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Original investigation

Serum Fetuin A and Secreted Phosphoprotein 24 as Diagnostic Markers in Inflammatory Bowel Disease

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ABSTRACT

Purpose: A completely satisfactory biomarker has yet to be identified for the diagnosis and characterization of inflammatory bowel disease (IBD). This study evaluated the utility of fetuin A and secreted phosphoprotein 24 kDa (Spp24) as novel biomarkers in IBD.

Methods: Ninety IBD patients, 54 with ulcerative colitis (UC) and 36 with Crohn's disease (CD), and 41 healthy controls (HC) were included. Serum fetuin A, Spp24, TNF-α, and IFN-γ levels were analyzed by ELISA. Serum C-reactive protein (CRP) levels were determined turbidimetrically by an automated procedure.

Results: There were no significant differences in serum Spp24 levels among the UC (17.15 pg/mL, mean), CD (18.88 pg/mL), and HC (14.77 pg/mL) groups (P>0.05). Median fetuin A levels were significantly lower in the UC (249 mg/L) and CD (254 mg/L) groups compared to HC (352 mg/L) (UC vs. HC P<0.001, CD vs. HC P<0.001). Fetuin A levels were not associated with disease activity, extent, or localization in UC or CD. In ROC curve analysis, a fetuin A cut-off value of 304 mg/L predicted IBD with better area under curve, sensitivity, and specificity (0.770, 76.1%, and 70.0%, respectively) compared to CRP (0.645, 32.2%, and 90.2%, respectively). TNF-α levels in IBD patients were not differ compared to HC (P>0.05), whereas IFN-γ levels were statistically significant between UC (3.94 pg/mL, median) and HC (2.65 pg/mL) groups (P=0.037).

Conclusion: Fetuin A may have an anti-inflammatory function in IBD and may be used as a potential biomarker for the discrimination of IBD patients from healthy individuals.

Keywords: Fetuin A, inflammatory bowel disease, secreted phosphoprotein 24

INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC) are relapsing inflammatory diseases of the gastrointestinal tract and are referred to as inflammatory bowel disease (IBD). Both CD and UC are associated with various pathogenic factors, including environmental changes, numerous susceptibility gene variants, abnormalities in the quality and quantity of the gut microbiota, and broadly irregular immune responses (1). CD is an idiopathic chronic regional enteritis characterized by transmural inflammation that affects the entire gastrointestinal tract, mainly the ileocecal region (2). The inflammatory process of UC is confined to the mucosa and the submucosa of the colon, and there is usually rectal involvement (3). This inflammation is the main cause of most of the signs and symptoms of the disease, so detecting and monitoring inflammation is of key importance in the clinical management of IBD. Because gastrointestinal inflammation cannot be adequately expressed by patients or detected by physicians, various methods have been investigated to categorize the severity and extent of ongoing inflammation.

Accurate diagnosis of IBD and differentiation between CD and UC are crucial for optimal treatment and prognosis. Currently, a combination of clinical, radiological, endoscopic, and histopathological diagnostic methods is used, most of which are invasive. Although endoscopy is considered the gold standard for

assessing disease activity in IBD (4), it is not ideal for many reasons including high cost, limited availability, and risk of complications. Furthermore, quantitative endoscopic indices of disease activity for CD and UC are difficult to calculate and interpret, which seriously limits their routine use. Noninvasive and micro-invasive laboratory biomarkers may provide more objective, rapid, and cost-effective diagnosis and thereby alleviate the physiological and financial burden on patients. For these reasons, identifying accurate, noninvasive biomarkers for disease activity has become a research priority.

