RESEARCH ARTICLE

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Received: 08.10.2023
Acceptance: 10.12.2023
DOI: 10.18521/ktd.1373002

Konuralp Medical Journal
e-ISSN1309–3878
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Evaluation of Serum Annexin A1 Values in Patients with Inflammatory Bowel Diseases

ABSTRACT

Objective: Annexin A1(AnxA1) is an anti-inflammatory mediator. In the current study,we aimed to evaluate whether or not serum Annexin A1 levels of inflammatory boweldiseases (IBDs) patients relate to the clinical and laboratory traits of IBDs.

Materials and Methods: This case-control study included 67 ulcerative colitis (UC) patients, 53 Crohn's disease (CD) patients and 60 healthy controls. The Mayo Clinical scoring system (MCS) was used for UC and the histological activity index (HAI) was determined by Truelove and Richards method. The Crohn's disease activity index (CDAI) was used for CD patients. Montreal classification was used for the localization of IBDs.

Results: The mean serum AnxA1 concentrations were not statistically significant in UC, CD and the control groups (26.36±17.30 ng/ml vs 22.98±12.74 vs 24.45±12.18 ng/ml respectively, *p=0.404*). The MCS, HAI of UC patients negatively correlated with the serum AnxA1 values (rho=-0.616, *p<0.001 vs* rho=-0.778, *p<0.001* respectively). UC patients with limited disease had higher values than those with extensive disease (19.5 (IQR:14.5–47.8) ng/ml vs.13.4 (IQR:10.8–18.4) ng/ml respectively, *p=0.002*). In CD patients, CDAI values negatively correlated to the serum AnxA1 values (rho=-0.770, *p<0.001*).

Conclusions: Serum AnxA1 values might be an auxiliary biomarker for the disease activity in patients with IBDs.

Keywords: Inflammatory Bowel Disease, Annexin A1.

İnflamatuvar Bağırsak Hastalıklarında Serum Annexin A1 Düzeylerinin Değerlendirilmesi

ÖZET

Amaç: Annexin A1 (AnxA1) anti-inflamatuar bir moleküldür. Çalışmamızda, inflamatuar barsak hastalıklarında (İBH) serum Annexin A1 düzeylerinin hastalıkların klinik ve laboratuvar özellikleri ile ilişkili olup olmadığı araştırılmıştır.

Gereç ve Yöntem: Bu vaka-kontrol çalışmasına 67 ÜK hastası, 53 Crohn hastalığı (CH) hastası ve 60 sağlıklı kontrol dahil edilmiştir. ÜK klinik aktivitesi için Mayo klinik skorlama sistemi (MKS) kullanıldı, histolojik aktivite indeksi (HAİ) Truelove ve Richards yöntemiyle belirlendi. CH için Crohn hastalığı aktivite indeksi (CHAİ) kullanıldı. İBH lokalizasyonu için Montreal sınıflandırması kullanıldı.

Bulgular: ÜK, CH ve kontrol grupları arasında ortalama serum AnxA1 konsantrasyonları yönünden fark saptanmadı (26,36±17,30 ng/ml vs 22,98±12,74 vs 24,45±12,18 ng/ml, sırasıyla *p=0,404*). ÜK'de MKS, HAİ ve serum AnxA1 değerleri arasında negatif korelasyon tespit edildi (rho=-0,616, p<0,001 vs rho=-0,778, sırasıyla *p<0,001*). Sınırlı hastalığı olan ÜK hastalarında, yaygın hastalığı olanlara göre daha yüksek serum AnxA1 değerleri bulundu (19,5 (IQR:14,5–47,8) ng/ml ve 13,4 (IQR:10,8–18,4) ng/ml, sırasıyla *p=0,002*). CH'da serum AnxA1 değerleri ile CHAİ arasında negatif korelasyon bulundu (rho=-0,770, *p<0,001*).

Sonuç: Serum AnxA1 düzeyleri İBH'da hastalık aktivite tespiti için yardımcı bir biyobelirteç olabilir.

Anahtar Kelimeler: İnflamatuar Barsak Hastalığı, Annexin A1.

INTRODUCTION

The prevalence of inflammatory bowel diseases (IBDs) is increasing and they result in growing socioeconomic burden. In recent years, researches on various prognostic, diagnostic and therapeutic molecules have gained interest based on the pathogenicity of IBDs (1,2).

