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Original Article

Colonic mucosal expression of heat-shock proteins may have a potential prognostic value in ulcerative colitis

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ABSTRACT

Background and study aims: Ulcerative colitis (UC) is a lifelong, chronic, progressive, and relapsing inflammatory disease. Endoscopy with biopsies is the mainstay in diagnosis and assessment. The development of biomarkers is important for the diagnosis and follow-up of UC. We investigated the expression of molecular chaperones/heat-shock proteins (HSP70 and HSP90) in relation to the grades of inflammation and dysplasia in patients with UC before and after treatment.

Patients and methods: A total of 104 naïve patients with UC of varying severity were admitted to the Department of Tropical Medicine and Infectious Diseases, Tanta University Hospital. Ten biopsies from the healthy mucosa of patients with irritable bowel syndrome (IBS) served as a control. Disease activity was assessed clinically using the Mayo score system. Endoscopic mucosal biopsies were taken at diagnosis and 6 months after treatment. Histopathological activity was graded for inflammation and dysplasia. Immunohistochemistry was used to determine the percentage of cells positive for HSPs. The results were expressed in a semiquantitative scale.

Results: The expression of both HSP70 and HSP90 increased in patients with UC at the time of disease activity, and it decreased after treatment and remission. There was a significant correlation between the expression of both proteins and the grades of dysplasia as well as inflammation ($P < 0.05$). Strong expression of HSPs that persisted after treatment has been associated with cases of true dysplasia.

Conclusions: The results indicated that HSP70 and HSP90 had the potential for assessment of the activity and prognosis of UC. They can also predict the presence of dysplasia and differentiate it from reactive atypia. Larger studies are needed to confirm this diagnostic and prognostic value of HSPs.

Introduction

Ulcerative colitis (UC) is a chronic, progressive, and relapsing inflammatory disorder characterised by damage of the large bowel mucosa and extraintestinal autoimmune comorbidities. It represents a lifelong disorder with high morbidity and potential mortality. It has a risk of transforming into colorectal cancer [1,2].

Endoscopic biopsies are the mainstay in diagnosis, assessment of disease activity, and monitoring treatment. However, the need for reliable surrogate markers of intestinal inflammation and/or malignant transformation still exists [3].

Heat-shock proteins (HSPs) play a significant role in cell proliferation, differentiation, and oncogenesis [4–6]. They are constitutively and gradually expressed in a broad range of normal tissues and neoplasms, and their expression has been assessed as markers of dysplasia [7–10].

The expression of certain HSPs can be correlated with the carcinogenic process [11,12] as well as with the degree of cell proliferation and differentiation [13]; moreover, they have been implicated in the regulation of apoptosis [14]. Therefore, our aim was, importantly, to study the expression of HSPs in relation to the degree of inflammation and dysplasia in patients with UC before and after therapy.

Patients and methods

The study was conducted at the Department of Tropical Medicine and Infectious Diseases, Tanta University. A total of 104 naïve patients diagnosed with UC of varying severity were included from October 2011 to August 2013. Patients with colorectal cancer and/or high-grade dysplasia were excluded and referred to surgery.

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Endoscopic mucosal biopsies were obtained for all patients at diagnosis and 6 months after treatment irrespective of the response.

Ten apparently healthy subjects served as the control. They underwent colonoscopy to rule out organic pathology in a diarrhoea-predominant irritable bowel syndrome (IBS). Biopsies were taken to rule out microscopic colitis.

Clinically, disease activity was assessed using the Mayo score from 0 to 12 [15]. Endoscopic activity was graded as mild, moderate, and severe according to the Mayo score [16].

Histopathological activity was graded according to a six-grade classification system for inflammation: 0 = structural change only; 1 = chronic inflammation; 2 = lamina propria neutrophils; 3 = neutrophils in epithelium; 4 = crypt destruction; and 5 = erosions or ulcers [17]. Dysplasia represented as HSP expression was graded as mild, moderate, or strong according to Riddell et al. [18].

Patients were treated with mesalazine (5-aminosalicylic acid, 5-ASA) 4 g/day until clinical remission, and then with 2 g/day for 6 months. No patient needed to be treated with steroids. Then, follow-up endoscopy and biopsy was performed for all 104 patients to assess their histological response to therapy.

Histopathology was performed at the Department of Pathology, Tanta University Faculty of Medicine.

Tissue preparations and immunohistochemistry

All biopsies were fixed in formalin and embedded in paraffin. Sections of 5-μm thickness were obtained from all paraffin blocks, de-waxed, and rehydrated for immunohistochemical analysis. Immunostaining was performed using an avidin–biotin complex kit (DAKO, Carpentaria, CA, USA), after incubation for 10 min with serum-free protein blocking. Primary antibodies such as anti-HSP70 or anti-HSP90 (dilution 1:200, Santa Cruz Biotechnology, Dallas, TX, USA) were added. Appropriate positive controls, as well as nonimmune serum for negative controls, were run concurrently. 3-3′-diaminobenzidine (DAB chromogen solution, DAKO, Glostrup, Denmark) was used as the developer chromogen. Nuclear counterstaining was performed using haematoxylin.

