PO Box 2345, Beijing 100023, China
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CLINICAL RESEARCH

Microproteinuria in patients with inflammatory bowel disease: Is it associated with the disease activity or the treatment with 5-aminosalicylic acid?

Androniki C Poulou, Konstantinos E Goumas, Dimitrios C Dandakis, Ioannis Tyrmpas, Maria Panagiotaki, Androniki Georgouli, Dimitrios C Soutos, Athanasios Archimandritis

Androniki C Poulou, Konstantinos E Goumas, Dimitrios C Dandakis, Ioannis Tyrmpas, Maria Panagiotaki, Androniki Georgouli, Dimitrios C Soutos, Gastroenterology Department of Red Cross Hospital of Athens, Athanasaki 1, 11521 Athens, Greece
Athanasios Archimandritis, 2nd Department of Internal Medicine, University of Athens Medical School "Hippokratio" General Hospital, 11521 Athens, Greece
Correspondence to: Dr Androniki Poulou, Red Cross Hospital of Athens, Spetson 25, Voula, 16673 Athens, Greece. ddandakis@yahoo.com
Telephone: +30-210-6414724 Fax: +30-210-6414635
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Abstract

AIM: To investigate whether microproteinuria in patients with inflammatory bowel disease (IBD) is associated with the disease activity or the treatment with 5-aminosalicylic acid (5-ASA).

METHODS: We prospectively studied microproteinuria in 86 consecutive patients with IBD, 61 with ulcerative colitis (UC) and 25 with Crohn's disease (CD), before as well as 2 and 6 months after their inclusion in the study. Forty-six patients received 5-ASA for a period of 28.8 months (range 1-168 mo). Microalbuminuria (mALB) and urine levels of the renal tubular proteins β2-microglobulin (β2mGLB) and β-*N*-acetyl-D-glucosamidase (β-NAG) as well as the creatinine clearance were determined in a 12-h overnight urine collection. Tumor necrosis factor-α (TNF-α) serum levels were also measured.

RESULTS: A total of 277 measurements (194 in UC patients and 83 in CD patients) were performed. The prevalence of abnormal microproteinuria in UC and CD patients was 12.9% and 6.0% for mALB, 22.7% and 27.7% for β2mGLB, and 11.3% and 8.4% for β-NAG, respectively. mALB was not associated with IBD activity. β2mGLB and β-NAG urine levels were correlated to UC activity (UCAI: $P<0.01$; UCEI: $P<0.005$). mALB in UC patients and β-NAG urine levels in CD patients were related to TNF-α serum levels. An association was noticed between microproteinuria and smoking habit. Treatment with 5-ASA was not correlated to the severity of microproteinuria or to the changes of creatinine clearance.

CONCLUSION: Microproteinuria is mainly associated with UC and its activity but not affected by 5-ASA.

Key words: Inflammatory bowel disease; Microproteinuria; 5-aminosalicylic acid

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INTRODUCTION

Complications of the urinary system are not uncommon in patients with inflammatory bowel disease (IBD). Their incidence has been reported to vary from 4% to 23% and is greater in patients with more severe and long-standing disease[1]. Apart from secondary complications, such as nephrolithiasis, hydronephrosis and amyloidosis, other associations between IBD and renal disorders have also been described, regardless of treatment[2-5]. Membranous glomerulonephritis, rapidly progressive glomerulonephritis, mesangiocapillary glomerulonephritis, IgA nephropathy, thin basement membrane disease and kidney granuloma are connected with IBD[6-10]. In addition, non-specific morphological changes in the glomeruli of patients with IBD, such as podocyte effacement and mesangial deposition of immunoglobulin and complement have also been well documented[11]. On the contrary, cases of interstitial nephritis are attributed to the nephrotoxic effect of aminosalicylates[11]. However, before the establishment of 5-ASA as a treatment of choice in IBD, studies have identified renal tubular lesions in a proportion of patients[12]. Till date, more than 37 cases implicating the drug in interstitial nephritis (mainly mesalazine but also sulfasalazine, balsalazide, and olsalazine) have been reported to the Committee on Safety of Medicines (CSM)[13].

