World Journal of Medical Sciences 7 (2): 72-76, 2012
ISSN 1817-3055

DOI: 10.5829/idosi.wjms.2012.7.2.62131

Study the Level of ASCA and Panca Antibodies in Inflammatory Bowel Diseases in Comparison with Biopsy Findings

[1]Shokouh Taghipour Zahir, [1]Fariba Binesh,
[2]Hassan Salmanroghani and [3]Horiossadate Paknejad

[1]Clinical and Surgical Pathology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[2]Internal Medicine and Gastroenterologist, Shahid, Sadoughi University of Medical Sciences, Yazd, Iran
[3]General Practitioner

Abstract: Introduction: To determine the accuracy of the assay using pANCA (perinuclear cytoplasmic antibody) and ASCA(anti saccharomyces cerevisiae antibodies) in diagnosing ulcerative colitis(UC) and crohn disease (CD).Methods: Serum samples were obtained from 62 patients with UC and11 with CD. Diagnosis was also, established by biopsy. Determination of ASCA and pANCA were performed using rapid ELISA methods and Immunometric Enzyme Immunoassay technique respectively.Results:1.6% of patients with UC against none patient with CD expressed p ANCA(pv=1),36.4% of patients with CD against 17.7%of UC patients had positive ASCA IgA (pv=0.221),9.1% of patients with CD and 30.6%of UC patients expressed positive ASCA IgG (pv=0.270) In patients with UC sensitivity, specificity, PPV and NPV of pANCA were as follows:1.61%,100%,100%and15.27% respectively.The use of ASCAIgA test in diagnosing CD yield a sensitivity, specificity, PPV and NPV as follows:36.36%,82.25%,26.66% and87.93% respectively. In patients with CD the sensitivity, specificity, PPV and NPV of ASCA IgG were as follows:9.09%,69.35%,5%,and81.13% respectively.Conclusion: pANCA and ASCA testing can not be considered as a serum differential diagnosis marker for inflammatory bowel disease.

Key words: Inflammatory Bowel Diseases · Panca, ASCA · Crohn's Disease · Ulcerative Colitis

INTRODUCTION

The inflammatory bowel disease (IBD), crohn disease (CD) and ulcerative colitis (UC) are heterogenous chronic inflammatory disorders of the gastrointestinal (GI) tract. The most widely accepted etiopathogenic hypothesis for these disorders suggests an immune mediated process originates from an inappropriate response of mucosal immune system to the normal enteric flora in a genetically susceptible individual [1, 2]. Epidemiological studies have shown that CD and UC each have a prevalence of approximately 100-200 per 100000 individuals in Europe and North America [2]. In Iran the prevalence of IBD is not well documented.A serological response to various microbial and autoantigens can develop in IBD and it has been suggested that this antibody production may be due to the loss of mucosal immune tolerance, rather than simply a consequence of increased bowel permeability [3]. Although an increasing amount of experimental data are available on newly discovered antibodies directed against various microbial antigens,the anti-Saccharomyces Cerevisiae antibody (ASCA) and perinuclear antineutrophilic cytoplasmic antibody (pANCA) remain the best-characterized serological markers in IBD [3, 4]. To date,the use of ASCA andpANCA as serological markers has been helpful in distinguishing IBD from functional disorders,in the characterization of cases of indeterminate colitis,and as subclinical markers in affected families [3-5]. Although individually ASCA andpANCA tests have moderate sensitivity and specificity,the combination of these markers may be helpful in patients in whom a distinction between CD from UC is not obvious from diagnostic tools based on clinical data, endoscopic and histopathologic examination. An ASCA+/pANCA- serologic pattern is mainly characteristic of CD, while an ASCA-/pANCA +

Correspondence Author: Shokouh Taghipour Zahir, Department of Pathology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. Tel: +98-3518113615.

phenotype is characteristic of UC (6). Several independent studies have determined that these combination had sensitivities ranging from 30% to 64%, specificities >90% and positive predictive value(PPV) of 77-96% for diagnosis of CDor UC [3, 7-10]. In addition emerging data support the use of serologic markers to stratify patients with IBD into more homogenous subgroup,with respect to response to therapy and disease progression [4, 6]. Since limited data on serologic markers in IBD are available in Iran, the aim of the present study was to determine the diagnostic value of serologic ASCA and pANCA in an Iranian population of IBD patients.