A quantitative analysis was used to determine the percentage of cells positive for HSPs in both the epithelium (Ep) and lamina propria (LP) of the colon mucosa. The results were expressed in a semi-quantitative scale (−: 0%; +: 1–33%; ++: 34–66%; and +++: 67–100%).

All the observations were made at a magnification of 400× and the means of duplicate counts were used for statistical analyses.

Statistical analyses

The collected data were organised, tabulated, and statistically analysed using SPSS version 12. For quantitative data, the mean and standard deviation were calculated. For qualitative data, the number and percentage of distribution were calculated. Chi-square was used as a test of significance. A correlation was performed between the immunohistochemical levels of HSP, disease activity, and epithelial dysplasia before and after treatment, using Pearson's test. A *P*-value <0.05 was considered significant.

Ethics committee approval was taken before the start of the study. Informed consent was signed by every patient before endoscopy.

Results

Most patients (77%) presented with bleeding per rectum, 88% suffered from various degrees of microcytic hypochromic anaemia, 67% suffered chronic diarrhoea, 43% complained of weight loss, and 53% experienced abdominal pain. Endoscopic findings ranged from mild disease with hyperemia and loss of vascular pattern to severe ulceration, pseudopolyps, and loss of haustrations. Colonic biopsies showed various grades of severity of UC (Figs. 1a, 1b, 2a and 2b).

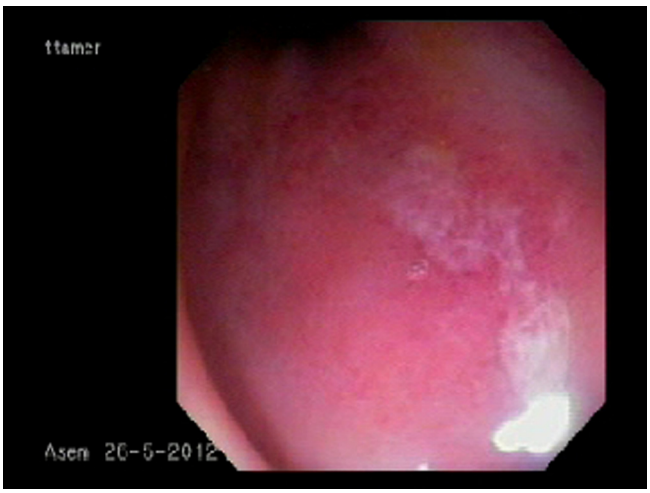

Fig. 1a. Endoscopic view of a case with mild UC.

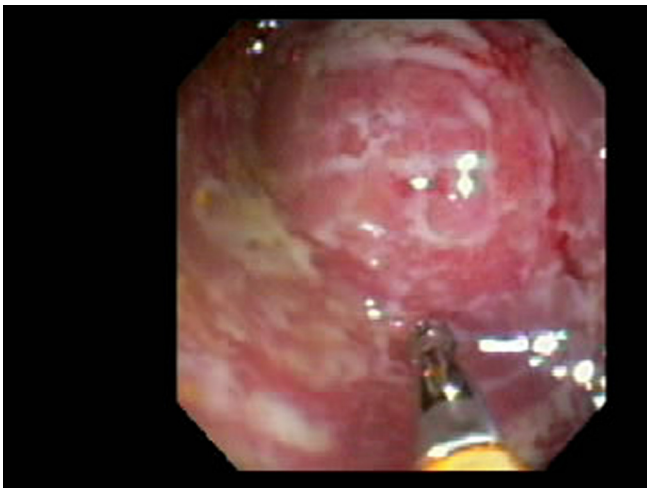

Fig. 1b. Endoscopic view of a case with moderate UC.

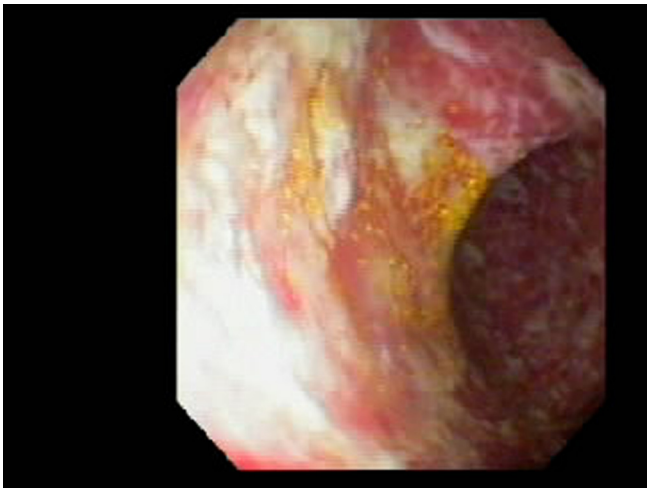

Fig. 2a. Endoscopic view of a case with severe UC.