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## Target Antigens for Perinuclear Antineutrophil Cytoplasmic Antibodies in Iranian Patients with Ulcerative Colitis

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### ABSTRACT

#### BACKGROUND

Patients with ulcerative colitis (UC) carry autoantibodies such as perinuclear antineutrophil cytoplasmic antibodies (p-ANCA).

**Objective:** The aim of the present study was to evaluate the target antigens for p-ANCA in Iranian patients with UC.

#### METHODS

p-ANCA target antigens including elastase, lactoferrin, cathepsin G, myeloperoxidase, lysozyme, and bactericidal permeability increasing protein (BPI) were determined in 113 patients with UC using enzyme-linked immunosorbent assay (ELISA).

#### RESULTS

59.2% of the patients were positive for at least one antigen and p-ANCA directed against lactoferrin, elastase, lysozyme, cathepsin G, Bactericidal permeability increasing protein, and myeloperoxidase in 31.5%, 25.9%, 8.3%, 7.4%, 5.6%, and 0% of the patients, respectively.

#### CONCLUSION

The highest prevalence of p-ANCA was observed against lactoferrin and elastase. Also, myeloperoxidase was not an antigen for p-ANCA among our patients.

#### KEYWORDS

Inflammatory bowel disease; Ulcerative colitis; Anti-neutrophil cytoplasmic antibody; Ttarget antigen

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### INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic disorder that affects the gastrointestinal tract and is subdivided into ulcerative colitis (UC) and Crohn's disease (CD). Although the etiology of IBD is unknown, it is believed to be an immunologically mediated disorder in genetically predisposed subjects.<sup>1</sup>

A subset of anti-neutrophil antibodies, commonly referred to as perinuclear anti-neutrophil cytoplasmic autoantibody (p-ANCA) has

been observed in the sera of patients with IBD. The prevalence of a positive p-ANCA varies from 40% to 80% in UC and from 0% to 20% in CD.<sup>2,3</sup> Several proteins such as elastase, cathepsin G, lysozyme, lactoferrin, catalase, bactericidal permeability increasing protein (BPI),  $\beta$ -glucuronidase, and  $\alpha$ -enolase have been described as the potential target antigens for p-ANCA in UC.<sup>4</sup> We have previously shown that they are not valuable diagnostic serologic markers for IBD alone or in combination, because of their low sensitivity.<sup>5</sup>

The aim of the present study was to investigate the prevalence of target antigens for p-ANCA in a sample of Iranian patients with UC.

## MATERIALS AND METHODS

### Patients

The study consisted of 113 Iranian patients with UC who referred to the Department of Gastroenterology in Taleghani University Hospital of Tehran. Diagnosis of UC was established on the basis of clinical, endoscopic, radiological, histological, and surgical criteria as described by Lennard-Jones.<sup>6</sup> We excluded patients with indeterminate colitis and patients in whom a diagnosis of UC had not been clearly established. The severity of disease was determined according to the Truelove and Witts index.<sup>7</sup>

Patients' data including smoking habit, familial history of IBD, duration of disease, extra-gastrointestinal manifestations, intestinal complications, and medications were obtained from the database of Research Center for Gastroenterology and Liver Diseases.

### Blood sampling

Fasting blood samples were obtained from the antecubital vein. The sera were separated and stored at -80 °C until analysis.

### p-ANCA target antigens

Auto-antibodies against lactoferrin, lysozyme, elastase, cathepsin G, and BPI were determined by enzyme immunoassay method using commercial

available kit (ORGENTEC Diagnostika GmbH, Hamburg, Germany) and autoantibody against myeloperoxidase (MPO) was determined by commercial available kit (GENESIS diagnostics Co, UK).

To detect p-ANCA, an indirect immunofluorescence (IIF) method was performed using commercially available kit (IMMCO Diagnostics, Inc. Canada) as described previously.<sup>5</sup>

### Ethics

This project was approved by the Ethics Committee of Shaheed Beheshti University of Medical Sciences and informed consents were obtained from all individuals participated in the study.

