

Original Article

Level of serum apolipoprotein m and its diagnostic significance in inflammatory bowel disease

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Abstract: Our study has investigated the level of serum apolipoprotein M (apoM) and its clinical significance in Inflammatory Bowel Disease (IBD) patients. In this study, serum apoM and tumor necrosis factor α (TNF- α) levels in newly diagnosed IBD patients (n = 101) and healthy individuals (n = 69) were measured by ELISA. Serum apoM levels in Ulcerative Colitis (UC) and Crohn's Disease (CD) groups were found to be 13.5 ± 8.8 $\mu\text{g/ml}$ and 10.2 ± 5.8 $\mu\text{g/ml}$ respectively, compared with 2.0 ± 1.3 $\mu\text{g/ml}$ in the control group. The expression of apoM in the UC group was significantly higher than the CD group (P < 0.05); however, its clinical significance needs to be further investigated. Interestingly, serum apoM concentrations in both UC and CD patients elevated with increasing disease activity in active period of IBD (P < 0.05). The levels of TNF- α were found to be 2.73 ± 1.68 pg/ml , 2.20 ± 1.64 pg/ml and 0.90 ± 2.03 pg/ml in the UC, CD and control groups, respectively. In addition, a positive correlation was found between the serum concentrations of apoM and TNF- α ($\gamma = 0.314$, P = 0.001); particularly, a significant correlation was obtained for the CD group ($\gamma = 0.365$, P = 0.010). Our results demonstrate that serum apoM levels were significantly higher in the IBD patients compared to the healthy individuals. Therefore, analysis of the apoM levels might serve as a novel diagnostic marker for the assessment of IBD activity.

Keywords: Apolipoprotein M, inflammatory bowel disease, tumor necrosis factor α , diagnostic marker

Introduction

Inflammatory Bowel Disease (IBD), which includes Ulcerative Colitis (UC) and Crohn's Disease (CD), is a chronic nonspecific intestinal inflammatory disease. The etiology and pathogenesis of IBD are still unclear. The epidemiological data indicate that the incidence of IBD is increasing every year in China. Moreover, clinical evaluation of IBD activity relies mainly on the clinical symptoms and endoscopically visible inflammation. Due to a lack of effective serological tests, studies identifying potential serological indices might prove to be useful in the diagnosis of IBD. Apolipoprotein M (apoM), a newly discovered member of the lipocalin superfamily, is a specific apolipoprotein present mainly in high density lipoprotein (HDL). Recent studies have shown that apoM is an acute phase protein, which is involved in inflammatory responses [1]. The aim of our present study was to evaluate serum apoM levels in 101 newly diagnosed IBD patients and 69

healthy controls, and determine its potential clinical significance in IBD.

Materials and methods

Study population

A total of 101 newly diagnosed IBD patients, including 52 and 49 UC and CD patients, respectively, were enrolled in this study. All the patients were recruited between June 2014 and December 2015, and were inpatients at the Third Hospital Affiliated to Soochow University. Patients who had coronary heart disease, diabetes, dyslipidemia, malignant tumor, rheumatoid disease, acute or chronic infections, liver or kidney dysfunction were excluded. Also, 69 healthy volunteers were selected as controls. Disease activity and severity were measured by using the Mayo score, Best-CDAI score referred by the Diagnosis and Treatment of Inflammatory Bowel Disease Consensus (2012, Guangzhou) [2]. The study was approved

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Table 1. Demographic and clinical characteristics of IBD and control groups

Parameters	UC	CD	Control	<i>p</i> *
n	52	49	69	-
Male/Female	31/21	31/18	44/25	0.886
Median age (years)	31	30	28	0.078
Mild	(17) 32.7%	(15) 30.6%	Pearson $\chi^2 = 9.225$	0.010
Moderate	(22) 42.3%	(26) 53.1%		
Severe	(13) 25.0%	(8) 16.3%		

Note: CD: Crohn's disease; UC: Ulcerative colitis; IBD: Inflammatory bowel disease. *calculated using chi-square test.