The resolution process makes acute inflammation unnoticeable and self-limited without progressing to the chronic phase and it is a normal protective response (3). The failure of resolution leads to chronic inflammation and tissue damage (4). Resolution is mainly directed by biochemical molecules and specialized pro-resolving mediators (SPMs) including resolvins, galectins, lipoxins, annexins and protectins. SPMs are synthesized by the effect of neutrophils and macrophages, and they exert anti-inflammatory activity (2).

Annexin A1 (AnxA1) is a resolution-associated calcium and phospholipid binding protein and in general, it is reported to have anti-inflammatory activity. AnxA1 mediates the majority of its effects through formyl peptide receptors (FPRs). It is related to mucosal regeneration and healing (5,6). AnxA1 has a well-defined anti-inflammatory role in the innate immune system but the pro-inflammatory role of AnxA1 is also pronounced (7,8).

The therapeutic efficacy of AnxA1 is also another concern (2,8). Growing evidence exists about the role of AnxA1 in chronic inflammatory diseases and cancer, but the role of AnxA1 in these diseases is not entirely clear (8).

There is a scarcity of data about the role of AnxA1 in IBDs and the results of the reports are variable. Furthermore, the role of AnxA1 in the disease activity of IBDs is not clear (5,9-13). With regard to this, we aimed at evaluating whether serum levels of AnxA1 in patients with IBDs could serve as a biomarker by using the different clinical and endoscopic disease activity assessment models along with the histological activity in UC patients according to Truelove and Richards method.

MATERIAL AND METHODS

Study Population: The study included 67 patients with ulcerative colitis (UC), 53 patients with Crohn's disease (CD) and 60 healthy controls, admitted to the Gastroenterology department of our institute between January 2023 and May 2023. The Local Ethics Committee approved the study (11.01.2023/05). Written informed consent was obtained from all participants. Participants with clinical conditions that can affect serum AnxA1 levels such as sepsis, any malignancies, cardiac failure, chronic renal disease were excluded from the study. Participants with severe organ failure, acute or chronic infections, autoimmune diseases, or gut resection were also excluded from the study. The healthy control group included participants who underwent a colonoscopy for indications other than IBDs and whose colonoscopy results were normal.

The disease duration, medications for IBDs, comorbidities, extra-intestinal manifestations, IBDs in first degree relatives in the patients were recorded. Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and other biochemical tests were measured before endoscopic examination**.**

Assessment of the Clinical and Endoscopic Activities: The Mayo Clinical score (MCS) was applied for the patients with UC and was scored between 0-12. Scores of ≤2 were classified as clinical remission whereas scores of >2 indicated an activation [14]. The Crohn's disease activity index (CDAI) was used to assess the disease activity in the patients with CD and scores of <150 were noted as clinical remission whereas scores of ≥150 were noted as activation (15).

The disease extent of the patients with IBDs was defined in agreement with the Montreal classification (16). In UC, proctitis and left-sided colitis were recorded as limited disease, whereas extensive pancolitis was recorded as extensive disease. Mayo endoscopic activity scoring (MES) index was used for the endoscopic activation of UC and was classified as remission (0), mild (1), moderate (2) and severe (3) colitis. Scores of (0) and (1) were recorded as inactive disease whereas (2) and (3) were recorded as active disease (14). The localization of CD was classified as ileal, colonic or ileocolonic disease (16).

Histopathologic Evaluation in Ulcerative colitis: The same pathologist who was blind to the participants evaluated the formalin-fixed, paraffin-embedded, and H&E-stained colonic biopsies of the UC patients and performed grading through a scale similar to that developed by Truelove and Richards. Active inflammation, chronic inflammation and crypt distortion were the components of the scale. The histopathologic activity index (HAI) was defined as the sum of the scores of these components (17).

Measurement of Serum Annexin-A1: The serum for AnxA1 was separated from venous blood samples and after centrifugation at 5000×g for 10 minutes at 30°C, the supernatant serum was stored at (−) 80°C until analysis for 6-9 months. The commercially available Human Annexin A1 Bioassay Technology Laboratory Kit (Cat. No. E3288Hu, Lot:202302004) was used for the ELISA measurement of the serum annexin A1 (Intra-Assay: CV <8 %, Inter-Assay: CV <10 %) with a microplate reader (Biotech Epoch 2 Microplate ELISA Reader, USA).