## RESULTS

Demographic characteristics of the patients with UC are shown in table 1. According to the colonoscopic and histological assessments, 29.8% of the patients had pancolitis, 32.4% had left colitis, 14.2% had distal colitis, and 23.3% had proctitis.

### Target antigens for p-ANCA

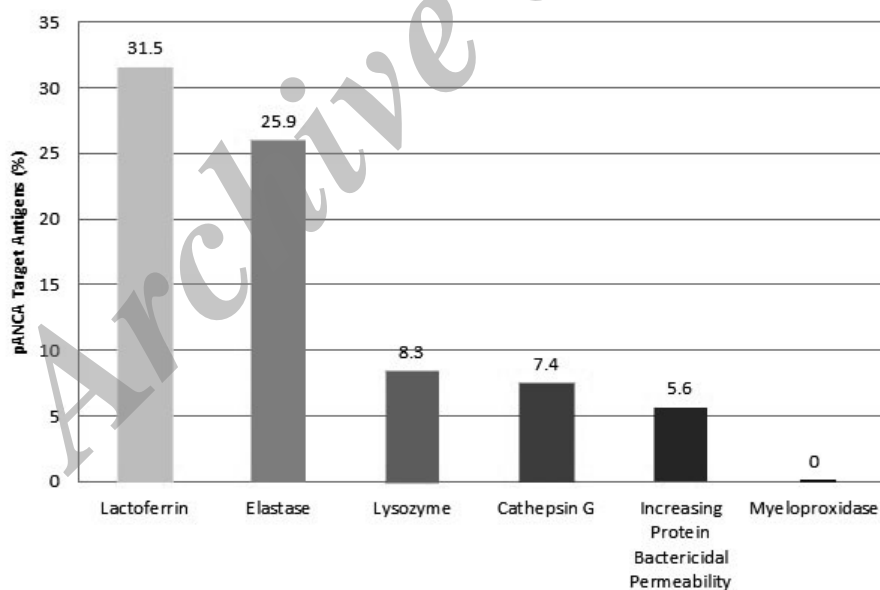
One hundred and eight patients completed the study. In order to find the target antigens for antineutrophil cytoplasmic antibody among the patients with UC, we did ELISA to find autoantibody against lactoferrin, elastase, lysozyme, cathepsin G, BPI, and myeloperoxidase (MPO). As shown in figure 1, the prevalence of auto-antibodies against lactoferrin, elastase, lysozyme, cathepsin G, BPI, and myeloperoxidase (MPO) were 31.5%, 25.9%, 8.3%, 7.4%, 5.6% and 0%, respectively.

In addition we found that 3 patients (2.7%) had positive tests for three antigens, and 15 (13.8%) patients had positive tests for two antigens. Also, 59.2% (64/108) of the patients had positive test for at least one antigen, however, the results of IIF showed that only 39.8% of the patients with UC had positive p-ANCA.

Further analyses showed no correlation between clinical severity of the disease and the mentioned target antigens. But dysplasia was more frequent as a complication in patients with positive p-ANCA against lysozyme (Fisher's test,  $p=0.01$ ). Furthermore, patients with pan-colitis or left-sided colitis

**Table 1: Demographic characteristics of patients with ulcerative colitis**

	No	%	
Patients	113	100	
Gender (male)	49	43.4	
Age (years)	39		
Age of onset (years)	33		
Disease duration (months)	69		
Current cigarette smokers	13	11.5	
History of IBD in first relatives	7	6	
Skin lesion	14	12.4	
Oral aphthous	9	8	
Musculoskeletal manifestations	12	10.6	
Arthritis and Sacristies	9	8	
Eye manifestations	5	4.5	
Liver manifestations	8	7	
Sclerosing cholangitis	6	5.3	
	Dysplasia	6	5.3
	Stenosis	39	34.5
Intestinal complications	Severe bleeding	20	17.7
	Abdominal abscess	0	0
	Pouchitis	1	0.9

**Fig. 1: Prevalence of autoantibody to specific antigens in patients with ulcerative colitis**

had more positive test results for antibodies against lactoferrin, elastase, and BPI, compared with those with distal colitis or proctitis ( $p < 0.05$ ). We found no correlation between other demographic characteristics of the patients and p-ANCA subtypes.