Table 2. Serum apoM levels in IBD and control groups

Group	apoM ($\bar{x} \pm SD, \mu\text{g/ml}$)	<i>F</i> ^a	<i>p</i>
UC	13.5 \pm 8.8**	63.107	0.000
CD	10.2 \pm 5.8		
Control	2.0 \pm 1.3		

Note: CD: Crohn's disease; UC: Ulcerative colitis; IBD: Inflammatory bowel disease. **comparison between UC and CD groups, *p* value 0.005; ^acalculated using ANOVA test.

by local Ethics Committee, and informed consents were obtained from all patients.

ApoM and TNF- α ELISA

Fasting peripheral vein blood samples (5 ml) were collected and centrifuged at 4000 rpm for 5 mins. Serum was then collected, and stored at -80°C. ApoM and TNF- α levels were measured by ELISA kits (Wuhan USCN Business Co., Ltd, Wuhan, Hubei Province, China) according to the manufactures' protocol [3]. The following were brief steps of apoM measurement.

- 1) 96-well plate coated with apoM monoclonal antibodies was obtained directly from apoM ELISA kit.
- 2) 100 μL standard (diluted in advance) and 100 μL sample were added to each corresponding pore. The plate was then incubated at 37°C for 2 h.
- 3) Dried the plate, no washing.
- 4) 100 μL biotinylated apoM antibody solution (made up in advance) was added to each pore, and the plates were incubated at 37°C for 1 h.
- 5) Washed the plate 3 times repeatedly with Wash Buffer (diluted in advance).
- 6) 100 μL HRP labeled avidin solution (made up in advance) was added to each pore after washing the unbound biotinylated antibodies, and incubated at 37°C for 30 minutes.

- 7) Washed the plate 5 times repeatedly with Wash Buffer (diluted in advance).
- 8) 90 μL TMB substrate solution was added to each pore and incubated at 37°C for 15-25 minutes away from light.
- 9) 50 μL Stop Buffer was added to each pore.
- 10) Sample absorbance values were read at 450 nm using the microplate reader (Thermo, America). ApoM concentration in each sample was calculated using the standard curve.

Statistical analysis

Statistical analysis was performed using SPSS Statistics 19.0 (IBM, USA). The chi-square tests were used to compare the demographic and clinical characteristics. One-way analysis of variance (ANOVA) and least significant difference test (LSD) test using group means were performed for pairwise comparisons. Correlation between the levels of apoM and TNF- α was obtained using Pearson's linear correlation coefficient. Data were expressed as mean \pm SD. *P* < 0.05 was considered to be statistically significant.

Results

Demographic and clinical characteristics

A total of 101 IBD patients (age: 13-79 years) were included in this study, of which, 62 patients were male and 39 patients were females. Among the 69 healthy controls (age: 22-64 years), 44 subjects were male and 25 were female (**Table 1**). The Mayo score, Best-CDAI score were used to calculate the disease activity. As shown in **Table 1**, 52 IBD patients were diagnosed with UC, among which, 17 cases had mild condition (32.7%), 22 cases moderate condition (42.3%) and 13 cases with severe illness (25.0%). There were 49 CD patients, and 15 of these had mild condition (30.6%), 26 cases moderate condition (53.1%) and 8 cases with severe illness (16.3%).

Serum apoM levels increase in IBD patients

As shown in **Table 2**, serum apoM levels were significantly elevated in both UC and CD groups

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Table 3. Serum apoM levels in IBD patients respective to their disease activity index

Group	Disease activity	n	apoM ($\bar{x} \pm SD, \mu\text{g/ml}$)	F ^a	p
UC	Mild	17	7.98 \pm 8.40	8.425	0.001
	Moderate	22	14.04 \pm 5.02 Δ		
	Severe	13	19.64 \pm 10.38 \blacktriangle		
CD	Mild	15	6.18 \pm 5.34	9.591	0.000
	Moderate	26	10.85 \pm 3.34**		
	Severe	8	15.36 \pm 7.93 \diamond		

Note: CD: Crohn's disease; UC: Ulcerative colitis; IBD: Inflammatory bowel disease. Δ comparison between UC moderate and mild, *p* value 0.019; \blacktriangle comparison between UC severe and moderate, *p* value 0.045; **comparison between CD moderate and mild, *p* value 0.005; \diamond comparison between CD severe and moderate, *p* value 0.029; ^acalculated using ANOVA test.