Abnormal microproteinuria [microalbuminuria (mALB) and/or tubular proteinuria] has been reported both in patients with ulcerative colitis (UC) and in those with Crohn's disease (CD)[13-17]. Increased mALB, equivalent to an albumin excretion rate of 20-200 μg/min, is generally regarded as a sensitive indicator of glomerular disease and has been widely used as a clinical marker of incipient diabetic nephropathy[18]. Mahmud *et al*.[14] have found that there is a significant correlation between mALB and IBD activity. Increased urine levels of some tubular proteins, such as β-*N*-acetyl-D-glucosamidase (β-NAG), α_1-microglobulin, β_2-microglobulin (β_2mGLB), alkaline phosphatase and gamma-glutamyltransferase, have been reported to be reliable indirect indices of renal tubular dysfunction[19]. Moreover, Riley *et al*.[15] have also found that the incidence of elevated urinary β-NAG is low in patients with quiescent UC, which is irrelevant to the dose and duration of mesalazine treatment. Kreisel *et al*.[17] reported that there is a strong correlation between abnormal tubular proteinuria and the activity of UC but not the activity of CD. On the contrary, Schreiber *et al*.[20] showed that increased prevalence of tubular proteinuria is attributed to high dosages of 5-ASA.

The etiology of abnormal microproteinuria in patients with IBD remains ambiguous. We conducted the present 6-month prospective study in order to investigate the incidence of mALB and tubular proteinuria in patients with IBD, emphasizing on the possible relationship between microproteinuria and the disease activity or the treatment with 5-ASA.

MATERIALS AND METHODS

Patients and sample selection

A total of 86 out of 166 screened consecutive Caucasian patients with confirmed chronic IBD (61 with UC and 25 with CD), treated at the Gastroenterology Department of the Red Cross Hospital of Athens from June 1998 to April 2001, were prospectively included in this study. Patients with signs of urinary tract infection and a history of acute or chronic nephropathy, diabetes mellitus, hypertension, severe liver disease, cardiac failure, use of non-steroidal anti-inflammatory drugs (NSAIDs) or other nephrotoxic drugs, patients with a present or recent pregnancy, patients treated with olsalazine, and patients who refused to participate in this study were excluded. Fifty patients were male, 36 were female and their mean age was 42.7 (range 15-77 years) years. Fifty-nine were out-patients and 27 were in-patients. At the time of their inclusion in this study, the IBD was active in 38 patients (27 with UC and 11 with CD) and inactive in 48 patients. Thirty out of eighty-six patients were first diagnosed with IBD (23 with UC and 7 with CD). The mean duration of the disease was 69.8 months (range 0-576 months). The group of patients with UC consisted of 16 patients with total colitis and 45 with left sided colitis (two of them with rectal disease only). The group of patients with CD included 11 patients with ileo-colonic disease, 8 with small bowel disease only and 6 with colonic disease only. Due to the small number of patients, two subgroups of patients with CD were considered in the subsequent analysis, one consisted of the patients with ileo-colonic or colonic disease and the other consisted of those suffering from small bowel disease only. Extra-intestinal manifestations at present or during the last 2 months before the first meeting or during follow-up, were recorded in 29 patients (14 with UC, 15 with CD). Forty-six patients received mesalazine (Salofalk 2-3 g/day or Asacol 1.6-2.4 g/day) for a mean period of 28.8 months (range 1-168 months). During the last year, 20 of them were treated with prednisolone for a mean period of 1.9 months (range 1-12 months) and 6 with azathioprine for a mean period of 4.9 months (range 1-10 months).

