Anti PR3 ANCA as a Marker of Diagnosis and Assessing the Severity in Ulcerative Colitis Patients

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Background and study aim: Ulcerative colitis (UC) belongs to the chronic inflammatory diseases with unknown etiology. At present, the diagnosis and monitoring of UC, tracking of responses to intervention, and detection of mucosal healing depend on endoscopy. However, it is invasive, expensive, and requires colonic preparation. We aimed to investigate the clinical roles of anti-PR3-ANCAs in the disease diagnosis and disease severity of ulcerative colitis patients.

Patients and Methods: This study was conducted on 50 UC patients attending Kafrelsheikh University Hospital, 13 intestinal control group, and 17 age and sex-matched healthy volunteers from May 2023 to May 2024, colonoscopy and evaluation of (Anti PR3-ANCAs) were done in each group.

Results: forty-one patients were PR3-ANCA positive, of them 18 patients were recently diagnosed, while 20 patients were treated for more than one year, and 3 patients were treated for less than one year. The cut-off value for PR3-ANCA to predict ulcerative colitis was >3.3 U/mL with sensitivity 84.0%, specificity 77.0%, area under the curve (AUC) 0.906, 95% confidence interval (CI) (0.839–0.972), p =0.000.

Conclusion: PR3-ANCA has an important role as a diagnostic biomarker for UC and in assessing the disease severity.

INTRODUCTION

One of the chronic inflammatory disorders whose etiology is uncertain is ulcerative colitis (UC). UC is currently believed to be the product of an uncontrolled immune response caused by a confluence of microbial, environmental, and genetic elements [1]. Diffuse mucosal inflammation limited to the intestinal area is a common feature of UC.

Bloody diarrhea, abdominal discomfort, fecal incontinence, and exhaustion are common symptoms of UC [2], which can cause individuals to experience crippling physical and psychological symptoms. Additionally, UC can have an impact on society by absenteeism, and medical expenses [3]. Currently, endoscopy is used for UC diagnosis and monitoring, intervention response tracking, and mucosal healing detection. However, colonic preparation is necessary; colonoscopy is expensive and intrusive. Furthermore, without any evidence of disease activity under endoscopy, at least one-third of UC patients in the remission period experience gastrointestinal symptoms such as diarrhea and abdominal pain. The "active period" and "remission period" of the disease must be predicted using basic markers that are reflective of endoscopic findings to ascertain whether the patient has experienced a "relapse". Non-invasive markers in particular are preferred due to their objectivity, repeatability, and ease of use [4].

Blood indicators that are frequently used to assess disease activity include C-reactive protein, erythrocyte sedimentation rate, hemoglobin, white blood cell count, albumin, and orosomucoid concentrations [5]. However, they are not very useful in the diagnosis of UC because UC inflammation is restricted to the intestinal mucosa and has little relationship to the activity of systemic diseases [6].

In previous years, immunologic markers have been used in clinical practice. In UC, the presence of certain antibodies can aid as an alternate diagnostic marker [7].

The following antibodies are among these markers: Antibodies against bacterial flagellin, anti-neutrophil cytoplasmic antibodies, anti-Saccharomyces cerevisiae antibodies, pancreatic antibodies, antibodies against the outer membrane porin C of Escherichia coli, antibodies against the pseudomonas fluorescens-associated sequence, and antibodies against CD peptide [8].

Anti-neutrophil cytoplasmic autoantibodies are collectively referred to as serum anti-neutrophil cytoplasmic antibodies or ANCAs. Two types of ANCAs that are clinically important are perinuclear ANCAs, also known as myeloperoxidase (MPO)-ANCA, and cytoplasmic ANCAs, also known as anti-proteinase 3 (PR3)-ANCA, which target PR3 [9]. According to recent research, PR3-ANCAs are found in a sizable percentage of IBD patients, especially those with UC in Spain and the UK [10].

The primary goal of this study was to evaluate the utility of PR3-ANCA in diagnosing and assessing the severity of the disease in UC patients, as the clinical importance of this test has not yet been thoroughly determined in our region. We aimed to investigate the clinical roles of anti-PR3-ANCAs in the disease diagnosis and disease severity of ulcerative colitis patients.

PATIENTS/MATERIALS AND METHODS

Subject: This study was carried out in the Medical Microbiology and Immunology Department of Kafrelsheikh Faculty of Medicine. It was conducted on 50 UC patients attending the Gastroenterology department of Kafrelsheikh University Hospital, 13 intestinal control group (patients with intestinal symptoms and normal colonoscopy), and 17 age and sex-matched healthy volunteers served as another control group after taking their consent from May 2023 to May 2024

Material and Methods:

All UC patients and controls were subjected to full history taking, thorough clinical examination, Colonoscopy, and laboratory assessment: CBC, ESR, CRP, Calprotectin, Liver and renal function & detection of (Anti PR3-ANCAs) by Enzyme-Linked immunoassay (ELISA).