MATERIALS AND METHODS

This cross sectional study was approved by committee for ethics of the shahid Sadoughi university.Written informed consent was obtained from all participants.A total of 90 consecutive patients suspected to IBD were included in the study. All patients were evaluated at the shahid Sadoughi general hospital and had their diagnosis confirmed by colonoscopy andhistologic criteria. Patients with indeterminate colitis were excluded from the study, finally there were 73 patients: 62 with UC and11 CD patients. Demographic and clinical data were obtained via a questionnaire and review of medical records. After formal consents were obtained, 5ml venous blood was collected from each subject. Samples were centrifuged and then serum separated, aliquoted and stored at -20°C until use.Evaluation of ANCA was performed using an immunometric enzyme immunoassay for the quantitative determination of IgG autoantibodies to myeloperoxidase by ORGENTEC Diagnostika GmbH kit, (lot number 519-0508-01-gb) which the principle antigen of test was highly purified myeloperoxidase (MPO) bounded to microwells. Antibodies against this antigen,if presented in diluted serum or plasma, bounded to the respective antigens. Washing of the microwells removed unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti human IgG immunologically detected the bounded patient antibodies forming a conjugate /antibody/antigen complex.Washing of the microwells removed unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzed to form a blue color. The addition of an acid stopped the reaction formed a yellow end product. The intensity of this yellow color was measured photometrically at 450nm. The amount of colour was directly proportional to the concentrattion of IgG antibodies presented in the original sample. The samples of >5U/ml Anti –MPO IgG considered positive. For detection the ASCA-IgG andIgA we used GENESIS Diagnostics kit,(product codes GD78 and 79, lot No 21391) which are rapid ELISA methods for the qualitative/semiquantitative detection of anti –saccharomyces cerevisiae antibodies and the principle of test was as detailed: diluted serum samples were incubated with mannan immobilised on microtitre wells.after washing away unbound serum components, rabbit anti human IgA conjugated to horseradish peroxidase was added to the wells and this bounded to surface bound antibodies in the second incubation. Unbound conjugate was removed by washing and a solution containing 3,3,5.5-tetramethylbenzidine (TMB) and enzyme substrate was added to trace specific antibody binding. Addition of stop solution terminated the reaction and provided the appropriate pH for color development. The optical densities of the standard, controls and samples were measured using a microplate reader at 450 nm. Optical density was directly proportional to antibody activity in the sample. A sample was considered positive if the optical density was greater than that of the 10U/ml standard. The results were documented both in absolute values and in frequency of positivity.

Statistical Analysis: Data were analyzed statistically using SPSS version 13, using Anova, Fischer and chi-square tests.Statistical significance was accepted for $p<0.05$.For the ASCA-IgA andIgG andANCA tests sensitivity, specificity, PPV and NPV were estimated.

RESULTS

A total of 73 consecutive patients were included in the study: 62(84%) with UC (mean age= 43.24 years, range 15-79) and 11 CD patients (mean age 27.36 years, range 18-40 years). According to gender 54% of CD patients were male and 45.4% female. 48.4% of patients with UC were male and 51.6% were female. The chief complaints were 1-diarrhea (the most frequent complaint) 2-bloody stool and the remainders were classified as the others. None of the patients had abdominal pain. Among CD patients (whom their diagnosis were confirmed by biopsy) 4 patients (36.4%) were positive for ASCA IgA and 11(17.7%) of UC patients were ASCA IgA positive (Table1). According to Pvalue=0.22 which has reached of Fischer test, we concluded that ASCA IgA is not useful for differentiation of CD from UC. In patients with CD

Table 1:The mean titer of ASCA IgA antibody according to diagnosis confirmed by biopsy.