Statistical Analysis: Statistical analyses were performed using the IBM SPSS software version 26.0. Descriptive analyses were presented using proportions for categorical variables and using medians and inter-quartile range (IQR) /mean±standard deviation for continuous variables.

The variables were investigated using Kolmogorov Smimirnov test to determine whether or not they were normally distributed. Comparisons were performed using the Mann-Whitney U test for continuous variables between two groups. Kruskal Wallis H tests were conducted to compare for continuous variables among three groups. Post hoc tests were performed using Bonferroni correction to adjust for multiple comparisons. Comparisons were performed using the chi-square test for categorical variables. The correlation coefficients and their significance were calculated using the Spearman test.

The capacity of serum AnxA1 values in predicting presence for the Mayo clinical scoring (Activation–Remission) of ulcerative colitis and for Crohn's Diaease Acitivity Index (Activation–Remission) of Crohn's disease was analyzed using ROC (Receiver Operating Characteristics) curve analysis. Their outcomes were presented as AUC (Area under the curve), criterion (cut off), sensitivity and specificity values. A p-value of less than 0.05 was considered to show a statistically significant result.

RESULTS

In total, 67 UC patients (47 males and 20 females), 53 CD patients (37 males and 16 females) and 60 healthy controls (36 males and 24 females) participated in the study. Demographic, clinical and laboratory characteristics of the participants are presented in Table 1. The groups were similar with respect to age and gender. The disease duration in the patients was also similar. CRP, ESR and neutrophil values were higher in the patients with IBDs but WBC values were not different in the three groups (*p>0.05*). It was determined that 35.8 % of the patients with UC were not under treatment whereas 41.5 % of the patients with CD were not taking any medication (Table 1).

Table 1. Demographic, clinical and laboratory characteristics of the study population.

	UC Patients n=67	**CD Patients n=53**	**Control Group n=60**	**p value**
Gender, n (%)				
Female	20 (29.9)	16 (30.2)	24 (40.0)	0.406*
Male	47 (70.1)	37 (69.8)	36 (60.0)	
Age (years), median (IQR)	35 (26-50)	36 (25-48)	39 (29-51)	0.302[#]
Disease duration (years), median (IQR)	2 (0.5-5)	1.5 (0-4.5)		0.189[&]
CRP (mg/L), median (IQR)	23.5 (2.7-40.1)	9.5 (2.5-30.8)	2.7 (0.9-4.6)	<0.001[1,#]
ESR (mm/h), median (IQR)	35.0 (14.0-60.0)	31.0 (18.0-50.0)	9.50 (3.0-17.8)	<0.001[2,#]
WBC ($x10^3/\mu L$), median (IQR)	8.0 (6.6-9.7)	8.4 (6.9-11.2)	7.6 (6.2-8.9)	0.081[#]
Neutrophils ($x10^3/\mu L$), median (IQR)	5.1 (3.8-6.7)	6.2 (4.5-8.7)	4.6(3.7-5.7)	0.003[3,#]
Serum Annexin A1 (ng/ml), mean(sd)	26.36 (17.30)	22.98 (12.74)	24.45 (12.18)	
Serum Annexin A1 (ng/ml) median (IQR)	17.1 (12.7-45.9)	20.4 (11.8-35.7)	18.2 (15.2-35.1)	0.404 [#]
Localization of UC, n (%)				
Limited disease	47 (70.1)			
Extensive disease	20 (29.9)			
Localization of CD, n (%)				
Ileal	35 (66.1)			
Colonic	6 (11.3)			
Ileocolonic	12 (22.6)			
Mayo Endoscopic Score of UC, n (%)				
Inactive disease	22 (32.8)			
Active disease	45 (67.2)			
Treatment of the patients, n (%)				
No treatment	24 (35.8)	22 (41.5)		<0.001*
Only 5-ASA	28 (41.8)	3 (5.7)		
5-ASA±Az±S	10 (14.9)	16 (30.2)		
BA+other agents	5 (7.5)	12 (22.6)		
IBDs in first degree relatives, n (%)	9 (13.4)	10 (18.9)		
Mayo Clinical Score of UC, median (IQR)				
Remission (score ≤ 2), n (%)	18 (26.9)			
Activation (score>2), n (%)	49 (73.1)			
Histological Acitivity Index in UC, median (IQR)	6 (3-7)			
Crohn's Diaease Acitivity Index				
Remission (score <150), n (%)	21 (39.6)			
Activation (score≥150), n (%)	32 (60.4)			
Extraintestinal Manifestations n (%)	8 (11.9)	18 (34.0)		0.004*

