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The Important role of Anti-Saccharomyces cerevisiae and Anti-neutrophil cytoplasmic antibodies in diagnosis of inflammatory bowel disease

Dr. Eman Shaker Al-Obeidy, Assis.Lect. Sarah Mousa Mahmoud**
* Ph. D Clinical Immunology. College of Al-Farabi University, Dept. of laboratory Technique.
** M.Sc Medical Virology, College of Pharmacy, Al-Mustansiriyah.
* Corresponding author e-mail- emanshaker944@gmail.com

الدور التشخيصي للأجسام المضادة لـ Saccharomyces cerevisiae والأجسام المضادة السيتوبلازمية في مرض التهاب الأمعاء

د. إيمان شاكر العبيدي، و م. م ساره موسى محمود**
* دكتوراه علم المناعة السريرية - كلية الفارابي الجامعة قسم تقنية المختبرات
** ماجستير احياء مجهريه طبيه -كلية الصيدلة الجامعه المستنصرية .

الخلاصه

التشخيص الصحيح لمرض تهيج الأمعاء (IBD)، وخاصة التمييز بين مرض كرون (CD) والتهاب القولون التقرحي (UC)، مهم للغاية تجاه العلاج والتشخيص. العلامات المصلية هي أدوات تشخيصية غير جراحية يمكن أن تكون ذات قيمة في التمييز بين مرض التهاب القولون التقرحي والتهاب القولون المزمن، وفي حالات التهاب القولون غير المحدد، وفي تحديد المجموعات الفرعية في مرض التهاب الأمعاء (IBD)**الهدف** هو تسليط الضوء على الدقة التشخيصية للأجسام المضادة لـ ASCA (Saccharomyces cerevisiae) لمرض التهاب الأمعاء. المرضى والطرق: تمت دراسة ASCA في مجموعة من مرضى IBD المتتاليين (CD، وUC)، والأصحاء الطبيعيين كمجموعة تحكم. تم إجراء ELISA موحد للكشف عن ASCA.**النتائج:** كان انتشار ASCA مرتفعا في مرضى CD (70.9٪) ومرضى جامعة كاليفورنيا (٢٦.٣٪)، على التوالي. بيانات الدقة (الحساسية، والنوعية) للتمييز بين IBD والسيطره هي كما يلي: 60% :+ASCA، 91%، 88% و٦٨٪. خاتمة؛ تعد خصوصية العلامات المصلية لمرض التهاب الأمعاء (IBD) عالية، لكن الحساسية المنخفضة تجعلها أقل فائدة كاختبارات تشخيصية.

الكلمات المفتاحية: الأجسام المضادة لخميرة (ASCA)، مرض التهاب الأمعاء (IBD)، التهاب القولون التقرحي (UC)، مرض كرون (CD)

Abstract

Background: Accurate diagnosis of inflammatory bowel disease, particularly the distinction between Crohn's disease and ulcerative colitis, is crucial for effective treatment and prognosis. Serological markers serve as noninvasive diagnostic instruments that may assist in differentiating Crohn's disease from ulcerative colitis, addressing cases of indeterminate colitis, and identifying specific subgroups within inflammatory bowel disease. **Aim** is to clarify the diagnostic precision of anti-Saccharomyces cerevisiae antibodies in relation to inflammatory bowel disease (IBD). **Patients and Methods:** anti-Saccharomyces cerevisiae antibodies was

studied in a cohort of consecutive inflammatory bowel disease patients (Crohn's disease and ulcerative colitis), and normal healthy as control. A standardized ELISA was performed for detection of anti-Saccharomyces cerevisiae antibodies. **Results:** The prevalence of anti-Saccharomyces cerevisiae antibodies was notably elevated among patients with Crohn's disease (70.9%) and ulcerative colitis (26.3%).**Conclusion;** Specificity of serological markers for inflammatory bowel disease is high, but low sensitivity makes them less useful as diagnostic tests. **Keywords:** anti-Saccharomyces cerevisiae antibodies, inflammatory bowel disease, ulcerative colitis, Crohn's disease.