According to the study design, all patients were evaluated at the time of their inclusion in the study (stage 1), two (stage 3) and six (stage 4) months afterwards. Twenty-seven patients (19 with UC, 8 with CD) with severely exacerbated disease at the time of their inclusion in the study were re-evaluated 10 days after intensive corticosteroid-based treatment (stage 2). A meticulous routine and more specific clinical and laboratory evaluation of the patients was conducted in each stage of the study, including determination of the hematocrit value, white blood cell and platelet count, serum levels of C-reactive protein (CRP) and tumor necrosis factor-alpha (TNF-α), usual urinalysis, levels of mALB and tubular proteinuria (β_2mGLB, β-NAG) and creatinine clearance. Measurements of microproteinuria and creatinine clearance were performed using a 12-h urine collection, which has been proved equivalent to a 24-h urine collection[21] or an 8-h urine collection[22]. The criterion of our choice was the better compliance of our patients. The clinical disease activity quantifications were performed on the day of urine collection, using reliable activity indices for UC[23] and CD[24]. All patients with UC underwent colonoscopy at the 1st, 3rd, and 4th stages of the study and an endoscopic index of the activity of UC (UCEI)[25] was calculated for these patients[25]. Finally, a detailed drug history was taken from all the patients to confirm the type, dose and duration of medication.

The blood samples for the measurement of serum levels of TNF-α after centrifugation were stored at -48 ° C. The urine samples for the determination of urine levels of microalbumin were stored at 2-8 °C for a time not exceeding 2 weeks, at -20 °C for β_2mGLB and at -48 °C for β-NAG, respectively.

Microproteinuria (mALB and tubular proteinuria) was correlated to patients' characteristics including sex, age, smoking habits; features of the diseases including activity, extent, duration, presence of extraintestinal manifestations; medication including type (mesalazine, corticosteroids, azathioprine) and duration. Furthermore, separate analyses of the courses of microproteinuria were conducted for patients with highly active disease at the time of their inclusion in the study and those with a first diagnosis of the disease, who did not receive any medication.

Analytical techniques (chemical analysis)

Urea and creatinine in serum as well as creatinine in urine were measured with standard techniques. Serum CRP concentrations were determined by nephelometry.

Urinary albumin concentrations were measured using a radioimmunologic assay (^{125}I radioimmunologic analysis, EURO/DPC, Glyn Rhonwy, UK), which could indicate

Table 1 Incidence of abnormal microproteinuria in patients with active and inactive IBD (%)

	Ulcerative colitis (n = 194)			Crohn's disease (n = 83)		
	Active (n = 49) UCAI ≥ 6	Inactive (n = 145) UCAI < 6	P	Active (n = 15) CDAI ≥ 150	Inactive (n = 68) CDAI < 150	P
Urine m-ALB (>18 μg/min)	16.3	11.7	NS	13.3	4.4	0.06
Urine β_2 mGLB (>120 μg/min)	40.8	16.6	<0.001	53.3	22.1	<0.05
Urine β-NAG (>5.9 U/L)	26.5	6.2	<0.001	13.3	7.4	NS

Table 2 Microproteinuria and creatinine clearance in active and inactive IBD (mean±SE)

	Ulcerative colitis (n = 194)			Crohn's disease (n = 83)		
	Active (n = 49)	Inactive (n = 145)	P	Active (n = 15)	Inactive (n = 68)	P
Urine m-ALB (μg/min)	11.89±3.19	9.0±1.03	NS	8.59±2.65	7.02±1.07	NS
Urine β_2mGLB (μg/min)	204±50.2	88.0±10.28	<0.01	359±130.9	92.2±16.26	<0.05
Urine β-NAG (U/L)	4.69±0.64	2.39±0.16	<0.001	3.07±0.90	2.36±0.25	NS
Urine Kcr (mL/min)	114.4±6.03	120.3±3.24	NS	135.6±8.34	127.7±3.96	NS

NS: Not significant.

subtle increases in albumin excretion above the normal range but below that was detected by standard "dipstick" analysis (equivalent to a urinary albumin excretion rate of 20-200 μg/min)[26].

Urinary β2mGLB concentrations were measured using a radioimmunologic assay (^{125}I radioimmunologic analysis, RADIM)[27].

Urinary β-NAG concentrations were determined colorimetrically using a commercially available kit (Boehringer-Mannheim, Germany). The optical density of the samples was measured at 580 nm (Cobas Bioanalyser, Roche). Results were calculated by reference to a standard curve[15,28].