Abbreviations: CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; IQR: Inter quartile range; WBC: White blood cells; UC: Ulcerative colitis; CD: Crohn's disease; 5-ASA:5-aminosalycilate; Az: Azathioprine, S: Steroid, BA: Biological agents, IBDs: Inflammatory bowel diseases. sd: standard deviation, IQR: İnterquartile range.

Footnotes: [1] Significant difference in comparison of UC vs controls, CD vs controls (p<0.001, p<0.001); [2]Significant difference in comparison of UC vs controls, CD vs controls (p<0.001, p<0.001); [3]Significant difference in comparison of CD vs controls (p=0.002).

* Chi-square test [&] Mann-Whitney U test [#] Kruskal-Wallis test

Although the mean serum AnxA1 concentrations were higher in the patients with UC compared to CD patients and the control group, the differences were not statistically significant (26.36±17.30 ng/ml vs 22.98±12.74 vs 24.45±12.18 ng/ml respectively, *p=0.404*). The mean serum AnxA1 value was the lowest in the patients with CD (Table 1).

The patients with UC who were in remission had higher serum AnxA1 concentrations than those having clinically active diseases (51.6 (IQR:44.1–56.2) ng/ml vs. 15.3 (IQR:11.5–19.3) ng/ml respectively, *p<0.001*). UC patients with limited disease also had higher AnxA1 values than those with extensive disease (19.5 (IQR:14.5–47.8) ng/ml vs.13.4 (IQR:10.8 – 18.4) ng/ml respectively, *p=0.002*). According to endoscopic activity scores, UC patients having inactive diseases had higher AnxA1 values than the patients having active diseases (47.4 (IQR:35.1-55.9) ng/ml vs. 15.3 (IQR:11.5–19.3) ng/ml respectively, *p<0.001*). The median serum AnxA1 values were similar in UC patients with respect to treatment status and modalities, extra-intestinal manifestations and family history of IBDs (Table 2).

Table 2. Serum Annexin A1 values according to the disease phenotype and treatment modalities in the patients with IBDs.

				Serum Annexin A1 (ng/ml)			
		n	**%**	**Median**	**IQR**		**p**
Ulcerative Colitis							
Treatment status	No treatment	24	35.8	17.8	14.3	45.4	
	Under treatment	43	64.2	17.1	12.7	45.9	0.969[&]
Treatment modalities	No treatment	24	35.8	17.8	14.3	45.4	
	Only 5-ASA	28	41.8	17.5	13.2	47.3	
	5-ASA±Az±S	10	14.9	15.2	12.4	23.9	
	BA+other agents	5	7.5	41.7	9.8	49.6	0.890[#]
Mayo clinical scoring	Remission (score ≤ 2)	18	26.9	51.6	44.1	56.2	
	Activation (score >2)	49	73.1	15.3	11.9	19.4	<0.001[&]
Localization of UC	Limited disease	47	70.1	19.5	14.5	47.8	
	Extensive disease	20	29.9	13.4	10.8	18.4	0.002[&]
Mayo endoscopic activity	Inactive disease	22	32.8	47.4	35.1	55.9	
	Active disease	45	67.2	15.3	11.5	19.3	<0.001[&]
IBDs in first degree relatives	Positive	58	86.6	16.4	12.6	42.8	
	Negative	9	13.4	22.8	16.0	55.5	0.162[&]
Extra-intestinal Manifestations	Positive	59	88.1	18.8	13.2	46.8	
	Negative	8	11.9	14.7	11.0	19.3	0.209[&]
Crohn's disease							
Treatment status	No treatment	22	41.5	17.7	9.4	26.7	
	Under treatment	31	58.5	20.8	12.3	37.4	0.112[&]
Treatment modalities	No treatment	22	41.5	17.7	9.4	26.7	
	Only 5-ASA	3	5.7	30.6	14.9	39.7	
	5-ASA±Az±S	16	30.2	20.6	13.4	38.5	
	BA+other agents	12	22.6	20.5	9.1	37.0	0.293[#]
Crohn's disease activity index	Remission (score<150)	21	39.6	36.3	31.4	40.8	
	Activation (score≥150)	32	60.4	12.2	9.1	18.9	<0.001[&]
Localization of CD	Ileal	35	66.1	22.3	12.1	36.0	
	Colonic	6	11.3	15.2	9.4	34.7	
	Ileocolonic	12	22.6	16.0	11.0	33.5	0.727[#]
IBDs in first degree relatives	Positive	43	81.1	20.8	12.1	36.0	
	Negative	10	18.9	12.5	8.5	33.1	0.211[&]
Extra-intestinal Manifestations	Positive	35	66.0	24.7	12.8	36.2	
	Negative	18	34.0	13.6	9.1	31.4	0.102[&]