Introduction

Inflammatory bowel diseases (IBD) represent a diverse array of disorders with an unclear cause. This category is mainly divided into two types: ulcerative colitis (UC) and Crohn's disease (CD). While CD and UC are typically regarded as separate entities within IBD, there can be significant overlap in their clinical manifestations, (Renata D'Incà and Giulia Sturniolo , 2023; Kaul, A, 2012).Crohn's disease (CD) and ulcerative colitis (UC) represent the two primary types of inflammatory bowel disease (IBD). Both conditions are chronic in nature, impacting individuals of all ages, including children and adults, with a nearly equal prevalence among men and women. These diseases are particularly prevalent, as noted by Cabrera-Abreu *et al*. (2004) and Elitsur *et al*. (2005).The precise diagnosis of inflammatory bowel disease (IBD) holds significant importance. Given that the treatment approaches for Crohn's disease (CD) and ulcerative colitis (UC) vary, particularly in instances where surgical intervention is necessary, considerable efforts have been made over the years to differentiate between these conditions (Eggena, M, *et al*, 2000; Horn MP, *et al*, 2018; Xu Y, 2020).Non-invasive tests are anticipated to play a significant role in the differential diagnosis. In the context of inflammatory bowel disease (IBD), researchers have been investigating for several years the presence of antibodies associated with different conditions. Two specific antibodies have been recognized for their utility in the clinical diagnosis of IBD: perinuclear antineutrophilic cytoplasmic antibody (ANCA) and anti-Saccharomyces cerevisiae antibody (ASCA) (Mizuochi T, *et al*, 2021; Torres J, *et al*, 2020).p-ANCAs represent a specific category of ANCA characterized by perinuclear staining observed through indirect immunofluorescence. Research indicates that these antibodies are present in approximately 60-70% of individuals diagnosed with ulcerative colitis (UC), in 5-10% of those with Crohn's disease (CD), and in 0-5% of healthy control subjects (Aoyama Y, *et al*, 2021).ASCAs are antibodies that target the oligomannosidic epitope found in the cell wall of Saccharomyces cerevisiae. Elevated levels of ASCAs have been observed in 6-70% of individuals diagnosed with Crohn's disease (CD), in 10-15% of patients with ulcerative colitis (UC), and in 0-5% of healthy individuals (Jaskowski *et al*., 2006; Imakiire S *et al*., 2022).The assessment of p-ANCA alone has demonstrated limited clinical diagnostic utility in inflammatory bowel disease (IBD) due to its inadequate sensitivity for diagnosing ulcerative colitis (UC) and Crohn's disease (CD). Consequently, the simultaneous evaluation of p-ANCA and ASCA has been suggested as an effective diagnostic strategy in IBD (Aoyama Y, *et al*, 2021; Laass M, *et al*, 2022).**The Aim** of the study is to search the value of detecting perinuclear antineutrophil cytoplasmic autoantibody, (P-ANCA) and anti-Saccharomyces cerevisiae antibody (ASCA) for the diagnosis of patients with inflammatory bowel disease in Baghdad.The present study consisted of 150 consecutive IBD patients.The diagnosis of UC or CD was based on accepted clinical and endoscopic criteria supported by histopathology, (O'Morain C, *et al*, 1989).According to their concordant diagnosis established by standard clinical criteria and with radiologic, endoscopic and histopathological confirmation, the patients were classified as CD (n=55) or UC (n=95). Clinical activity in CD was measured by Crohn disease activity index (CDAI) and in UC by Rachmilewitz endoscopic index, (REI). CDAI higher than 150 was predicted as active disease in CD. Rachmilewitz index equal to or higher than 4 was predicted as active disease in UC, (**Cabrera-**Abreu, *et al*, 2004).**REI index**; This indexing system comprises seven items;Frequency (0-3), blood stool, (0-4); general well-being, (0-3); acdominal discomfort, (0-3); fever, (0-3); extra intestinal manifestation, (0-9), and laboratory findings, (ESR and Hb), (0-4). The score is the sum of those seven items ranging from 0 to 29. Clinical emission was defined as CAI $\leq$ 4, while a decrease of $\geq$ 4 point in CAI relative to baseline was defined as clinical response, (**Lennarad**-Jones, JE, 1989).Sera from 50 healthy persons without gastrointestinal complaints or a familial history of IBD served as controls.The enzyme-linked immunosorbent assay (ELISA) (Euroimmune, Germany) was employed to assess p-ANCA and ASCA levels. According to the manufacturer's guidelines, the cut-off value for each test is set at 20 IU/ml, with results exceeding this threshold classified as positive, (Elitsur, Y, *et al*, 2005; **Goldschmeding R,** *et al*, 1989).**Inclusion** criteria was indicated any patients have a chronic diarrhea. While **exclusion** criteria were;

-No family history of IBD.

-No immune mediated disorder.

-All patients free from intestinal complains, (**Mosli M**., *et al*. 2014).

Statistical analysis; All data are present as percentage. Chi square was used to compare between data. P value less than 0.05 was used as significant value.

Results

The demographic information of the study population is presented in Table 1. This study involved 55 patients diagnosed with Crohn's disease (CD), comprising 33 females and 25 males. Additionally, 95 patients with ulcerative colitis also participated in the research, as detailed in **Table 1**.