Blood sampling: Venous blood samples were taken under sterile conditions in serum separator tubes from each participant. Samples were centrifuged for 15 minutes at 1000 x g. Separated serum samples were stored at -80°C until further use for quantification of (Anti PR3-ANCAs) IgG antibodies by (ELISA) technique

Quantification of (Anti PR3-ANCAs) IgG antibodies serum levels:

IgG(Anti PR3-ANCAs) antibodies serum levels were quantitated by using (DRG® cANCA (EIA-3596) ELISA Kit and the procedure was done according to the manufacturer's instructions. Briefly, The microwells of the ELISA plate were pre-coated with highly purified PR3 antigen. Standard and serum samples were added to pre-coated wells and after incubation, (Anti PR3-ANCAs)IgG antibodies present in the sample or standard were bound to respective antigens in pre-coated wells. The ELISA plate was washed several times to remove unspecific serum components. Horseradish peroxidase (HRP) conjugated anti-human IgG was added to detect the bound patient antibodies forming a conjugate/antibody/antigen complex. The ELISA plate was incubated and washed again to remove unbound conjugate. An enzyme substrate was added the plate was incubated and a blue color was formed in the wells had bound conjugate. The addition of an acid stopped the reaction forming a yellow end-product. The intensity of this yellow color was measured photometrically at 450 nm. The amount of color was directly proportional to the concentration of IgG antibodies present in the original sample. The cutoff value was >5 U/mL

Statistical analysis:

RESULTS

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The age of ulcerative colitis groups was 29 (22-35) years, 23(22-30) for the intestinal control group and 26(23-33) for healthy control. The ulcerative colitis group included 25 females and 25 males, while the intestinal control group included 7 males and sex females, and the healthy control group included 9 males and 8 females. Regarding hemoglobin there was a highly significance difference between the three groups more between the ulcerative colitis group and healthy control group (p:0.000). For platelet count there was no significant difference between the three groups. Regarding WBC count there was a highly significant difference between the three groups more between the ulcerative colitis group and healthy control group (p:0.000). Also for ESR there was a highly significant difference between the three groups between the ulcerative colitis group and healthy control group (p: 0.000). For serum albumin, SGPT, and serum creatinine there was highly significance difference between the three groups more between ulcerative colitis group and healthy control group (p:0.000) (table 1). The ulcerative colitis group patients included 50 patients. 41 patients (22 males and 19 females) of them were PR3-ANCA positive, while 9 patients (3 males and 6 females) were PR3-ANCA negative. Regarding the PR3-ANCA positive group, 18 patients were recently diagnosed, 20 patients were treated for more than one year, and 3 patients were treated for less than one year. WBC count showed a significant difference between the PR3-ANCA positive group and the PR3-ANCA negative group (p: 0.010). Also for ESR and Fecal calprotectin, there was a significant difference between PR3-ANCA positive group and PR3-ANCA negative group (p: 0.000. As regards serum albumin and SGPT there was no significant difference between PR3-ANCA positive group and PR3-ANCA negative group (p: 0.936) (p:0.667) respectively. Also for hemoglobin level, serum creatinine, and blood urea, there was no significant difference between the PR3-ANCA positive group and PR3-ANCA negative group (p: 0.889) (p: 0.328) (p: 0.399) respectively (table 2). In Fig. 1. The optimum cut-off value for PR3-ANCA to predict ulcerative colitis was >3.3 U/mL with sensitivity 84.0%, specificity 77.0%, area under the curve (AUC) 0.906, 95% confidence interval (CI) (0.839–0.972), p =0.000. (Table 2).

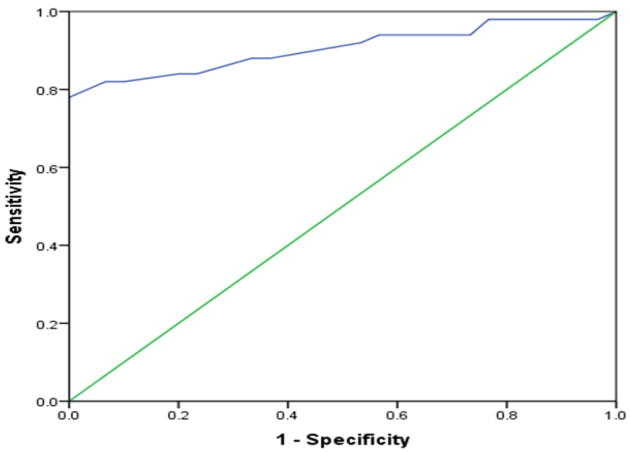

Figure 1: Receiver operating characteristic (ROC) curves for prediction of ulcerative colitis by proteinase 3 antineutrophil cytoplasmic antibody (PR3-ANCA).