Serum apoM levels of IBD patients correlates with that of TNF- α

The levels of TNF- α were found to be 2.73 \pm 1.68 $\mu\text{g/ml}$, 2.20 \pm 1.64 $\mu\text{g/ml}$ and 0.90 \pm 2.03 $\mu\text{g/ml}$ in the UC, CD and control groups, respectively. The difference was statistically significant (*P* < 0.05). Using Pearson analysis, a positive correlation was found between apoM and TNF- α concentrations in the serum samples of the IBD group (γ = 0.314, *P* = 0.001). Furthermore, this correlation was insignificant in the UC group (γ = 0.254, *P* = 0.069), but highly significant in the CD group (γ = 0.365, *P* = 0.010).

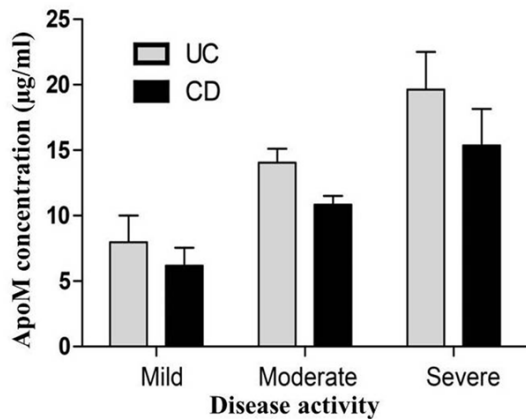


Figure 1. Serum apoM levels in IBD patients with different stages of disease activity. The levels of serum apoM were quantified using an ELISA. The disease activity of the IBD patients (*n* = 101) was measured using the Mayo score, Best-CDAI score, and categorized into mild, moderate and severe. ANOVA test and LSD test was used to calculate the differences. Serum apoM concentrations elevated as the disease activity increased from mild to severe.

compared to the control group (*P* < 0.05). Mean serum apoM levels in the UC, CD and control groups were 13.5 \pm 8.8 $\mu\text{g/ml}$, 10.2 \pm 5.8 $\mu\text{g/ml}$ and 2.0 \pm 1.3 $\mu\text{g/ml}$, respectively. Additionally, serum apoM levels were higher in the UC group than the CD group (*P* < 0.05).

Serum apoM levels correlate with the disease activity

Serum apoM concentrations in the IBD patients elevated as the disease activity increased from mild to severe. Also, differences among disease activity indexes were statistically significant (*P* < 0.05) (Table 3 and Figure 1).

Discussion

It is well known that both environmental factors and genetic susceptibility contribute to the pathogenesis of IBD, ultimately resulting in persistent and systemic intestinal immune responses. ApoM, a 26-kDa apolipoprotein, was recently identified by Xu et al. [4]. ApoM is expressed mainly in the hepatocytes and renal tubular epithelial cells, where a majority of serum apoM is secreted from the liver [5]. Serum apoM is usually combined with HDL; however, only about 5% of HDL carries apoM. ApoM functions by facilitating the formation of pre β -HDL, and enhancing the atheroprotective effects exerted by HDL. Moreover, in patients with diabetes, augmented apoM levels can not only decrease plasma glucose levels, but also magnify insulin secretion [6]. Therefore, apoM has been considered to be a potential protective factor in atherosclerosis, coronary heart disease, and diabetes mellitus [7, 8]. The human apoM gene is situated on chromosome 6p21.3 within the major histocompatibility complex class III (MHC III) region, which is closely related to human immune functions [9]. Recently, apoM was found to be abnormally expressed in some inflammatory diseases such as viral hepatitis, sepsis, rheumatoid arthritis, systemic inflammatory response syndrome. Additionally, serum apoM levels positively correlated with the disease activity [1, 10, 11].