Abbreviations; IQR: Inter quartile range; UC: Ulcerative colitis; CD: Crohn's disease; IBDs: Inflammatory bowel diseases. 5-ASA:5-aminosalycilate; Az: Azathioprine, S: Steroid, BA: Biological agents,
Footnotes: [&] Mann-Whitney U test [#] Kruskal-Wallis tes

There were strong and negative correlations between CRP and HAI values of UC patients and serum AnxA1 values (rho=-0.723, *p<0.001* vs rho=-0.778, *p<0.001* respectively). ESR, leucocyte, neutrophil and MCS values of UC patients were also inversely correlated to the serum AnxA1 concentrations whereas the disease duration was positively correlated (Table 3).

Table 3. Correlations between the serum Annexin A1 values and the clinical, laboratory variables of the patients with inflammatory bowel diseases

Serum Annexin A1 **(ng/ml)**	**rho***	**p**
Ulcerative Colitis (n=67)		
CRP (mg/L)	-0.723	<0.001
ESR (mm/h)	-0.546	<0.001
Leucocyte (x10^3/μL)	-0.425	<0.001
Neutrophil (x10^3/μL)	-0.413	0.001
Mayo clinical scoring	-0.616	<0.001
Histological activity index	-0.778	<0.001
Disease duration (years)	0.248	0.043
Crohn Disease, (n=53)		
CRP (mg/L)	-0.583	<0.001
ESR (mm/h)	-0.558	<0.001
Leucocyte (x10^3/μL)	-0.189	0.175
Neutrophil (x10^3/μL)	-0.206	0.139
Crohn's disease activity index	-0.799	<0.001
Disease duration (years)	0.214	0.124

Abbreviations: CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate.
Footnotes: *Spearman correlation coefficient

The patients with CD who were in remission had higher serum AnxA1 values than the patients having clinically active disease (36.3 (IQR:31.4-40.8) ng/ml vs. 12.2 (IQR:9.1-18.9) ng/ml respectively, *p<0.001*). In terms of disease localization, treatment status and modalities, extra-intestinal manifestations and family history of IBDs in CD patients, there was no statistically significant difference with respect to median serum AnxA1 values (*p>0.05*) (Table 2). There was strong and negative correlation between CDAI of CD patients and serum AnxA1 values (rho=- 0.799, *p<0.001*). CRP and ESR values also inversely correlated with serum AnxA1 concentrations in CD patients (Table 3).

The receiver operating characteristic curve (ROC) analysis revealed that the area under curve (AUC) for AnxA1 concentrations had a 0.901 (95%CI: 0.805-0.998, *p<0.001*) diagnostic accuracy for the clinical activity of UC (MCS). The sensitivity and specificity for the cut-off level of ≤34.9 ng/ml were 88.9% and 89.8 %, respectively (Figure 1).