The SPP2 gene spans about 27 kb on chromosome 2q37.1, contains 8 exons, and encodes secreted phosphoprotein 24 kDa (Spp24), a member of the cystatin superfamily. Spp24 consists of 211 amino acids, includes a signal peptide (the first 29 residues), a cystatin-like domain, and a variable C-terminal region (5). Studies on Spp24 have mostly focused on its role in the regulation of bone metabolism. There have also been a limited number of studies investigating its association with retinitis pigmentosa and its inhibitory effect on the growth of prostate, hepatocellular, and pancreatic cancer cells. In addition to its association with the diseases mentioned above, it has been suggested that Spp24 may trigger inflammatory pathways in response to increased Tumor necrosis factor (TNF)-α levels (4).

Fetuin A, also called α2-heremans-schmid glycoprotein (AHSG), is a multifunctional plasma glycoprotein with a molecular weight of approximately 60 kDa and is a member of the cystatin superfamily (6). Fetuin A has been studied as an inhibitor of ectopic calcium deposition, an important promoter of insulin resistance, and a negative acute phase reactant. Acting as a negative acute phase protein, fetuin A binds macrophage deactivating cations and the resulting fetuin-cation complex also transmits an anti-inflammatory signal (7).

TNF-α, is a multifunctional cytokine that plays a role in promoting inflammatory responses and increased levels of TNF-α have been demonstrated in IBD (8). Similar to TNF-α, interferon (IFN)-γ is a T helper (Th) 1 type cytokine and its role in the onset and progression of IBD has been shown in previous studies (9). In addition to both being members of the cystatin superfamily, fetuin A and Spp24 were also shown to be strongly associated with TNF-α (4, 10).

Based on these limited but promising data, the present study was conducted to evaluate the utility of Spp24 and fetuin A as novel biomarkers in IBD. In addition to the assessment of novel markers, levels of TNF-α and IFN-γ which play a role in the pathogenesis of IBD and which are on the same pathways with the markers we investigated were also evaluated.

METHODS

Subjects

A total of 90 IBD patients, 54 with UC and 36 with CD, who had been followed for at least 6 months in the IBD outpatient clinic of the Department of Gastroenterology were recruited for the study. Another group consisting of 41 healthy individuals/controls (HC) was also formed. The CD and UC groups included subjects over 18 years old diagnosed based on clinical, endoscopic, and histological criteria. Exclusion criteria were history of major IBD-related surgery such as colectomy or ileal resection; pregnancy; comorbidities such as chronic kidney disease, cirrhosis, cancer, or diabetes mellitus; and use of nonsteroidal anti-inflammatory drugs. The HC group included subjects over 18 years old who were examined for nonspecific dyspeptic complaints and for whom IBD was ruled out by endoscopic examination. The individuals in the HC group were not pregnant and had no known disease such as chronic kidney disease, cirrhosis, cancer, or diabetes mellitus.

UC and CD clinical activity scores were assessed using the Truelove-Witts Clinical Activity Index (11) and Crohn's Disease Activity Index (CDAI), respectively (12). Patients were categorized into those in remission and those with active disease (mild, moderate, or severe). Truelove-Witts index for UC includes six items: frequency of stool, blood in stool, temperature, pulse, hemoglobin (anemia), and erythrocyte sedimentation rate. The CDAI consists of eight items: watery stool, abdominal pain, and general well-being in the last seven days, anti-diarrhea drug usage, abdominal mass, hematocrit, percentage deviation from standard weight, and one point for each extra-intestinal manifestation. CDAI score ≤150 was accepted as inactive disease (remission), 150–220 as mild, 221–450 as moderate, and 451–600 as severe/fulminant disease. Disease location and behavior were determined for CD using the Vienna classification, while disease extent in UC was determined using the Montreal classification (13, 14).

Sample collection and preparation

The local ethics committee approved the study protocol and written informed consent was obtained from each subject according to the Declaration of Helsinki and using the principles of Good Clinical Practice. Blood samples from each participant were collected in a clot-activating tube containing gel separator (BD Vacutainer® SST II Advance tube, 5 mL, 13 x 100 mm, NJ, USA). The tubes were centrifuged at 1500 x g for 10 minutes and the serum was stored at −80°C until analysis.