Serum TNF-α was measured using a sandwich enzyme-linked immunosorbent assay (ELISA; Quantikine, R&D Systems Inc., Minneapolis, MN, USA: sensitivity 0.35 pg/mL)[29,30].

Quantification of disease activity

The activity of UC was quantified using the UC activity index (UCAI) introduced by Rachmilewitz[23]. A score greater than or equal to six was considered to be suggestive of active UC. The activity of CD was estimated according to the Best CD activity index (CDAI)[24]. CD was considered to be active in cases with CDAI greater than 150. Finally, the endoscopic activity of UC was also evaluated according to a reliable endoscopic index established by other researchers[25].

Statistical analysis

The Mann-Whitney *U*-test, the Wilcoxon rank-sum *W*-test and the Spearman's rank correlation test were used for statistical evaluation of the non-parametric data. Student's *t*-test was applied for statistical analysis of all parametric data. $P<0.05$ was considered statistically significant.

RESULTS

Eighty-two out of eighty-six patients participating in three stages of the study (time: 0, 2nd, and 6th month) completed the follow-up. Twenty-seven of them participated in one more stage (time: 10th day). Two out of eighty-six patients participated in two stages (time: 0, 10th day) and two in one stage only (time: 0). A total of 277 distinct evaluations were performed in 86 IBD patients, 194 in patients with UC and 83 in patients with CD. The IBD was active in 38 out of 86 patients at the 1st stage, in none out of 27 patients at the 2nd stage, in 7 out of 82 patients at the 3rd stage and in 6 out of 82 patients at the 4th stage of the study.

Abnormal urine levels of albumin (>18 μg/min), β2mGLB (>120 μg/min) and β-NAG (>5.9 U/L) were found in 12.9%, 22.7%, and 11.3% of the patients with UC and in 6%, 27.7%, and 8.4% of those with CD, respectively, more frequently in active than in inactive disease (Table 1). The mean values of mALB, tubular microproteinuria and creatinine clearance in active and inactive IBD are shown in Table 2. No differences in albuminuria were found between active or inactive UC and CD. In contrast, significantly higher urine levels of β_2-mGLB were detected both in patients with active UC and in those with active CD. Urine levels of β-NAG were

Table 3 Correlation of microproteinuria and creatinine clearance of IBD patients after 5-ASA treatment (mean±SE)

	Ulcerative colitis ($n=194$) 5-ASA treatment		P	Crohn's disease ($n=83$) 5-ASA treatment		P
	No ($n=37$)	Yes ($n=157$)		No ($n=15$)	Yes ($n=68$)	
Urine m-ALB (μg/min)	8.99±2.10	9.90±1.29	0.794	8.08±2.57	7.13±1.08	0.562
Urine β_2mGLB (μg/min)	163±61.16	107±12.07	0.852	274±129.2	111.0±20.15	0.083
Urine β-NAG (U/L)	4.22±0.82	2.67±0.18	0.118	2.79±0.91	2.42±0.26	0.845
Urine Kcr (mL/min)	117±7.25	119±3.11	0.987	130±7.62	129±4.05	0.713

Table 4 Correlation between microproteinuria in patients with ulcerative colitis and disease activity parameters ($n=194$ measurements)

		Ulcerative colitis activity parameters					
		UCAI	UCEI	CRP	ESR	Platelets	Serum TNF-α
Urine m-ALB (μg/min)	r_S	0.0283	0.0420	0.0232	0.0091	0.0826	0.1665
	P	0.6954	0.5612	0.7479	0.9001	0.2520	0.0203
Urine β_2mGLB (μg/min)	r_S	0.1957	0.1037	0.1059	0.0033	0.0540	0.0123
	P	0.0062	0.1501	0.1414	0.9634	0.4543	0.8648
Urine β-NAG (U/L)	r_S	0.2633	0.2335	0.1438	0.1224	0.2336	0.1057
	P	0.0002	0.0010	0.0455	0.0891	0.0010	0.1426

r_S: Spearman's correlation coefficient; r_P: Pearson's correlation coefficient.

higher in active UC, but not in active CD.