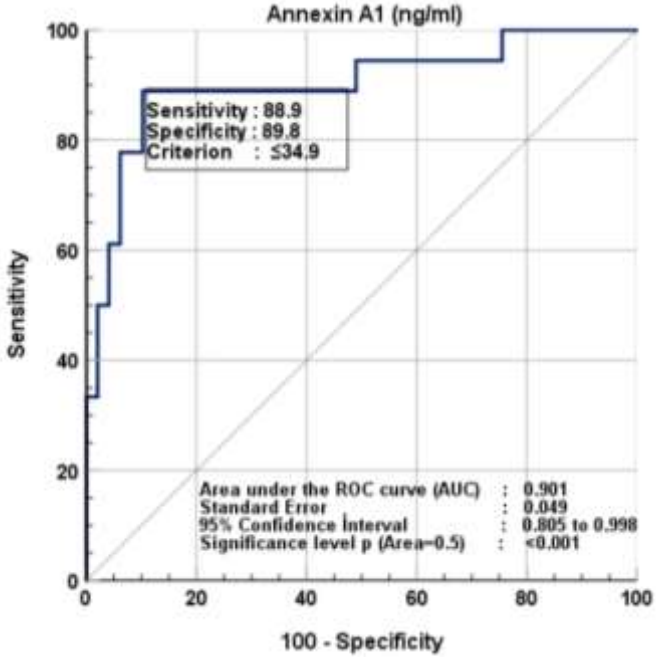

Figure 1. ROC curve analyses of the predictive values of serum Annexin A1 concentrations for the Mayo clinical scoring of ulcerative colitis.

The ROC analysis also revealed that the AUC for AnxA1 concentrations had a 0.972 (95%CI: 0.931-1.000, *p<0.001*) diagnostic accuracy for the clinical activity of the disease in CD (CDAI), and the sensitivity and specificity for the cut-off level of ≤25.2 ng/ml were 95.2 % and 93.7 %, respectively (Figure 2).

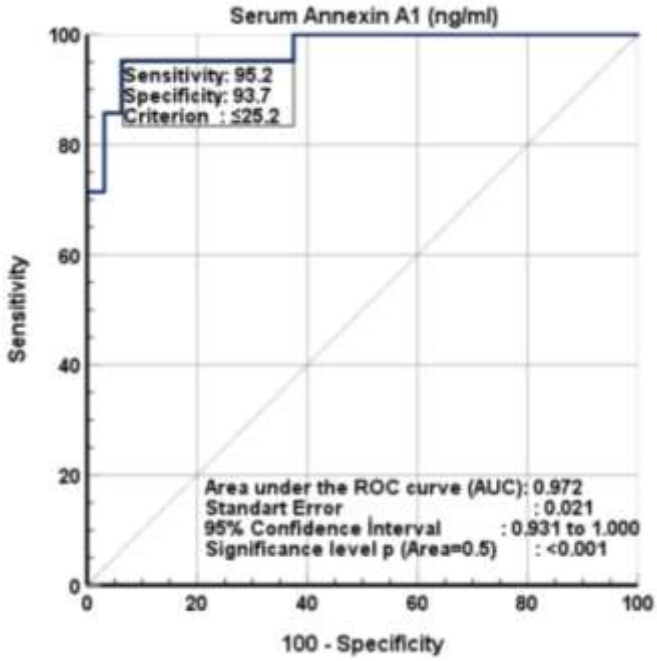

Figure 2. ROC curve analyses of the predictive values of serum Annexin A1 concentrations for the Crohn's Diaease Activity Index of Crohn's disease.

DISCUSSION

The pathogenesis of inflammatory bowel diseases (IBDs) is not exact and heterogeneous. The immune dysregulation of the enteric microbiota is believed to be the major pathogenic mechanism in IBDs. It is worthy of note that IBDs have relapsing and remitting phases (18).

Annexin-A1 (AnxA1) is a 37 kDa protein and it is also known as lipocortin 1. This molecule has diverse biological actions. The expression of AnxA1 is induced by glucocorticoid signaling and it inhibits the activity of cytosolic phospholipase A2 and cyclooxygenase 2, thus exhibiting anti-inflammatory, anti-pyretic, and anti-hyperalgesic activities. It is abundant in monocytes, macrophages and neutrophils (19).

Neutrophilic infiltration into the gut wall is the mainstay of the histopathological features in IBDs (5,18,20). Neutrophils are reported to be the main source of AnxA1 in the inflamed mucosa of experimental colitis model in rats (21)**.** As a pro-resolving mediator, AnxA1 enhances mucosal wound repair via inhibiting neutrophil recruitment to the inflamed area. It also induces neutrophil clearance (8,19).