Diagnosis confirmed by biopsy	number	The mean titer of ASCA IgA(u/ml)	SD	Min	max
Ulcerative colitis	62	7.05	9.26	0.7	49
Crhon's disease	11	8.25	5.44	1.5	17.20
sum	73	7.24	8.78	0.7	49

Table 2: The mean titer of ASCA IgG antibody according to diagnosis confirmed by biopsy

Diagnosis confirmed by biopsy	number	The mean titer of ASCA IgG(u/ml)	SD	Mean	MAX
Ulcerative colitis	62	8.42	7.26	0.1	34.2
Crhon's disease	11	5.95	4.82	0.8	18.5
sum	73	8.05	6.98	0.1	34.2

Table 3: The mean titer of pANCA antibody according to diagnosis confirmed by biopsy

Diagnosis confirmed by biopsy	number	The mean titer of pANCA(u/ml)	SD	Mean	Max
Ulcerative colitis	62	1.10	0.94	0.3	7.50
Crhon's disease	11	1.37	1.00	0.3	3.20
sum	73	1.14	0.95	0.3	7.50

(whom their diagnosis were confirmed by biopsy) only one patient (9.1%) was positive for ASCA IgG and 19 (30.6%) patients with UC were positive for ASCA IgG (Table 2). Because of p-value =0.270 which has reached of Fischer test, ASCA IgG is not useful for differentiation of CD from UC. Of the 62 patients with UC (whom their diagnosis were confirmed by biopsy) only one (1.6%) was positive for pANCA and none of CD patients was positive (Table 3). According to p-value=1.0 which has reached of Fischer test pANCA is not useful for differentiation of UC from CD. In patients with UC sensitivity, specificity, PPV and NPV of pANCA were as follows: 1.61%, 100%, 100% and 15.27% respectively. The use of ASCAIgA test in diagnosing CD yield a sensitivity, specificity, PPV and NPV as follows: 36.36%, 82.25%, 26.66% and 87.93% respectively. In patients with CD the sensitivity, specificity, PPV and NPV of ASCA IgG were as follows: 9.09%, 69.35%, 5% and 81.13% respectively.

DISSCUSION

pANCA and ASCA are serologic markers that have been proposed as tools to assist in diagnosing IBD, in differentiating UC from CD, in indeterminate colitis and in determining therapy and monitoring response to treatment. Elevated levels of serum pANCA in UC patients are believed to be caused by pANCA production in the colonic mucosa. ASCA is an antibody that react to a component of yeast commonly found in food. ASCA has been detected in serum of a majority of CD, but fewer UC patients. The origin of ASCA antibody is not clear. Besides IBD, ASCA is found in some other diseases, such as primary biliary cirrhosis,sclerosing cholangitis and spondyloarthropathy. In addition less than of 1%of normal blood donor are ASCA positive. (11) Although pANCA and ASCA are the most well studied serological markers in IBD, limited data have been reported from the Iranian population. We therefore sought to assess the utility of pANCA and ASCA as diagnostic markers in a central Iranian (Yazd) patients. These antibodies have been studied extensively in different countries and their results are different: In one study 206 unrelated patients (UC, n=152, CD, n=54), 60 patients with other GI diseases and 80 healthy controls were studied. Sera pANCA and ASCA titer were determined. The sensitivity, specificity, positive andnegative predictive values andpositive likelihood ratio of pANCA were calculated for differentiating UC from healthy controls (43.4%, 96.3%, 95.7%, 47% and 11.7% respectively) and ASCA for differentiating CD from healthy controls were 46.3%, 96.3%, 89.3%72.6% and 12.5% respectively. They concluded that pANCA and ASCA are useful in confirming the diagnosis of IBD and differentiating between UC and CD [12]. Anad *et al*. conducted a retrospective study to evaluate the diagnostic accuracy