Serum creatinine and its clearance

The levels of serum creatinine were normal in all the patients. Although creatinine clearance values varied widely, its mean values did not differ significantly between the patients with active and those with inactive UC (114.4 ±6.03 mL/min *vs* 120.3±3.24 mL/min, $P>0.10$) as well as between the patients with active and those with inactive CD (135.6±8.34 mL/min *vs* 127.7±3.96 mL/min, $P>0.10$). Our male IBD patients showed higher creatinine clearance values than female IBD patients (UC: 134±3.19 mL/min *vs* 95±4.07 mL/min, $P<0.001$; CD: 137.1±5.16 mL/min *vs* 118.8±4.22 mL/min, $P<0.05$). Creatinine clearance values were inversely correlated with the age of UC patients (ulcerative colitis: $r_P=-0.374$, $P<0.001$; CD: $r_P=-0.164$, $P>0.10$).

In addition, creatinine clearance values were higher in smokers with UC (smokers: 127±4.04 mL/min; non-smokers: 114±3.81 mL/min, $P<0.05$) and in those with CD (smokers: 137.3±4.50 mL/min; non-smokers: 113.1±4.50 mL/min, $P<0.001$). Finally, no correlation of creatinine clearance values with 5-ASA treatment was observed (UC patients treated with and without 5-ASA: 119±3.11 mL/min and 117±7.25 mL/min, respectively, $P=0.987$; CD patients treated with and without 5-ASA: 129±4.05 mL/min and 130±7.62 mL/min, respectively, $P=0.713$).

Microproteinuria and IBD patients' parameters

No correlation was found between microproteinuria (mALB or tubular proteinuria) and the age or the sex of our IBD patients (data not shown). However, albuminuria was significantly lower in smokers with UC than in non-smokers (smokers: 8.22±2.16 μg/min; non-smokers: 10.6 ±1.23 μg/min, $P=0.021$) and the urine levels of β-NAG were significantly lower in non-smokers with CD than in smokers (non-smokers: 1.62±0.19 U/L; smokers: 2.93± 0.37 U/L, $P=0.023$).

Microproteinuria and IBD activity

In our patients with UC, urinary concentrations of β-NAG were significantly correlated with the UCAI ($r_S=0.26$, $P<0.0005$), the UCEI ($r_S=0.23$, $P<0.005$) and the serum CRP levels ($r_S=0.14$, $P<0.05$). Similarly, in these patients urinary levels of β_2 mGLB were significantly associated with the UCAI ($r_S=0.2$, $P<0.01$) but not with the UCEI or the serum CRP levels (Table 4). On the contrary, the patients with CD did not show any correlation of tubular microproteinuria with the parameters of their disease activity (Table 5).

No significant relationship was found between the levels of mALB and the disease activity in patients with UC neither in those with CD (Tables 4 and 5). A significant association between mALB and serum levels of TNF-α was noticed only in UC patients. Finally, a history of recent or present extra-intestinal manifestations did not influence the levels of mALB and tubular microproteinuria in IBD patients (data not shown).

Microproteinuria and IBD duration and extent

A significant inverse relationship was found between the levels of mALB and the duration of UC ($r_S=-0.24$, $P<0.001$). In contrast, a tendency towards a positive association was noticed between the severity of mALB and the duration of CD ($r_S=0.19$, $P=0.078$). No significant differences were found between the levels of microproteinuria (mALB and tubular microproteinuria)

Table 5 Correlation between microproteinuria in patients with Crohn's disease and disease activity parameters ($n=83$ measurements)

		Crohn's disease activity parameters				
		CDAI	CRP	ESR	Platelets	Serum TNF-α
Urine m-ALB (μg/min)	r_S	0.1186	0.0326	0.0352	0.1483	0.1537
	P	0.2856	0.7686	0.7522	0.1810	0.1654
Urine β$_2$mGLB (μg/min)	r_S	0.0813	0.0187	0.0624	0.1727	0.2100
	P	0.4648	0.8669	0.5751	0.1184	0.0568
Urine β-NAG (U/L)	r_S	0.1106	0.0319	0.0947	0.0527	0.2676
	P	0.3196	0.7744	0.3347	0.6359	0.0145

r_S: Spearman's correlation coefficient; r_P: Pearson's correlation coefficient.