Vong et al. (9) examined the colonic mucosal biopsies of inactive and active UC patients, and healthy controls by using fluorescence microscopy. After biopsies were performed on patients with active disease, Vong et al reported that the neutrophils in the biopsies were stained by marked AnxA1. In this study, AnxA1 expression was not limited to cells infiltrating the lamina propria but was also detected in epithelial cells lining the intestinal crypts in the biopsies of active UC patients. The over-expression of the molecule was ascribed to the protective function of AnxA1 for mucosal homeostasis (9). We also reported higher serum neutrophil values in the patients with IBDs compared to the healthy controls and this results confirm the increased neutrophil activity in IBDs.

Although AnxA1 is generally reported to have anti-inflammatory properties in some diseases, it has alternating roles being discussed within the same disease, as well as within different disease subsets (8). There are a limited number of studies evaluating the role of AnxA1 in IBDs. The methods used in and the results of these studies are different (5,9-13).

Kourkoulis et al. (10) evaluated the serum AnxA1 values of UC patients (n=42) and healthy controls (n=14). They reported higher serum AnxA1 values compared to the healthy controls and proposed serum AnxA1 values as a diagnostic biomarker of UC. On the other hand, Sena et al. (11) detected lower levels of plasma AnxA1 in CD patients (n=28) compared to the healthy controls (n=12). Vong et al. (9) reported that immunofluorescence detection of AnxA1 in colonic biopsies of the participants demonstrated increased expression in patients with UC, whether active

(n=8) or in medically-induced remission (n=16) compared to healthy controls (n=20).

In our study, the mean serum AnxA1 concentrations were higher in the patients with UC but lower in CD patients compared to the healthy control group. However, these results are not statistically significant. Different AnxA1values in three studies may partly be due to the different numbers of the subjects evaluated in the studies and we think that larger sample-sized cohorts might affect the statistical significance and they can reveal significant results which could be clinically important.

Medications aim to suppress the immune activation in IBDs and they might affect AnxA1 activation because AnxA1 expression is induced by glucocorticoid signaling and depends on the neutrophilic activity which has a key role in AnxA1 expression (8,19). 64.2 % of UC patients and 58.5 % of CD patients in our study were under treatment. In the study of Sena et al. (11), CD patients who were successfully treated with infliximab were reported to have higher regulated plasma AnxA1 expressions and it was concluded that loss of AnxA1 expression may support inflammation during CD and can serve as a biomarker of disease progression. Also, changes in AnxA1 levels may be predictive of therapeutic efficacy for infliximab.

In another study, it was also concluded that infliximab induces AnxA1 expression and secretion in activated intestinal leukocytes (13). We think that AnxA1 expression might be altered by these medications (19,21). Neutrophil counts in the patients with IBDs were higher than the healthy controls in our study but serum AnxA1 values in three groups were not statistically different. This result may be a pointer to the effect of medications on the neutrophil activity which plays a role in AnxA1 expression. In addition, the ratios of the patients with IBDs who were not under treatment could not be ignored in the current study but no statistically significant differences were noted in the patients who were not under treatment and those who were.

We also did not detect any differences according to serum AnxA1 concentrations in the patients using different treatment modalities. In the current study, most of the patients with UC were on 5-ASA therapy while most CD patients were on immuno-suppressive therapy, and the ratio of the patients taking biological agents was the lowest in both groups, especially in UC. The groups of patients were heterogeneous according to the treatment modalities and we think that larger sample sized cohorts including equal numbers of patients with respect to different treatment modalities could reveal significant results. To exclude the effect of medications on the serum values of AnxA1, newly diagnosed patients with IBDs not taking any medications can be evaluated for the diagnostic accuracy of serum AnxA1, and this may be another subject for further investigations.

Kourkoulis et al. (10) also evaluated the association between UC endoscopic activity scores according to MES index and serum AnxA1 concentrations as in our study. They detected no statistical difference between the endoscopic activity and serum AnxA1 concentrations, but in that mentioned study, the number of UC patients especially in the patients with active endoscopic disease (MES 2 and 3) was very small. We reported higher serum AnxA1 values in UC patients who had inactive diseases than those with active diseases. In terms of endoscopic findings, UC patients with limited disease also had higher values. These results may be attributed to the anti-inflammatory activity of AnxA1which limits the disease progression in UC. In CD patients, according to the localization of the disease, there was no statistically significant results in serum AnxA1 concentrations.