of pANCA and ASCA as single agents and in combination for diagnosis of CD and UC including cases of indeterminate colitis. The authers concluded that this combination of serological markers provides generally high specificity but low sensitivity, especially in the CD [13]. A retrospectivestudy by Sabery and Bass evaluated the use of serologic markers as a screening tool compared with elevated ESR and anemia in patients referred to a GI clinic for suspected IBD. The authers concluded that the measurement of elevated ESR and hemoglobin value has a higher PPV and is more sensitive andmore specific than commercial serologic tests [14]. Reese *et al*. conducted a meta-analysis to assess the diagnostic precision of ASCA and pANCA in IBD. Sensitivity,specificityandlikelihood ratio were calculated for different test combinations for CD, UC andcompared with controls. The authers concluded that ASCA and ANCA testing are specific but not sensitive for CD and UC. They stated ASCA and ANCA testing may be useful for differentiating UC from CD in the pediatric population but this needs to be the subject of further research [15]. In one study the accuracy of pANCA andASCA in diagnosing UC and CD were evaluated. The sensitivity, specificity and PPV of combination of positive ASCA andnegative pANCA to diagnosis CD was 0,89.7% and 0 respectively and those of combination of positive pANCA and negative ASCA to diagnosis of UC was 20.7%, 64.7% and 33.3% respectively. They concluded that positivity of either ASCA or pANCA are not enough sensible to screen IBD but useful to diagnosis IBD [16]. Their results are somewhat similar to results identified in our study. A prospective multicenter study conducted by Joosens *et al*. evaluated the value of ANCA and ASCA to increase diagnostic accuracy in categorization indeterminate colitis. Because only 31 patients had a confirmed diagnosis andonly 21 of these patients were included in a evaluation of specificity andsensivity, it is difficult to draw conclusion regarding the accuracy of serological testing in the study [17]. Dubinsky coducted a prospective study of pediatric patients to determine if accuracy of diagnosing IBD versus functional chilhood disorder was improved by the use of pANCA and ASCA testing. The authers concluded that the incorporation of sequential noninvasive testing into a diagnostic strategy may avoid unnecessary and costly evaluation [18]. In presented study sensitivity, specificity, PPV and NPV of positive pANCA to diagnosis UC were 1.61%, 100%, 100% and 15.27%, those of positive ASCAIgA were 36.36%, 82.25%, 26.66% and 87.93% respectively andthose of ASCAIgG were 9.09%, 69.35%, 5% % 81.13% which are somewhat lower than other studies. The cause of these differences may be explained hat: 1-It has been suggested that pANCA and ASCA response may be influenced by several distinct genetic determinants and or environmental risk factor.Also age of diagnosis may be important [6, 19]. So different antibody response among different population is acceptable.

2-Our study was limited by small numbers in CD patients and more extended research is needed. In addition The American college of gastroenterology (updated in 2010) state that these tests are evolving to provide adjunctive support for the diagnosis of CD but are not sufficiently sensitive or specific be recommended for use as a screening tools. Patients with negative results would still need to undergo the standard diagnostic testing. Patients with a positive results would still need to undergo additional tests to distinguish CD from UC.Finally the clinical utility of these tests have not been established.

CONCLUSION

These tests have several limitation,therefore clinicians must be aware of the evidence on serologic markers, interpret them with caution and always correlate with the clinical picture.

ACKNOWLEDGEMENT

The authors declare that they have no conflict of interest.Special thanks to Dr Anticchi, Dr Falahzadeh and Mr Atashi for their assistance in this research.

REFERENCES

1. Bamias, G. and F. Cominelli, 2007. Imunopathogenesis of inflammatory bowel disease: Current concepts. Curr Opin Gastroenterol; 23: 365-369.
2. Duerr, R.H., 2003. Update on the genetics of inflammatory bowel disease. J. Clin Gastroenterol, 37: 358-367.
3. Papp, M., G.L. Norman, I. Altorjay and P.L. Lakatos, 2007. Utility of serological markers in inflammatory bowel diseases: Gadget or magic? World J. Gastroenterol, 13: 2028-2036.
4. Beaven, S.W. and M.T. Abreu, 2004. Biomarkers in inflammatory bowel disease. Curr Opin Gastroenterol, 20: 318-327.