Table 1: Clinical Characteristics of Ulcerative Colitis Patients

Clinical Characteristics variables		Ulcerative colitis (N=50)	Intestinal control (N=13)	Healthy control (N=17)	p-value
Age (years)		29(22-35)	23(22-30)	26(23-33)	0.496
Sex	Male	25(50.0%)	7(53.8%)	9(52.9%)	0.958
	Female	25(48.0%)	6(46.2%)	8(47.1%)	
Duration of treatment	Not treated	18(36.7%)			
	Less than one year	9(18.4%)			
	More than one year	23(46.9%)			
Hb / gm		9.0(8.0-10.0)	11.6(11.0-13.0)	12.5(11.2-13.0)	0.000*
PTT/c mm		2(1.5-2.5)	3(1.5-3.3)	2(1.8-4)	0.380
WBCs /c mm		12000(110000-14000)	7000(5500-10000)	5000(4000-6000)	0.000*
ESR		59(30-73)	10(10-20)	10(10-20)	0.000*
Fecal calprotectin Mg/g		250(157-600)	22(19-25)	40(25-55)	0.000*
Serum Albumin gm/dl		3.2(3.0-3.5)	4.0(4.0-5.0)	4.0(4.0-4.5)	0.000*
Prothrombin time/sec		11.0(11.0-13.0)	12.0(11.0-13.0)	12.0(11.0-13.0)	0.189
SGPT U/L		55(44-70)	24(19-30)	33(30-35)	0.000*
Creatinine mg/dl		.9(0.8-1.0)	0.8(0.7-0.9)	.8(0.6-0.8)	0.000*
Urea mg/dl		25(20-30)	20(20-25)	20(20-25)	0.204
PR3-ANCA	Median (IQR)	9.3(6.0-23.2)	2.0(3.0-3.4)	2.4(1.6-3.3)	0.000*
	High Positive	41(82.0%)	2(15.4%)	0(0.0%)	0.000*
	Negative	9(18.0%)	11(84.6%)	17(100.0%)	

Hb: hemoglobin, WBCs: white blood cells, ESR: erythrocyte sedimentation rate, PTT: partial thromboplastin time Values are presented as number (%) and Median (IQR). *Significant.

Table 2: Comparison of Clinical Characteristics between PR3-ANCA Positive and PR3-ANCA Negative Groups in Ulcerative Colitis Patients:

Clinical Characteristics variables		PR3-ANCA positive group (N=41)	PR3-ANCA negative group (N=9)	p-value
Age (years)		30(22-35)	25(23-30)	0.657
Sex	Male	22(53.7%)	3(33.3%)	0.463
	Female	19(47.3%)	6(66.7%)	
Duration of treatment	Not treated	18(43.9%)	0(0.0%)	0.000* MC
	Less than one year	3(7.3%)	3(33.3%)	
	More than one year	20(48.8%)	6(66.7%)	
Hb / gm		9.0(8.0-10.0)	9.0(8.0-10.0)	0.889
PTT/c mm		2(1.5-3)	19(16-20)	0.577
WBCs /c mm		13000(11000-14000)	10000(7000-12000)	0.010*
ESR		65(50-75)	15(10-30)	0.000*
Fecal calprotectin Mg/g		300(200-900)	100(90-100)	0.000*
Serum Albumin gm/dl		3.2(3.0-3.5)	3.3(3.0-3.5)	0.936
Prothrombin time/sec		11.0(11.0-13.0)	12.0(11.0-12.0)	0.716
SGPT U/L		55(44-70)	50(49-70)	0.667
Creatinine mg/dl		.9(0.8-1.0)	0.9(0.8-1.0)	0.328
Urea mg/dl		25(20-30)	22(17-25)	0.399
Time of diagnosis	Recently diagnosed	18(43.9%)	18(43.9%)	0.018* FE
	Previously diagnosed	23(56.1%)	9(100.0%)	

DISCUSSION

A multitude of studies have focused on the crucial need to develop UC biomarkers which could have numerous clinical applications including helping disease diagnosis by distinguishing between UC and other intestinal diseases like infectious gastroenteritis or irritable bowel syndrome, monitoring of disease activity non-invasively, and predicting UC severity [11].