We noted negative correlations between CRP, ESR values and serum AnxA1 concentrations in the patients with IBDs. These correlations were stronger in UC patients. Sena et al. (11) also observed an inverse correlation between plasma CRP and plasma AnxA1. CRP and ESR are traditional acute phase reactants and inverse correlations between these tests and these results can be due to the lack of AnxA1 to exert an anti-inflammatory activity in IBDs.

The European Crohn's and Colitis Organization (ECCO) guidelines state that the treatment of IBDs should not only control the symptoms and that mucosal healing is the best therapeutic goal (22). Inflammation in the gut wall is also an indicator for the disease activity in patients with IBDs (23). De Paula-Silva et al**.** (13) evaluated the colon biopsies from CD untreated (n=4) and treated positive (n=3) or negative (n=2) responders to infliximab. They analyzed the colon biopsies by fluorescence intensity of staining and performed a histological grading. The dextran sulfate sodium (DSS) induced experimental colitis model was also used, and the healthy controls were also included in this group. In the study of de Paula-Silva et al**.** (13), the subjects were assigned into infliximab treated and non-treated groups. Histological grading was designed according to changes on crypts, architecture, edema, ulceration and presence of immune cells at the gut wall. Grades of 0, 1, 2, 3, and 4 were respectively attributed to normal, mild, mild–moderate, moderate–severe, and severe conditions. Results were expressed as the mean of total grading both in CD patients and the experimental colitis models.

The results of this study revealed that colonic AnxA1 expressions presented a strong negative correlation with the histological grading which means that the decrease of these markers is

associated with more tissue damage (13). We also reported strong negative correlations between the HAI scores and serum AnxA1 concentrations according to Truelove and Richards methods. In both studies, results can be ascribed to the protective effect of AnxA1 in IBDs. As a limitation, we did not apply histological grading system in the patients with CD. With regard to infliximab response in CD patients, de Paula-Silva et al. (13) reported that AnxA1 in blood did not correlate with CDAI and plasma levels of serum AnxA1 and might not be a reliable biomarker for remission or failure after infliximab treatment (responders, n=3 and non-responders, n=2). However, the number of the patients in that study was very small.

To the best of our knowledge, we firstly investigated the relations between the clinical activity scores and serum AnxA1 concentrations in the patients with IBDs. There were inverse correlations between these scores and serum AnxA1 values. These correlations were stronger in CD patients. With respect to MCS values in UC and CDAI in CD patients, serum AnxA1 concentrations in the patients who were in the clinical remission phases were higher. We think that serum AnxA1 values might be a good determinant of clinical activity in patients with IBDs.

For IBDs, AnxA1 was also declared as a therapeutic target (2,8). Today, current medical treatments for IBDs focus on the inhibition of immune activation but they cannot achieve complete remission (18). Topical delivery of AnxA1 into the gut mucosa might be an adjunctive treatment modality.

Several investigations have focused on the identification of biomarkers of disease progression that could be valuable in the diagnosis and treatment of IBDs. The patients with IBDs usually undergo invasive endoscopic procedures which can cause discomfort. The clinical, endoscopic and biochemical findings can be inconsistent with each other in IBDs. Searching for the ideal biomarkers correlating to all disease activity parameters, like fecal calprotectin, is important in IBDs (24).

The major limitation of the current study was the small number of the study population as it was a single-centered trial. Larger cohorts might reveal significant results about the diagnostic accuracy of serum AnxA1 values and they might also exhibit the effects of therapeutic agents on serum AnxA1 values. Comparing fecal calprotectin values with serum AnxA1 concentrations could be more valuable for the assessment of diagnostic and prognostic accuracy of serum AnxA1.

Diagnostic strategies with the possibility of therapeutic interventions can be developed by identifying new, practical and objective biochemical markers in IBDs. Serum AnxA1 can be a valuable biomarker for the clinical and laboratory traits of IBDs and it might be an auxiliary test for the assessment of disease activation. Further studies are needed to delineate the diagnostic and the therapeutic accuracy of serum AnxA1 in IBDs.

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