Laboratory assays

Spp24, fetuin A, TNF-α, and IFN-γ levels were measured using the enzyme-linked immunosorbent assay (ELISA) method with commercially available kits (Human secreted phosphoprotein 24 ELISA kit, Abbkine Inc., Wuhan, China; Human Fetuin-A ELISA kit, Sunred Biotechnology, Shangai, China; Human TNF-α High Sensitivity ELISA kit, eBioscience Inc., CA, USA; Human IFN-γ ELISA kit, eBioscience Inc., CA, USA, respectively). C-reactive protein (CRP) was analyzed turbidimetrically by an automated procedure (AU5800 autoanalyzer, Beckman Coulter Inc., CA, USA).

Total coefficient of variation was <11% for Spp24, <12% for fetuin A, <9.8% for TNF-α, <5.7% for IFN-γ, and <3.79% for CRP. The minimum detectable concentration of Spp24 assay was less than 1.0 pg/mL. The sensitivity of fetuin A assay was 7.115 mg/L and CRP assay was 0.014 mg/dL. The limit of detection of TNF-α and IFN-γ assays were 0.13 pg/mL and 0.99 pg/mL, respectively.

Statistical analysis

The SPSS version 20.0 (SPSS Inc., Chicago, USA) software package was used for statistical analyses. Normality of the variables was analyzed with the Shapiro-Wilk test. For normally distributed data, statistical differences between groups were evaluated using parametric tests (independent t-test and one-way ANOVA); data not normally distributed were evaluated with nonparametric tests (Mann-Whitney U test and Kruskal-Wallis test). The results were given as mean ± standard deviation or median (interquartile range). Diagnostic cut-off values and their sensitivity, specificity, and area under curve (AUC) values were determined by receiver operating characteristic (ROC) curve analysis. Results with $P<0.05$ were considered statistically significant.

Ethical considerations

The study was approved by the clinical research ethics committee (Resolution Number 2017/0350, dated December 04, 2017).

RESULTS

The demographic and clinical characteristics of the IBD patients and HC were summarized in Table 1.

Serum Spp24 was higher in the UC and CD groups than in the HC group, but the differences were not statistically significant (UC vs. HC $P=0.603$, CD vs. HC $P=0.312$) (Table 2).

Fetuin A levels in both the UC and CD groups were significantly lower than in HC (UC vs. HC $P<0.001$, CD vs. HC $P<0.001$); however, there was no difference between the UC and CD groups ($P=0.720$).

CRP was included in our study as one of the most widely accepted biomarkers for evaluating disease status in IBD (15). Therefore, we used CRP to compare the diagnostic performance of our candidate biomarkers. CRP levels in the UC and CD groups were significantly higher than in the HC group, as expected (UC vs. HC $P=0.042$, CD vs. HC $P=0.008$). Similar to fetuin A, CRP could not distinguish between UC and CD (UC vs. CD $P=0.358$).

Table 1. Demographic and clinical characteristics of patients with ulcerative colitis, Crohn's disease, and healthy controls

	UC (n=54)	CD (n=36)	HC (n=41)
Age, years (mean ± SD)	50.6±16.5	44.2±13.7	38.5±10.9
Sex			
Male, n (%)	27 (50%)	14 (38.9%)	24 (58.5%)
Disease extent (UC)			
Proctitis, n (%)	8 (14.8%)	–	–
Left-sided colitis, n (%)	28 (51.9%)	–	–
Pancolitis, n (%)	18 (33.3%)	–	–
Disease location (CD)			
Ileum, n (%)	–	17 (47.2%)	–
Colon, n (%)	–	6 (16.7%)	–
Ileocolon, n (%)	–	13 (36.1%)	–
Disease activity			
Active, n (%)	30 (55.6%)	26 (72.2%)	–
Remission, n (%)	24 (44.4%)	10 (27.8%)	–

UC: ulcerative colitis; **CD:** Crohn's disease; **HC:** healthy controls; **SD:** standard deviation; **n:** number.