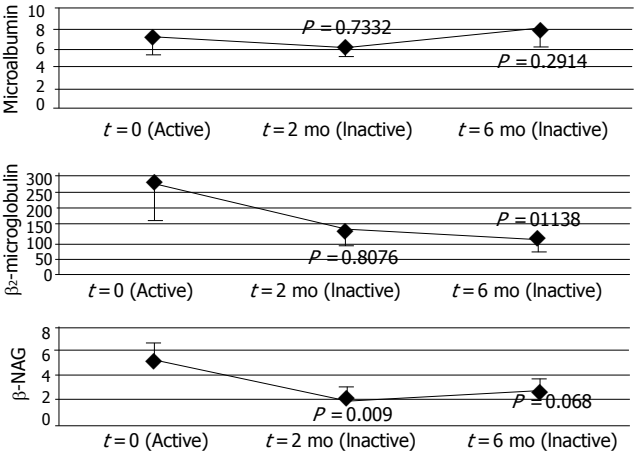

Figure 2 Changes of microproteinuria in the first-diagnosed patients with active IBD ($n=22$) before and after inducing remission.

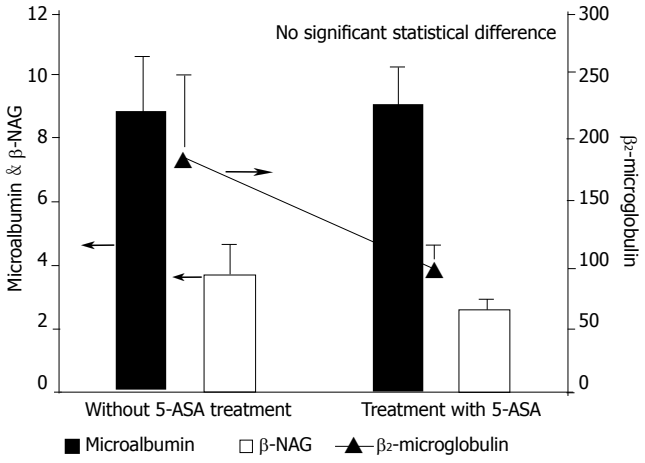

Figure 1 Correlation of IBD patients' microproteinuria (mALB, β_2mGLB, β-NAG) with 5-ASA treatment.

and the extent of UC (total *vs* left side colitis) as well as the extent of CD (small intestine+colon *vs* small intestine only).

Microproteinuria and IBD therapy

No differences were found in the levels of mALB and tubular microproteinuria between the IBD patients who received or did not receive 5-ASA therapy (Table 3 and Figure 1). Moreover, no differences were found between the duration of 5-ASA treatment and the levels of mALB (UC: $r_s=-0.06$, $P=0.43$; CD: $r_s=0.05$, $P=0.70$), the urinary levels of β_2mGLB (UC: $r_s=0.06$, $P=0.42$; CD: $r_s=0.17$, $P=0.16$) and β-NAG (UC: $r_s=-0.02$, $P=0.77$; CD: $r_s=0.02$, $P=0.88$). The levels of microproteinuria were also similar in the patients treated and those not treated with corticosteroids and/or azathioprine (data not shown).

Microproteinuria in the first-diagnosed patients with active IBD

Among the 30 IBD patients with a first diagnosis of the disease at the time of their inclusion in this study, 22 had active IBD (17 had UC, 5 had CD). The 6-month course of mALB and tubular microproteinuria in the patients with an initially active IBD never treated with 5-ASA, exhibited a particular interest (Figure 2). In these patients, microproteinuria showed a tendency of gradual reduction by the time when IBD remission was achieved, although this reduction was statistically significant for β-NAG only.