## DISCUSSION

We found p p-ANCA in 59.2% of the patients with UC by ELISA and the most common target antigens for p-ANCA in such patients were lactoferrin (31.5%), and elastase (25.9%). In addition

we found that MPO was not a target antigen for p-ANCA among our patients.

However, there was a difference between the results obtained by IIF and ELISA, so that only 39.8% of the patients with UC were positive for p-ANCA using IIF method and only about 65.1% (28/43) of the patients with a *p* p-ANCA IIF pattern reacted to at least one of the six antigens used in ELISA. This might be due to several factors such as avidity of autoantibodies, epitope exposure, conformational changes, and denaturation of the antigens. Substances other than these six antigens such as catalase,  $\alpha$ -enolase, histon H1 (HupB), High Mobility Group non-histone chromosomal proteins (HMG1/HMG2), and Proteinase 3 (PR3) have also been shown as targets antigens for p-ANCA in IBD.<sup>4,8-10</sup> Furthermore, unknown antigens were recognized as p-ANCA target antigens by some studies.<sup>11,12</sup>

On the other hand, 55.4 % (36/65) of the patients with negative IIF reacted to at least one target antigen, which was used in ELISA. These target antigens could probably have another pattern in IIF. Savige and colleagues showed that target antigens such as MPO, BPI, and cathepsin G might have c-ANCA pattern in IIF.<sup>13</sup> Cooper and co-workers also reported that BPI-ANCA is common in sera containing antinuclear (ANA) or antidouble-stranded (ds) DNA antibodies.<sup>14</sup> The vulnerability of some of target antigens such as BPI to be destructed by serine protease is another probable reason for negative IIF in some patients with positive ELISA.<sup>15</sup>

Another finding of our study was that in only 15% of the samples more than one p-ANCA antigen were detected using ELISA. This is in contrast with some studies, in which 25-50% of the patients with UC had more than one p-ANCA antigen.<sup>11,16</sup>

In other studies, BPI,<sup>15,17-19</sup> lysozyme<sup>20</sup> and lactoferrin<sup>8,21</sup> are reported as the most common target antigens for p-ANCA in patients with IBD, respectively. However, in our study, the most p-ANCA target antigen was lactoferrin. We also showed that none of patients had MPO as a target antigen for p-ANCA. This is in concordance to the studies which reported that MPO is not a target antigen for ANCA in patients with IBD.<sup>22</sup> Two studies from London

showed that only minority of sera had MPO as a p-ANCA target antigen.<sup>15,20</sup> However, according to a study performed in Japan, Sugi and colleagues reported that 69.7%, 51.5%, and 33.3% of their 33 patients with UC and p-ANCA positive sera showed reactivity with lactoferrin, MPO, and cathepsin G, respectively.<sup>8</sup> It has also been illustrated that some of MPO-ANCA positive sera have c-ANCA/ANA pattern in IIF.<sup>13</sup>

There was no correlation between demographic characteristics of our patients, with clinical severity of the disease, and p-ANCA target antigens. Flowaczny and colleagues performed a study on 61 patients with UC in Germany. They also found no correlation between clinical characteristics of the patients and p-ANCA subtypes, except for antikathepsin G antibody that was negatively correlated with using immuno-suppressive therapy.<sup>23</sup>

In summary, we showed that 59.2% of our patients with UC had positive test results for at least one antigen and the highest prevalence of p-ANCA was observed against lactoferrin and elastase. None of the patients had MPO as a target antigen for p-ANCA. Also, dysplasia was more frequent as a complication in patients with positive p-ANCA against lysozyme. Besides, colonic extension of the disease was positively correlated with the presence of antibodies against Lactoferrin, Elastase and BPI.

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#### CONFLICT OF INTEREST

The authors declare no conflict of interest related to this work.

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