Serum TNF-α level was higher in the UC and CD groups than in the HC group, but the differences were not statistically significant (UC vs. HC $P=0.108$, CD vs. HC $P=0.064$).

Although serum IFN-γ levels were significantly elevated in the UC group compared to HC group, no significant difference was found between CD and HC groups (UC vs. HC $P=0.037$, CD vs. HC $P=0.297$).

Evaluation of the relationship between fetuin A level and disease characteristics revealed no significant differences in

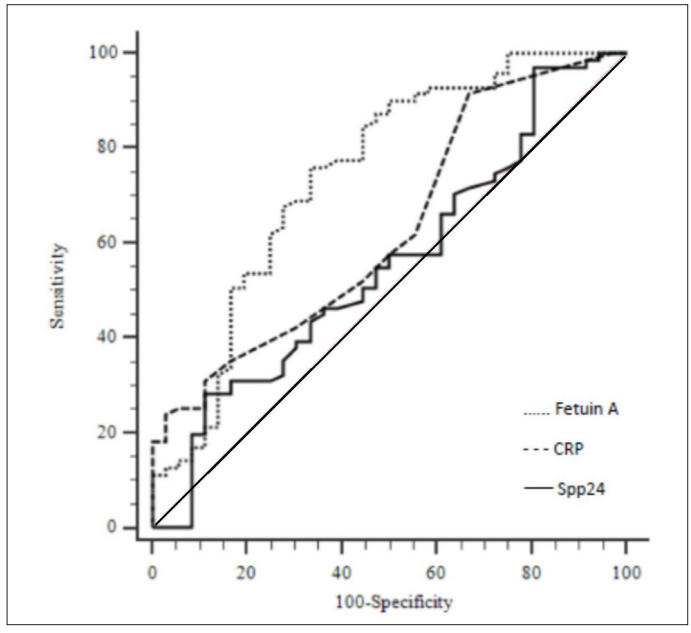

Figure 1. Receiver operating characteristic curves of fetuin A, C-reactive protein (CRP), and secreted phosphoprotein 24 (Spp24) for the differentiation of inflammatory bowel disease (ulcerative colitis and Crohn's disease) patients and healthy controls.

Table 2. Levels of secreted phosphoprotein 24, fetuin A, C-reactive protein, tumor necrosis factor-α, and interferon-γ in healthy controls and in patients with ulcerative colitis and Crohn's disease

	UC	CD	HC	*P* value
Spp24 (pg/mL), mean ± SD	17.15±10.57	18.88±13.69	14.77±10.92	0.603* 0.312† 0.784‡
Fetuin A (mg/L), median (IQR)	249 (183–318)	254 (230–285)	352 (274–1157)	**<0.001*** **<0.001†** 0.720‡
CRP (mg/dL), median (IQR)	0.45 (0.20 – 1.08)	0.55 (0.20 – 1.40)	0.30 (0.10 – 0.60)	**0.042*** **0.008†** 0.358‡
TNF-α (pg/mL), median (IQR)	1.67 (0.78–2.51)	1.82 (0.63–2.53)	1.01 (0.37–2.17)	0.108* 0.064† 0.993‡
IFN-γ (pg/mL), median (IQR)	3.94 (1.92–5.06)	2.62 (1.33–4.96)	2.65 (0.87–4.57)	**0.037*** 0.297† 0.356‡

*UC vs. HC; †CD vs. HC; ‡UC vs. CD; Bold values indicate statistical significance ($P<0.05$); **UC:** ulcerative colitis; **CD:** Crohn's disease; **HC:** healthy controls; **Spp24:** secreted phosphoprotein 24; **CRP:** C-reactive protein; **TNF-α:** tumor necrosis factor-α; **IFN-γ:** interferon-γ; **SD:** standard deviation; **IQR:** interquartile range (25th-75th percentile).

fetuin A according to UC extent or CD localization. Fetuin A levels were not associated with disease activity in UC or CD (Table 3).

