

## **Anticarbohydrate antibodies in patients with inflammatory bowel disease.**

**Mohammed Fakhraldein, Lecturer of internal medicine,  
Faculty of medicine, Mansoura University  
Huseen Saad Mostafa, Consultant clinical pathology,  
King Fahd hospital, Riyadh, Saudi Arabia**

### **Abstract**

#### **Objective:**

Evaluating the prevalence of antichitobioside carbohydrate antibody (ACCA), antilaminaribioside carbohydrate antibodies (ALCA), antimannobioside carbohydrate antibodies (AMCA) and anti-Saccharomyces cerevisiae antibodies (ASCA), in patients with inflammatory bowel disease (IBD).

#### **Subjects and methods:**

268 serum samples were used; 115 Crohn's disease (CD), 83 ulcerative colitis, and 70 healthy control samples. All samples were evaluated using enzyme-linked immunosorbent assay for the following four anticarbohydrate antibodies: ACCA, ALCA, AMCA, and ASCA.

#### **Results:**

In patients with Crohn's disease the prevalence of the anticarbohydrate antibodies was: ASCA 69%, AMCA 32%, ACCA 28% and ALCA 24% with the highest prevalence being for ASCA (P-value<0.0001) while in patients with ulcerative colitis the prevalence was: ACCA 46%, AMCA 35%, ALCA 23% and ASCA 15% with the highest prevalence being for ACCA (P-value<0.001).

#### **Conclusion:**

Anticarbohydrate antibodies are significantly present in patients with IBD. The use of a panel of anticarbohydrate antibodies may provide additional help in distinguishing IBD from non-IBD disease patterns and narrow the range of differential diagnosis in these patients.

### **Introduction**

In the past decade, significant advances have been made in the discovery of immunological processes related to inflammatory bowel disease (IBD); evaluation of these processes is the subject of many publications, which report correlation between different serological markers and IBD; however, relying exclusively on serum antibodies for IBD diagnosis is not justified yet, because the available humoral immunological markers are neither adequately sensitive nor

sufficiently specific [Marteu P, 2002]. Currently, a serology panel that includes perinuclear antineutrophil cytoplasmic antibodies (p-ANCA) and anti-Saccharomyces cerevisiae antibodies (ASCA) is used as an auxiliary diagnostic tool of IBD [Vermeire **et al**, 2004]. A number of studies have reported that ASCA titers are significantly greater and highly specific for Crohn's disease (CD) [Quinton **et al**, 1998 and Nielsen **et al**, 2000]. Recently, immune responsiveness to some microbe-derived antigens such as outer

membrane porin C (OmpC) of *Escherichia coli* [Ferrante **et al**, 2007], I2 DNA fragment of *Pseudomonas fluorescens* [Ferrante **et al**, 2007], or flagellin-derived protein Cbir1 [Targan **et al**, 2005 and Papp **et al**, 2007] have been described as contributing factors in the pathogenesis of IBD. Dotan **et al**. [2006] indicated that patients with CD might have elevated serum levels of antibodies specific for certain carbohydrate structures and these anticarbohydrate assays are based on the oligosaccharides; chitobioside carbohydrate, laminaribioside carbohydrate, and mannobioside carbohydrate, which are the predominant cell wall surface components in many saprophytic and pathogenic fungi, yeast, bacteria, as well as protozoa and viruses. In humans, these carbohydrates can trigger essential immunological processes, including T-cell activation, differentiation, and survival [Perzer **et al**, 1991 and Schwarz **et al**, 2003]. It was reported that anti-Cbir1, anti-I2, and anti-OmpC are associated with more complex CD course and patients expressing anti-I2 are significantly more likely to have fibrostenosing CD and require small bowel surgery [Ferrante **et al**, 2007]. A serum response to flagellin-like antigen Cbir1 is associated with small-bowel, penetrating, and fibrostenosing disease features [Targan **et al**, 2005]. ASCA, ALCA, and anti-

OmpC are linked to the ileal involvement; anti-OmpC and p-ANCA in CD can indicate UC-like behaviour [Papp **et al**, 2007]. It is hypothesized that anticarbohydrate antibodies could improve the diagnostic accuracy of serological testing in IBD and, in particular, could contribute to refining the differential diagnosis of CD and UC and IBD/non-IBD involvement [Targan **et al**, 2005]. The aim of this study was to investigate the prevalence of ACCA, ALCA, AMCA and ASCA in IBD patients.

## Subjects and Methods

This study was conducted at the gastroenterology and endoscopy unit, King Fahd hospital, Riyadh, Saudi Arabia. A total of 268 serum samples were included in the study: 115 CD, 83 UC and 70 healthy age and sex-matched blood donors. All serum samples were analyzed for ASCA, ACCA, ALCA, and AMCA levels using the ELISA method. All patients included in the study were already diagnosed to have IBD (Crohn's disease or ulcerative colitis) and are under regular follow up. Data about age, disease duration and disease location were collected from the medical records of the patients.