Microproteinuria in patients with severely active IBD

Twenty-seven of the patients (19 with UC, 8 with CD) were admitted to our hospital due to severely active disease. In these patients, we also evaluated the levels of microproteinuria in one more stage 10 days after the initiation of an intensive therapy (including corticosteroids). An early (10 days after) reduction of mALB (10.56 ± 2.27 μg/min *vs* 6.13 ± 1.43 μg/min, $P<0.01$) as well as urine levels of β-NAG (5.60 ± 1.10 U/L *vs* 3.17 ± 0.60 U/L, $P<0.05$) and β_2mGLB (241.2 ± 96.82 μg/min *vs* 99.6 ± 33.33 μg/min, $P>0.10$) was observed.

DISCUSSION

Although the incidence of renal complications is relatively low in patients with IBD, none of our patients developed clinically significant renal disease. No significant changes in serum creatinine and its clearance were recorded during the 6-month follow-up period. Furthermore, creatinine clearance was not correlated with the type, activity or the extent of IBD, which is in accordance with the majority of the published studies[13-17].

We observed a moderately abnormal mALB in 12.9% and 6% of our patients with UC and CD, respectively, which is in agreement with Fraser *et al*[13], Riley *et al*[15], Bonnet *et al*[31] and Zehnter *et al*[32]. Increased mALB reliably predicts diabetic nephropathy and an increased incidence of cardiovascular-related morbidity and mortality in diabetic and hypertensive patients as well as in the elderly[18,33,34]. Moreover, mALB has been described as a non-specific marker for acute illness[35], most probably as a result of the acute phase response to inflammatory

mediators. It has been suggested that mALB in IBD patients may result from increased renal microvascular permeability in response to increased circulating cytokines[14,30,36]. This hypothesis is compatible with our finding of a significant relationship between mALB and serum TNF-α levels in our UC patients ($P < 0.05$), a thesis which is also supported by other researchers[13,15,31,32].

We did not find any significant correlation between the levels of mALB and the IBD activity, which is in agreement with many published studies[13,15]. In contrast, Mahmud *et al*.[14,37] found that increased mALB in the vast majority of IBD patients is related to both clinical and histopathological activities of the disease. Differences between these studies may be attributed to the varied disease activity. In our study, a relatively small proportion of the patients had a considerable disease activity.

Abnormal tubular microproteinuria, reflecting subclinical tubular damage, is a quite common finding among IBD patients[15,17]. However, its incidence varies widely[13-17,31,32,38], from 0% in the studies by Biddle *et al*[38] (β-NAG and β2mGLB) and Mahmud *et al*[14] (glutathione-*S*-transferase) to 75% in the study by Bonnet *et al*[31] (γ-glutamyl transferase and alkaline phosphatase). Hitherto, it has been difficult to prove whether tubular microproteinuria results from the effect of 5-ASA or is an extra-intestinal manifestation of the IBD itself[13]. Its structural similarity to other salicylates and non-steroidal anti-inflammatory drugs has led to the speculation that 5-ASA may cause tubulointerstitial damage of the kidney[11].

A significant correlation between tubular microproteinuria and IBD activity was found only in our patients with UC. This might be due to the fact that our patients with severely active CD were much fewer than those with UC. Kreisel *et al*.[17] have observed an increased urinary β-NAG excretion in 28% of the patients with UC and in 19% of those with CD, especially higher in active disease. Schreiber *et al*.[28] acknowledged their inability to determine whether high levels of tubular microproteinuria are attributable to the severity of the disease's activity or 5-ASA treatment (or both), since 11% of their patients not receiving 5-ASA also had a tubular microproteinuria. Fraser *et al*.[13] found that urinary excretion of β-NAG and α_1-microglobulin was increased in 48% and 52% of patients with IBD at diagnosis, respectively. A positive relationship between the urine levels of several tubular proteins and the IBD activity has been proposed by the majority of the inv-estigators[13-17,31,39]. In contrast, Zehnter *et al*.[32] showed that increased urine levels of β_2-mGLB, alanine aminopeptidase and β-NAG in IBD patients were not correlated with the disease activity.