The diagnostic capacities of the novel biomarkers were evaluated using ROC curve analysis (Figure 1). As Hosmer and Lemeshow suggested (16), the AUC of fetuin A for the discrimination between HC and IBD patients was 'acceptable' (AUC=0.770, *P*<0.001). Cut-off values and diagnostic performance characteristics of CRP, fetuin A, and Spp24 for the discrimination of HC and IBD were presented in Table 4. ROC curve analysis showed that the optimal cut-off value of fetuin A for the prediction of IBD was 304 mg/L (76.1% sensitivity and 70.0% specificity).

There was no difference in the serum fetuin A levels of IBD patients according to sex (Table 5).

Table 3. Fetuin A levels according to disease characteristics and activity

Disease characteristics	Fetuin A (mg/L), mean ± SD	*P* value
Extent (UC)		
Proctitis	224±120	0.495*
Left-sided colitis	275±124	0.976†
Pancolitis	234±66	0.500†
Location (CD)		
Ileum	257±65	0.835§
Colon	281±29	0.726‖
Ileocolon	234±85	0.503¶
Disease activity	**Fetuin A (mg/L),** median (IQR)	*P* value
UC		
Active	267 (204–318)	0.271**
Remission	217 (123–320)	
CD		
Active	249 (205–277)	0.107**
Remission	273 (244–356)	

*proctitis vs. left-sided colitis; †proctitis vs. pancolitis; ‡left-sided colitis vs. pancolitis; §ileum vs. colon; ‖ileum vs. ileocolon; ¶colon vs. ileocolon; **active vs. remission in each group; **SD:** standard deviation; **UC:** ulcerative colitis; **CD:** Crohn's disease.

DISCUSSION

UC and CD are the two main disorders included under the umbrella of IBD. Early detection of active disease is important to develop an effective treatment protocol and to reduce mortality in patients with a severe course. Research is ongoing to enable noninvasive diagnosis with high sensitivity and specificity. In this study, we investigated serum Spp24 and fetuin A levels in IBD patients as well as associations between fetuin A and disease activity, localization, extent, and patient sex. TNF-α and IFN-γ levels were also analyzed to evaluate the cytokine pathways of novel candidate markers.

Although the etiology of IBD remains unclear, pro– and anti-inflammatory cytokines play an important role in its pathogenesis, akin to immunological disorders. Mucosal T lymphocytes, B lymphocytes, and macrophages are increased in active IBD. Immunoglobulin production, granulocyte activation, and secretion of cytokines may also occur. Numerous biomarkers have been developed over the years to detect this inflammation and are currently used in clinical practice. Biomarkers are involved in many aspects of IBD management, from diagnosing and differentiating CD from UC to determining disease activity and predicting response to therapy. These biomarkers include CRP, erythrocyte sedimentation rate, platelet count, mean platelet volume, red blood cell distribution width, fecal calprotectin, fecal lactoferrin, fecal neopterin, and S100A12. An ideal biomarker for IBD should be simple, convenient, noninvasive, cost-effective, rapid, and reproducible. Unfortunately, none of the existing markers fulfill all of these criteria. For example, CRP test was described by European Crohn's and Colitis Organization (ECCO) as a useful biomarker in laboratory follow-up of patients with IBD (17, 18). However, elevated CRP level is not specific to IBD and may be seen in various viral and bacterial infections, autoimmune disorders, malignancy, and other disorders resulting in tissue necrosis (19). In addition, there is remarkable heterogeneity in CRP response between CD and UC (20). Some authors have reported a strong relationship between CRP and CD (20), but many patients with established CD do not have increased levels of CRP despite

Table 4. Cut-off values and diagnostic performance characteristics of C-reactive protein, fetuin A, and secreted phosphoprotein 24 in the differential diagnosis of healthy controls vs. inflammatory bowel disease