**Table 1 shows criteria of Crohn's disease patients:**

Number	115
Male/ Female	65 50
Mean age (years)	30.7±6.5
Disease duration (years)	6-15
Disease location:	
terminal ilium	21(18%)
Colon	28(24%)
Ileocolon	66(58%)

**Table 2 shows criteria of ulcerative colitis patients:**

Number	83
Male Female	46 37
Mean age (years)	38.8±9.8
Disease duration (years)	7-15
Disease location:	
Proctitis	24(29%)
Distal and left-sided colitis	40(48%)
Pancolitis	19(23%)

*Serum ASCA, ACCA, ALCA, and AMCA were measured using ELISA method*

The  $\chi^2$ -test and Mann–Whitney's *U*-test were used for assessment of prevalence rates and evaluation of the differences in antibody titers . *P-values* of less than 0.05 were considered significantly different.

## Results

### ASCA, AMCA, ALCA, and ACCA reactivity in the IBD and healthy groups:

#### **In the CD sample group;**

1-79 serum samples out of 115 were ASCA-positive, equalling a prevalence of 69%.

2-32 samples out of 115 were ACCA positive, equalling a prevalence of 28%.

3-28 samples out of 115 were ALCA positive equalling a prevalence of 24%.

4- 37 samples out of 115 were AMCA positive equalling a prevalence of (32%).

5-25% of the CD serum samples were positive for more than one serological marker.

ASCA	79	69%
ACCA	32	28%
ALCA	28	24%
AMCA	37	32%

#### **In the UC sample group;**

1-12 serum samples out of 83 were ASCA positive equalling a prevalence of 15%.

2-38 serum samples out of 83 were ACCA positive, equalling a prevalence of 46%.

3- 19 serum samples out of 83 were ALCA positive, equalling a prevalence of 23%.

4- 29 serum samples out of 83 were AMCA positive equalling a prevalence of 35%

5-20% of the UC serum samples were positive for more than one serological marker.

ASCA	12	15%
ACCA	38	46%
ALCA	19	23%
AMCA	29	35%

### IN the healthy controls;

1-4 serum samples out of 70 were ASCA positive equalling a prevalence of 6%.

2-8 serum samples out of 70 were ACCA positive, equalling a prevalence of 12%.

3- 4 serum samples out of 70 were ALCA positive, equalling a prevalence of 6%.

4- 8 serum samples out of 70 were AMCA positive equalling a prevalence of 12%.

ASCA	4	6%
ACCA	8	12%
ALCA	4	6%
AMCA	8	12%

The differences in the prevalence of the anticarbohydrate antibodies between the patient group and the control group were highly significant, with the highest prevalence is for ASCA in the Crohn's disease patients (69% with the P-value <0.0001) and for ACCA in the ulcerative colitis patients (46% with the P-value <0.001).

### Discussion

Similar to many systemic and organ-specific autoimmune disorders, IBD is associated with various immunological abnormalities and can represent a process of immune dysfunction [Mow *et al*, 2004]. However, unlike most autoimmune disorders, IBD occurs at a mucosal site facing the intestinal lumen, thus the primary immune target in IBD may be an extrinsic antigen such as a chronic microbial colonist [Lennard-Jones, 1989]. The human gut microflora is composed of hundreds of bacterial species [Satsangi *et al*, 2006], the behavior of the intestinal microflora may be either commensal or pathogenic [Silverberg *et al*, 2005]. One of the numerous factors that determine a particular host-microbial relationship is the protein-glycan interaction, which control essential immunological responses, including lymphocyte activation, differentiation and survival [Schwarz *et al*, 2003]. It is possible that carbohydrate (glycan) antigens of endogenous microflora can contribute to the immune-mediated damage of the intestinal mucosa seen in IBD [Langholz E, 1999].

In our study, we evaluated the prevalence of 4 anticarbohydrate antibodies (ASCA, ACCA, ALCA and AMCA) in patients with

inflammatory bowel disease; in patients with CD, we found a prevalence of 69% for ASCA, 28% for ACCA, 44% for ALCA and 32% for AMCA with the highest prevalence being for ASCA (P-value<0.0001). In patients with UC, we found a prevalence of 15% for ASCA, 46% for ACCA, 23% for ALCA and 35% for AMCA with the highest prevalence being for ACCA (P-value<0,001). Thus, identification of novel antibodies associated with IBD would provide an advance to the current diagnostic tools [Perzer *et al*, 1991].

Anticarbohydrate antibody assays are based on the oligosaccharides chitobioside, laminaribioside, and mannoside carbohydrates, these glycans are cell-wall surface components in many saprophytic and pathogenic microbes [Silverberg *et al*, 2005]. The immune system in the intestinal mucosa of healthy individuals is tolerant to microbial carbohydrate (glycan) antigens [Wen and Fiocchi, 2004]. In patients with IBD, there is a loss of immunological tolerance to their 'own' resident microbes present on the mucosal surface, however, whether antibodies to these carbohydrate antigens are directly causative of IBD with the participation in the inflammatory cascade, or are simply an epiphenomenon of the immunopathologic inflammation, is uncertain [Duchmann *et al* 1995]. Similar

results regarding the association between IBD and anticarbohydrate antibodies were obtained in other studies [Papp *et al*, 2008]. However, none of the current available biomarker tests/ assays can be used as stand-alone tools in the diagnosis of IBD; therefore, it can only be recommended as an adjunct to conventional methods for diagnosis of the disease [Vermeire *et al*, 2006].