In this study, 22 first-diagnosed patients with active IBD had increased levels of tubular microproteinuria at diagnosis, which decreased after treatment with corticosteroids and 5-ASA. Moreover, 27 IBD patients with severely active disease manifested an early significant decrease of both microalbuminuria and tubular microproteinuria after being treated with corticosteroids. In addition, we found that there was a positive relationship between microalbuminuria and serum TNF-α levels, as well as between tubular microproteinuria and serum CRP and TNF-α levels, suggesting that microproteinuria in IBD patients may be related to a systemic inflammatory response and that it is an extra-intestinal manifestation of the active disease rather than a consequence of a subclinical renal injury due to 5-ASA treatment[13,15,17,20,31,32,37]. However, We did not find any differences in the severity of microproteinuria between the IBD patients with and those without a recent or present extra-intestinal manifestation of the disease, which is in agreement with Kreisel *et al*[17] and Mahmud *et al*[30].

Activation of the intestinal immune system, production of inflammation mediators or imbalance between activating and suppressing mediators play a pivotal role in the pathogenesis of IBD. In particular, TNF-α may play an important role in the inflammatory cascade of IBD and increased serum TNF-α levels have been reported in active IBD[40]. It has been reported that interleukin-1 and TNF-α inhibit the synthesis and induce a shedding of cell surface glycosaminoglycans[41], which might lead to an increased microvascular permeability and tissue damage[36,42].

No relationship was found between the severity of microproteinuria and the extent as well as the duration of IBD in our study, which is in concordance with other studies[14,20,32,43]. It is worth emphasizing on our finding of a significant correlation between microproteinuria and the smoking habit, which was negative in UC and positive in CD, because smoking alters the colonic mucosal blood flow[44] and decreases its permeability. Epidemiologic data suggest that smoking exerts a protective effect against UC[45] and is harmful to CD[45,46]. Consequently, differences in microproteinuria between smokers with UC and those with CD may be related to these pathophysiologic and epidemiologic data.

In this study, mALB was not correlated with the IBD treatment as it is almost universally accepted[13,15,31,32,37]. In addition, using sensitive markers of renal tubular toxicity[19], we were unable to find any evidence that treatment with mesalazine, corticosteroids or azathioprine was related to an increased tubular damage. However, this lack of correlation does not necessarily exclude nephrotoxicity. A potential nephrotoxicity of aminosalicylates, although rare, remains an existent possibility[11].

Riley *et al*[15] showed that there was no evidence of nephrotoxicity in a cohort of patients with quiescent UC receiving mesalazine or sulfasalazine. Furthermore, Kreisel *et al*[17] have demonstrated that there is no positive relationship between the cumulative dose of 5-ASA and the severity of tubular microproteinuria. In accordance to these studies, we observed that tubular microproteinuria in patients with active IBD at diagnosis decreased after inducing remission of the disease, even though the patients received the treatment with 5-ASA and corticosteroids. Fraser *et al*[13] have demonstrated that both the prevalence and degree of tubular microproteinuria in patients with a new diagnosis of IBD are relatively unaffected by 5-ASA treatment, which is in agreement with a number of studies[14,38,43,47]. In contrast, Schreiber *et al*[20] have reported that there is a positive relationship between tubular microproteinuria and cumulative 5-ASA exposure, but they questioned whether their study could provide a definite answer to the possible impact of chronic inflammation on

microproteinuria. A similar positive relationship between tubular microproteinuria and 5-ASA treatment has also been reported[31,32].

In conclusion, abnormal mALB and tubular microproteinuria are quite frequent in IBD patients. Subclinical renal tubular and/or glomerular damage are related to the IBD itself, representing an extra-intestinal manifestation of the disease. Moreover, treatment with 5-ASA does not affect microproteinuria in IBD patients, microproteinuria in IBD patients does not predict an adverse renal response to 5-ASA.

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