	Cut-off value	Sensitivity (%)	Specificity (%)	AUC (95% CI)	*P* value*
CRP (mg/dL)	0.80	32.2	90.2	0.645 (0.557–0.727)	**0.004**
Fetuin A (mg/L)	304	76.1	70.0	0.770 (0.680–0.844)	**<0.001**
Spp24 (pg/mL)	24.89	31.0	89.2	0.578 (0.485–0.668)	0.166

*Significance of AUC; Bold values indicate statistically significance (*P*<0.05); **CRP:** C-reactive protein; **Spp24:** secreted phosphoprotein 24; **AUC:** area under curve; **CI:** confidence interval.

Table 5. Variations of fetuin A (mg/L) according to the patient's gender

	UC	n (%)	*P* value	CD	n (%)	*P* value
Sex						
Male	249 (211–313)	14 (38.9%)	0.765	248 (202–315)	27 (50%)	0.689
Female	267 (231–287)	22 (61.1%)		262 (249–318)	27 (50%)	

Data were presented as median (25–75% quartile); **n:** number; **UC:** ulcerative colitis; **CD:** Crohn's disease.

evidence of active disease (21). The inability to reliably assess disease status in IBD patients using CRP, one of the most widely accepted biomarkers in clinical practice, has led many researchers to seek new potential biomarkers.

Spp24 was originally cloned from bone by Hu et al., but the role of this protein in biological systems was not fully understood at the time of its initial discovery (22). Spp24 has since been studied in various disorders, but only one study in the literature has examined Spp24 levels in IBD patients. Spp24 is present in smooth muscle and epithelial cells and may be released from macrophages and T-cells in response to heightened levels of TNF-α. Subsequently, increased levels of Spp24 may trigger macrophage infiltration and release of IFN-γ and interleukin-12 (4). Despite these promising findings regarding the use of Spp24 in the IBD patient group and the possible mechanisms of Spp24 level changes, we did not observe a significant difference in Spp24 levels related to IBD. Although Spp24 levels were higher in both UC and CD compared with HC, the differences were not significant. This may be attributed to the measurement technique used; Western blot analysis of Spp24 may yield a statistically significant discrimination between IBD patients and HC.

Fetuin A, which was first described in 1944 and is also called AHSG, is synthesized by hepatocytes and its concentration in the serum falls by about 10-fold after birth. In humans, fetuin A is a negative acute phase protein, as serum concentrations are significantly reduced after major surgical procedures, trauma, burns, and severe inflammation (23). Despite many studies, the physiological role of fetuin A in humans is not fully understood. In our study, we found that fetuin A levels in the IBD group were significantly lower compared to the HC group. This means that fetuin A test can be used to differentiate UC and CD patients from HC. We determined that a cut-off of 304 mg/mL was able to distinguish IBD patients from HC with 76.1% sensitivity and 70% specificity. This finding was consistent with the limited data from previous studies (10, 24).

Ma et al. reported that fetuin A may act as a negative inflammatory mediator in the pathophysiology of IBD (24). There are two possible mechanisms for the regulatory function of fetuin A in IBD. The first is related to the important role of meprin-α, a zinc metalloproteinase, in the pathogenesis of IBD by activating cytokines such as TNF-α. It should be remembered that fetuin A is an endogenous inhibitory regulator of meprin-α activity in plasma. The second possible mechanism relates to the high mobility group box 1 (HMGB1), an endogenous Toll-like receptor ligand that can be secreted from intestinal macrophages (24). HMGB1 may activate the immune cells by activating the Toll-like receptor pathway to produce various cytokines including TNF-α, and thus maintain a potentially harmful inflammatory response (24, 25). Fetuin A has been shown to inhibit intestinal inflammatory response by inhibiting active HMGB1 release (24). According to the study by Ma et al., fetuin A levels differed significantly between active and inactive IBD patients, but we observed no such relationship in the present study.