The objective of the current study was to investigate the clinical role of anti-PR3-ANCA in disease diagnosis and severity in UC patients.

Previous research found that the prevalence of p-ANCAs in UC patients exhibits geographical variation. For instance, p-ANCAs have been reported at higher rates in UC patients from Western nations compared to their Asian counterparts. This was exemplified by Prideaux and colleagues who noted a 70% positivity rate for p-ANCAs in Caucasian UC patients, in contrast to a 33% rate in Asian patients [12]. This disparity has been corroborated by research conducted across various Asian countries [13-16].

In the context of Western populations, PR3-ANCAs have been identified in a higher proportion of UC patients than Crohn's Disease (CD) patients, exhibiting a sensitivity of 52.1% and a specificity of 97.3% [17]. Also in Switzerland, a study among pediatric patients reported a 58% sensitivity and 93% specificity for PR3-ANCAs in diagnosing UC [18]. Similarly, in China, PR3-ANCAs showed a sensitivity of 57.1% and a specificity of 98.9% [19].

In the present study, conducted within the Egyptian demographic, a pronounced increase in serum anti-PR3-ANCA was observed among patients with Ulcerative Colitis (UC). The study noted an 82% positivity rate for PR3-ANCA in UC patients, compared to 15.4% in individuals with other intestinal conditions and 0% in the healthy control group. A cut-off value of 3.3 U/mL for PR3-ANCA was identified, demonstrating a sensitivity of 84% and a specificity of 77% in the prediction of UC. These findings emphasize the significance of PR3-ANCAs as a valuable diagnostic biomarker for

UC across wider populations and geographical regions.

Furthermore, this study demonstrated that 56.1% of the PR3-ANCA positive group were recently diagnosed while all PR3-ANCA negative group were previously diagnosed. Inflammatory markers, WBC count, and ESR, along with the marker for disease activity, fecal calprotectin, were significantly elevated in the PR3-ANCA positive group compared to the negative one. Notably, there was a strong positive correlation between PR3-ANCA levels and fecal calprotectin ($r=0.868$, $p<0.001$), colonoscopy score ($r=0.839$, $p<0.001$), and ESR ($r=0.803$, $p<0.001$). These findings underscore PR3-ANCA's potential as a non-invasive marker for assessing disease severity, including disease activity, the extent of gastrointestinal involvement, and the degree of inflammation.

These outcomes align with the findings of previous studies. A study by Imakiire demonstrated that elevated PR3-ANCA titers in UC patients were indicative of a greater necessity for steroid therapy to achieve remission [20]. In addition, Junxiang Zeng conducted a study where they created a scoring system incorporating various serum biomarkers to predict the severity of UC and found that PR3-ANCA emerged as the most significant indicator of disease severity [21].

This study still had some limitations. First, the study demonstrated strong correlations between PR3-ANCA levels and disease severity markers, so further studies to evaluate this role are recommended. However, the mechanistic link between PR3-ANCA and UC pathogenesis was not investigated and needs further exploration. In addition, the cut-off value of 3.3 U/mL for PR3-ANCA was identified in this study; however, determining the optimal threshold for diagnosis and prognosis requires further validation on a larger sample size. Finally, the study didn't assess the performance of PR3-ANCA over the course of the disease. Further research is needed to validate and refine the use of PR3-ANCA in the clinical management of UC.

CONCLUSION:

In conclusion, the findings of the current study reveal a high prevalence of PR3-ANCA among UC patients, emphasizing its role as a diagnostic biomarker for UC. Moreover, the study's demonstration of significantly elevated WBCs, ESR, and fecal calprotectin levels and their strong correlation with PR3-ANCA levels in UC patients further positions this biomarker as a non-invasive indicator of disease severity. Future research should focus on discovering the underlying mechanisms by which PR3-ANCA contributes to the pathogenesis of UC**.**

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All authors contributed equally to this work.

Ethical consideration: This study was approved by Kafrelsheikh university ethical committee and informed consent was taken.

HIGHLIGHTS:

- The findings of the current study reveal a high prevalence of PR3-ANCA among UC patients, emphasizing its role as a diagnostic biomarker for UC.
- The study's demonstration of significantly elevated WBCs, ESR, and fecal calprotein levels and their strong correlation with PR3-ANCA levels in UC patients further positions this biomarker as a non-invasive indicator of disease severity.
- Future research should focus on discovering the underlying mechanisms by which PR3-ANCA contributes to the pathogenesis of UC.

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