ApoM expression is directly modulated by a series of transcription factors such as liver X receptor (LXR), liver receptor homolog-1 (LRH-1), hepatocyte nuclear factor-1 α , 4 α (HNF-1 α , 4 α) and fork head box A2 (Foxa2). Moreover,

hormones and cytokines as well as various inflammatory factors have likewise been implicated in the regulation of apoM expression [6]. Xu et al. [12] demonstrated that platelet activating factor (PAF) upregulated apoM expression in HepG2 cells; and these effects may be reversed by a PAF-receptor antagonist, lexipafant. Feingold et al. found that apoM expression decreased under inflammatory conditions as well as during infections. Stimulation with agents such as zymosan, turpentine and LPS that generate systemic inflammation resulted in downregulated apoM mRNA levels. Furthermore, treatment with inflammatory cytokines like TNF- α and IL-1, but not IL-6 correlated inversely with apoM transcription levels [13]. In contrast, Xu et al. reported that IL-1 and TNF- α did not influence the levels of apoM [12]. Discrepancies between these results warrant further investigations of the underlying mechanisms. The above mentioned studies on the regulation of apoM were mainly performed *in vitro*. In the present study, we used clinical samples to detect the expression of apoM; our results showed that the levels of apoM in UC and CD were 13.5 ± 8.8 $\mu\text{g/ml}$ and 10.2 ± 5.8 $\mu\text{g/ml}$, respectively, which were significantly higher compared with the healthy controls. CD4⁺T lymphocytes play a key role in the pathogenesis of human IBD, as evidenced by their influx into the inflamed mucosa, the effectiveness of depleting anti-CD4 antibody therapies [14], or the suppression of Crohn's disease symptoms observed in individuals with concomitant HIV infection [15]. Thus CD4⁺T lymphocyte-associated cytokines occupy an important place in IBD. Traditionally, CD was predominantly characterized by Th1 T cells secreting IFN- γ , IL-2, TNF- α , etc.; whereas, UC was believed to be mediated by Th2 T cells secreting IL-4, IL-5 and IL-13 [16]. We speculate that multiple cytokines may act on the liver and lead to the upregulation of apoM during IBD activity.

TNF- α is the main proinflammatory factor involved in cell proliferation and apoptosis during inflammation, and it plays a very important role in IBD pathogenesis [17]. That is why we decided to assess TNF- α levels in our study, and obtain a correlation between TNF- α and apoM levels. Our study found that serum TNF- α levels were higher in the IBD patients compared with the healthy controls. Remarkably, we

obtained a positive correlation between serum apoM and TNF- α levels in the IBD groups ($\gamma = 0.314$, $P = 0.001$), and this correlation was highly significant in CD group ($\gamma = 0.365$, $P = 0.010$). The mechanism of a positive correlation between TNF- α and apoM concentrations in our serum test was unclear, although TNF- α downregulated apoM expression *in vitro* experiment as previously mentioned. Therefore, additional studies are required in order to fully elucidate the role of TNF- α on apoM in IBD. Our data also showed that elevated levels of serum apoM were associated with increased disease activity in both UC and CD patients during the acute stage. These results suggest that serum apoM may be a promising indicator of the IBD activity.

Recent studies have demonstrated that apoM functions via inhibiting the immune response [18, 19]. Circulating sphingosine-1-phosphate (S1P) is a key regulator of lymphocyte egress, and majority of plasma S1P is bound to apoM associated with HDL. It has been reported that ApoM-S1P-S1P1 signaling axis not only restrains the lymphocyte compartment, but also the ensuing adaptive immune responses [19]. However, the role of apoM in the regulation of the immune system in IBD needs to be further explored.

In conclusion, our data demonstrated that serum apoM levels were significantly elevated in IBD patients as compared to healthy individuals, and significantly correlated with IBD activity. These results will prompt the clinicians to further explore the importance of apoM in assessing the IBD activity. However, future studies should focus more on enrolling large number of patients and patients in remission in order to yield convincing data. Also, mechanisms underlying the function of apoM in the pathogenesis of IBD still need to be further explored.

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Disclosure of conflict of interest

None.

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