5. Israeli, E., I. Grotto, B. Gilburd, R.D. Balicer, E. Goldin, A. Wiik, *et al.*, 2005. Anti-Saccharomyces cerevisiae and antineutrophil cytoplasmic antibodies as predictors of inflammatory bowel disease. Gut, 54: 1232-1236.
6. Nisihara, R.M., W.B. de Carvalho, S.R. Utiyama, H. Amarante and M.L. Baptista, 2010. Diagnostic role and clinical association of ASCA and ANCA in Brazilian patients with inflammatory bowel disease. Dig Dis Sci., 55: 2309-2315.
7. Quinton, J., B.D. Sendid, P. Reumaux, A. Duthilleul, B. Cortot, Grandbastien, *et al,* 1998. Anti-Saccharomyces cerevisiae mannan antibodies combined with antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease: Prevalence and diagnostic role. Gut, 42: 788-791.
8. Peeters, M., S. Joossens, S. Vermeire, R. Vlietinck, X. Bossuyt and P. Rutgeerts, 2001. Diagnostic value of anti-Saccharomyces cerevisiae and antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease. Am. J. Gastroenterol, 96: 730-734.
9. Sandborn, W.J., E.V. Jr Loftus, J.F. Colombel, K.A. Fleming, F. Seibold, HA Homburger, *et al*., 2001. Evaluation of serologic disease markers in a population-based cohort of patients with ulcerative colitis and Crohn's disease. Inflamm Bowel Dis., 7: 192-201.
10. Koutrobakis, I.E., E. Petinaki and I.A. Mouzas, 2001. Anti-Saccharomyces cerevisiae mannan antibodies and antineutrophil cytoplasmic autoantibodies in Greek patients with inflammatory bowel disease. Am J. Gastroenterol., 96: 449-454.
11. Muratori, P., L. Muratori, M. Guidi, S. Maccariello, G. Pappas, R. Ferrari, *et al.*, 2003. Anti-Saccharomyces cerevisiae antibodies (ASCA) and autoimmune liver diseases.Clin. Exp. Immunol., 132(3): 473-6.
12. Zhou, F., B. Xia, F. Wang, U.K. Shrestha, M. Chen, H. Wang, *et al*., 2010. The prevalence and diagnostic value of perinuclear antineutrophil cytoplasmic antibodies and anti-Saccharomyces cerevisiae antibodies in patients with inflammatory bowel disease in mainland China.Clin Chim Acta, 9;411(19-20): 1461-65.
13. Anand, V., A.S. Russell, R. Tsuyuki and R. Fedorak, 2008. Perinuclear antineutrophil cytoplasmic autoantibodies and anti-Saccharomyces cerevisiae antibodies as serological markers are not specific in the identification of Crohn's disease and ulcerative colitis. Can J. Gastroenterol., 22(1): 33-6.
14. Sabery, N. and D. Bass, 2007. Use of serologic markers as a screening tool in inflammatory bowel disease compared with elevated erythrocyte sedimentation rate and anemia. Pediatrics, 119(1): 193-9.
15. Reese, G.E., V.A. Constantinides, C. Simillis, A.W. Darzi, T.R. Orchard, V.W. Fazio *et al.*, 2006. Diagnostic precision of anti-Saccharomyces cerevisiae antibodies and perinuclear antineutrophil cytoplasmic antibodies in inflammatory bowel disease. Am J. Gastroenterol., 101(10): 2410-22.
16. Gao, X., J.P. Hu, Y. He, S.Y. Liao, S. Peng and MH. Chen, 2005. [Diagnostic role of anti-saccharomyces cerevisiae and antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease.]. Zhonghua Nei Ke Za Zhi., 44(6): 428-30.
17. Joosens, S., W. Reinisch, S. Vermeire, B. Sendid, D. Poulain, M. Peeters*, et al.*, 2002. The value of serologic markers in indeterminate colitis: a prospective follow-up study. Gastroenterology.; 122(5): 1242-47.
18. Dubinsky, M., J. Ofman, M. Urman, S. Targan and E. Seidman, 2001. Clinical utility of serodiagnostic testing in suspected pediatric inflammatory bowel disease. Am. J. Gastroenterol., 96(3): 758-65.
19. Markowitz, J., S. Kugathasan, M. Dubinsky, L. Mei, W. Crandall, N. LeLeiko, *et al*., 2009. Age of diagnosis influences serologic responses in children with Crohn's disease: a possible clue to etiology? Inflamm Bowel Dis., 15(5): 714-9.