The distribution of Crohn's disease (CD) is as follows: 65.5% in the small bowel, 21.8% in the colon, and 12.7% in both the small bowel and colon. In contrast, ulcerative colitis is distributed as follows: 21.1% in proctitis, 31.6% in left-sided colitis, and 47.3% in pancolitis.Table-1: Dimorphic data in Crohn's disease (CD) and ulcerative colitis (UC), and healthy control group

	CD patients N=55	UC Patients N=95	Healthy control group N=50
Female/male	30/25	40/55	22/27
Mean age (years)	33	39.2	38
Range of age (years)	14-72	15-75	19-69
Disease duration (months)	(3-95)	(2-135)	
Disease location: Small bowel Colon Small bowel+ colon	 36 (65.5%) 12 (21.8%) 7 (12.7%)		
Proctitis Left-sided Pancolitis		20 (21.1%) 30 (31.6%) 45 (47.3%)	

Table 2 shows the results of perinuclear antineutrophil cytoplasmic autoantibody, (P-ANCA) and anti-Saccharomyces cerevisiae antibody (ASCA) in the serum of patients and normal healthy control subjects.

p-ANCA was detected in 10 of 55 (18.2%) samples from CD patients and in 64 of 95 (67.40%) samples from UC patients (**Table-2**). **Table-2:** Prevalence of P-ANCA and ASCA among the studied groups

Parameters	CD patients N=55	UC Patients N=95	Healthy control group N=50	P-value
P-ANCA	**10 (18.2%)**	**64 (67.4%)**	**1 (2.0%)**	**0.05***
ASCA	**39 (70.9%)**	**25 (26.3%)**	**1 (2.0%)**	**0.07***

The findings regarding perinuclear antineutrophil cytoplasmic autoantibody (P-ANCA) in patients with Crohn's disease (CD) and ulcerative colitis (UC) indicate that only 18.2% of the overall patient population tested positive for P-ANCA. In contrast, a significantly higher percentage, 67.4%, of UC patients exhibited positive results for this antibody. Additionally, the analysis of anti-Saccharomyces cerevisiae antibody (ASCA) levels in the serum of both CD and UC patients revealed that 70.9% of individuals with CD tested positive for ASCA. Conversely, only 26.3% of UC patients showed a positive result for the ASCA antibody, as detailed in **Table 2**.Only one of 50 (2.0%) samples from healthy controls was found positive for p-ANCA. The difference between the prevalence of p-ANCA positivity in UC and CD and control groups was statistically significant.Moreover, ASCA at low titers (20-30 IU/ml) was identified in 25 out of 95 (26.3%) samples from patients with ulcerative colitis, while high titers (≥ 80.0 IU/ml) were found in 39 out of 55 (70.9%) samples from patients with Crohn's disease (**Table 3**).Also, 95 patients with UC: 40 patients with active disease and 55 patients with inactive disease: 75%from the first group and 37% from the second group were ANCA positive (**table- 3**) the difference was statically significant. **Table-3: Show disease activity in UC patients**

UC patients (n=95)	P-ANCA positivity	P-value
-Disease activity: Active disease (n=40) Inactive disease (n=55)	 30% 45%	 0.05*
Disease location: Proctitis	 25%	

Left-sided	29%	NS
Pancolitis	26%	

The present study found a non-significant difference among the three groups of UC classified according to disease localization (**Table 3**).

Discussion

Previous study found that Proteinase 3 antineutrophil cytoplasmic antibody (PR3-ANCA) is a serologic marker for granulomatosis with polyangiitis. (Sandborn W, *et al*, 2001; Peeters M, *et al*, 2001). These results agree with the present finding.

Anti-Saccharomyces cerevisiae antibodies (ASCA) are antibodies. This antibody occurs in the serum in Crohn's disease, but not in ulcerative colitis, (Mokrowiecka A, *et al*, 2009).

In the present study, 70.9 % was found positive for ASCA in CD patients. However, only 26.3% of UC patients have a positive result for ASCA antibody, (Table 2). This result agrees with previous finding regarding ASCA sensitivity, (Xu Y,and Xu F, Li W, 2020; Sastegni R, *et al*, 2001).

In studies conducted as part of a meta-analysis, serum levels of ASCA demonstrated a sensitivity of 56% and a specificity of 88% in differentiating Crohn's disease from ulcerative colitis (Forcione D, *et al*., 2004; Kvehaugen A, *et al*., 2017; Saadah O, *et al*., 2013).

The frequency of ASCA in CD patients and ANCA in UC patients may reach 80% in some reports, (Sandborn W, *et al*, 2001; Imakiire, S , *et al*, 2022).

A number of studies have identified a correlation between ASCA positivity and various factors, including the early onset of the disease, prolonged disease duration, involvement of the ileum, complicated disease presentations, and the necessity for IBD-related surgical interventions (Ouadii Abakarim, *et al*, 2024; Renata D, *et al*, 2023).

The present study **concludes** that, 70.9 % was found positive for ASCA in CD patients. However, only 26.3% of UC patients have a positive result for ASCA antibody. Also, the influence of disease location, symptom duration, type of therapy, and the baseline inflammation all contribute to the variability of the results.

Limitation; of the study are small number of patients, because they attended private clinic, and many patients dropped from the study.

The present study **recommends** the following;

-Measurement of IL-1, IL-6 and IL-10.

-Measurement of CRP.

- Anti-glycan antibodies.

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