatin and catestatin, could have inhibitory modulating properties on inflammatory processes and antagonize some deleterious effects of high TNF-α levels.[7,36] Interestingly, in endothelial cells CgA and vasostatin-1 have been reported to inhibit the cytoskeleton rearrangement induced by TNF-α and to contribute to the control of the endothelial barrier function by protecting vessels against plasma leakage that occurs in inflammatory diseases.[37–39]

The source of circulating CgA in patients with IBD remains to be elucidated. In our UC patients there was no evidence for an increased CgA production from the colon, as the number of CgA-positive cells in mucosal biopsies was normal. This finding is consistent with some previously reported data,[40] although several other studies have demonstrated hyperplasia of colonic neuroendocrine cells, involving not only the mucosa but also the lamina propria and the submucosa.[23–28,41,42] In this context, surgical specimens rather than endoscopic biopsies are mandatory to adequately evaluate the proliferative changes of colonic endocrine cells in UC.[41] Moreover, there is evidence that microcarcinoids can occur in hyperplastic areas.[28] These lesions are usually benign and have been considered a reactive process to the chronic inflammation.[25,26,41] This interpretation is consistent with the recent report by Quinn and Platell[26] of a UC patient with vanishing rectal microcarcinoids after treatment and resolution of the inflammation.

The occurrence of carcinoid tumors in patients with IBD has been considered rare,[19,20] even if the current literature might underestimate their actual incidence. Indeed, in CD patients an incidence of carcinoids markedly higher than that in the general population has recently been clearly described[21] and 34 UC patients with carcinoids have been reported up to 2004.[19,20,28,40,42] In our 10-year series of GI carcinoid patients, 4 out of 83 (4.8%) had IBD, whereas the prevalence of IBD in the general population could be estimated as 1–2:1.000.[43] From a clinical point of view, the possibility that CgA levels are high in IBD should be taken into account when a carcinoid is suspected in such patients. Indeed, in our study only CgA values above 100 U/L can safely distinguish patients with neuroendocrine tumors from those with IBD alone, but most carcinoid patients without metastases have CgA levels below this cutoff, thus requiring other diagnostic tools. In this respect, it should be mentioned that treatment with aminosalicylates can give false-positive results in the assay of urinary 5-hydroxyindoleacetic acid, which is the other biochemical parameter usually employed in carcinoid diagnosis.[44]

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