Consistent with findings reported by Monalakis et al., fetuin A levels in our study were not associated with UC extent or CD location (10). This suggests that fetuin A may help to generally detect IBD but cannot be used to determine the extent or location of disease. We also evaluated whether fetuin A levels varied in IBD patients according to sex and observed no significant sex difference.

Fetuin A has been shown to be involved in various immunological events, including regulation of macrophage-associated lipopolysaccharide-induced opsonization, and modulation of the TNF-α and transformed growth factor levels (10). On the other hand, several studies have identified changes in cytokine levels including TNF-α and IFN-γ in IBD, and additional evidence from the genetic studies suggests that cytokines and cytokine-producing immune cells play a crucial role in the pathogenesis of IBD (26). High levels of TNF-α detected in the IBD group were not statistically significant, and this was an obstacle to emphasizing the mechanism involving the link between fetuin A and TNF-α in our study. In addition, the fact that we did not detect a significant change in Spp24 levels in our study prevents us from associating high IFN-γ levels in the UC group with Spp24.

Cascades involving inflammatory mediators, particularly cytokines, are key players in innate and adaptive immune responses. Cytokines and other mediators regulate important biological cellular functions such as initiation of downstream signaling pathways, and triggering immune cell proliferation and differentiation. Impaired immunological responses in IBD reflect an imbalance of the cytokine profile at different stages of the disease process in both UC and Crohn's disease. It is widely accepted that this imbalance in the cytokine profile observed in IBD is represented by a Th1 and Th2 polarization model. CD is thought to be associated with a disorder in the Th1 system mediated by TNF-α, IFN-γ and IL-12, whereas UC has a disturbance in the Th2 system mediated by IL-5, but with no increase in IFN-γ levels. However, the concept of polarization has been questioned by studies pointing Th2 profiles in CD and Th1 profiles in UC. Alex et al. demonstrated elevated levels of IFN-γ in colitis caused by chronic dextran sodium sulfate (27). Rovedatti et al. found high IFN-γ levels in ex vivo cultured UC biopsies (28). The high IFN-γ levels that we found in the UC group were consistent with the results of Alex and Rovedatti. Apart from that, in relation to cytokine levels in our study, the issue that changes in peripheral blood concentrations do not reflect local changes is an ongoing debate in the literature (29).

The results of our ROC curve analysis demonstrated that fetuin A had superior AUC, sensitivity, and specificity values compared to CRP. Although CRP is commonly used in the follow-up of IBD patients, our results contribute to the debate regarding its diagnostic value.

This study has certain limitations. The first was the relatively small number of volunteers included in the IBD and HC groups. We were only able to recruit this number of follow-up patients within our predetermined research period. Secondly, we were unable to evaluate Spp24 levels with a more sensitive analysis method. Western blot analysis may have been useful, but we were not

equipped to implement this technique in our center. Third, the lack of analysis of cytokine levels in tissue homogenates while analyzing the analytes in serum, made it difficult to interpret the results of TNF-α and IFN-γ parameters.

In conclusion, the results of our study suggest that fetuin A may have a possible anti-inflammatory function in IBD and that it may be used as a potential biomarker for the discrimination of these patients from HC.

Informed Consent: Written informed consent was taken from all participants

Compliance with Ethical Standards: Approval was obtained from the ethics committee of Istanbul Medeniyet University, Goztepe Training and Research Hospital (Resolution Number 2017/0350, dated December 04, 2017)

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Author Contributions: Concept - IK, FKI, BIB; Design - IK, FKI, BIB, FDA, CU; Supervision - IK, FKI, BIB, GA; Fundings - FKI; Materials - NGK, BC, CU; Data Collection and/or Processing - NGK, BC, CU; Analysis and/or Interpretation - IK, BIB, FDA, CU; Literature Search - IK, NGK, BC; Writing Manuscript - IK, BIB; Critical Review - FKI, GA, CU.

Conflict of Interest: No conflict of interest was declared by the authors.

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