Anti-Saccharomyces cerevisiae antibodies (ASCA) and perinuclear antineutrophil cytoplasmic antibodies (pANCA) were the first extensively characterized serological IBD markers; ASCA is more associated with Crohn's disease (CD) while pANCA is more associated with ulcerative colitis (UC) [Duchmann *et al*, 1995]. Three additional serum biomarkers were introduced later, including antibodies against outer membrane porin C (anti-OmpC), Pseudomonas fluorescens bacterial sequence I2 (anti-I2), and bacterial flagellin (anti-Cbir 1) [Langholz E, 1999]. Although data from different studies vary, combinations of more than one of the 5 serological markers have been shown to have the most clinical value for example; ASCA and pANCA together have a specificity of approximately 90% for both CD and UC [Vermeire *et al*, 2006]. These markers have been demonstrated as not only being useful for differentiating IBD from healthy control or CD from UC, but also as potential indicators and/or predictors for disease activity/location, disease course/complication, need for surgery, and prognosis of therapy. For example:

**1**-CD patients who are positive for multiple anti-microbial antibodies (ASCA, anti-OmpC, anti-Cbir1, and anti-I2) have increased risk of having more complicated disease

**2**-patients who are positive in all four of these biomarkers have 11-fold increased risk to develop penetrating and/or stricturing disease

**3**-CD patients positive for three markers (anti-OmpC, anti-Cbir1, and anti-I2) are more likely to have small bowel surgery

than those who were negative for these markers [Buhner *et al*, 2006].

Another importance of these serological biomarkers include their potential value as subclinical biomarkers and their inherent geographic/ethnic heterogeneity: different studies have shown that the prevalence of ASCA positivity is significantly higher (20%-25%) in unaffected first-degree relatives of patients with CD compared to general healthy populations (0%-10%), indicating a familial association denoting that ASCA may be a potential subclinical biomarker for CD [Lawrance *et al*, 2004].

Ferrante *et al* [2007] reported a study that involved a large cohort, including 1225 IBD patients. In this study, ACCA, ALCA, AMCA, ASCA and anti-OmpC were analyzed. 76% of CD patients are positive for at least one of the 5 markers. Simondi *et al* [2008] stated that, while the level of ASCA appeared to be associated with ileal disease and penetrating/structuring disease, level of ALCA has a similar trend, but did not reach statistic significance ( $P = 0.07$  and  $P = 0.09$ , respectively) also they reported that CD patients with at least one affected first-degree relative exhibited significantly higher levels of ALCA than those without familial cases ( $P = 0.0005$ ), suggestive of a familial association of ALCA. Similar results on antiglycan antibodies were reported by Papp *et al* [2007]. In another study [Pang *et al*, 2005] that involved 652 patients with IBD, 66.2% of CD patients were positive for at least one of the 5 biomarkers tested, including ASCA, ALCA, ACCA, AMCA, and anti-OmpC.

Results from our study and all other studies indicated the importance of anticarbohydrate antibodies as an adjunct tool in the diagnosis of IBD and narrowing the range of differential diagnosis in such patients, however none of these serological markers can be used as a sole diagnostic method for IBD and their value is confirmatory to other conventional methods of diagnosis.

## Conclusion

Anticarbohydrate antibodies are significantly present in patients with IBD. ASCA is present in 69% of patients with CD and ACCA is present in 46% of patients with UC. The use of a panel of anticarbohydrate antibodies may provide additional help in distinguishing IBD from non-IBD disease patterns and narrowing the range of differential diagnosis in such patients.

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## مضادات الكربوهيدرات فى مرض القنائة الهضمية الالتهابى

محمد فخر الدين - مدرس الباطنة-كلية الطب-جامعة المنصورة

حسين سعد مصطفى-استشارى الباثولوجيا الاكلينيكية  
-مستشفى الملك فهد-الرياض-المملكة العربية السعودية

يهدف البحث الى معرفة نسبة وجود مضادات الكاربوهيدرات (ACCA,ALCA,AMCA,ASCA) فى مرض القنائة الهضمية الالتهابى. ولقد تم البحث على 268 عينة دم من : 115 مريض بمرض كرون- 83 مريض بالقولون التقرحى- 70 شخص سليم كمجموعة ضابطة. وتم قياس مضادات الكاربوهيدرات الاربعة عن طريق تقنية ELISA ووجد فى مرضى كرون ان نسبة وجود ASCA = 69% , AMCA = 32% , ACCA = 28% , وفى مرضى القولون التقرحى كانت النسب كالاتى : ALCA = 24% , AMCA = 35% , ALCA = 23% , ACCA = 46% , ASCA = 15%. ونستخلص من البحث ان مضادات الكاربوهيدرات توجد فى نسبة عالية من المرضى ويمكن ان لها فائدة تشخيصية كبيرة.