The Usefulness of Angiotensin Converting Enzyme in the Differential Diagnosis of Crohn's Disease and Intestinal Tuberculosis

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Background : Since the pathologic findings of Crohn's disease (CD) and intestinal tuberculosis (IT) overlap to a large degree, the development of other biomarkers will be of great help for making the differential diagnosis of these 2 diseases. The aim of the present study is to examine the clinical efficacy of using the tissue angiotensin converting enzyme (ACE) assay in making the differential diagnosis between CD and IT.

Methods : Tissue specimens were obtained from 36 patients who were diagnosed with CD or IT by the colonoscopic biopsy, as well as by the clinical findings. The expression of tissue ACE was detected by immunohistochemical staining. The optimal cut-off value of the immunoreactive scoring (IRS) system we used to differentiate CD from IT was determined by analysis of the ROC curve and AUROC.

Results : Granuloma was present in 15 of 19 patients with CD (78.9%) and in 15 of 17 patients with IT (88.2%). ACE was present in the cytoplasm of the epithelioid cells in the granulomas from 13 of 15 patients with CD and in 14 of 15 patients with IT. The IRS scores of ACE were greater in the patients with CD than that of the patients with IT (8.07±4.38 vs. 4.13±2.47, respectively, p=0.006). In differentiating CD from IT, the AUROC curve for the IRS of ACE was 0.767 with a sensitivity of 66.7%, a specificity of 93.3% and the cut-off point was 7.5.

Conclusions : The results of our study suggest that the assessment of the tissue ACE expression can be helpful for making the differential diagnosis between CD and IT.

Key Words : Angiotensin converting enzyme, Crohn disease, Gastrointestinal tuberculosis, Granuloma

INTRODUCTION

In Korea, there are several problems to diagnose Crohn's disease because of the high incidence of intestinal tuberculosis and the 2 diseases have clinical, radiologic, endoscopic and histopathologic similarities[1-4]. Therefore, in many cases, a trial of anti-tuberculosis therapy may often be prescribed before the definite diagnosis of Crohn's disease is made[2-5].

PCR (polymerase chain reaction) is recently being increasingly used for making the diagnosis of tuberculosis. It has very high sensitivity and specificity for the diagnosis of pulmonary tuberculosis[6, 7]. However, there is scant evidence to show the usefulness of this assay for making the differential diagnosis of intestinal tuberculosis from Crohn's disease[8-11].

The serum levels of ACE (angiotensin converting enzyme) are elevated in a variety of granulomatous diseases, including

sarcoidosis, silicosis and military tuberculosis. It has generally been accepted that ACE has only a particular value to indicate the disease activity of sarcoidosis. However, there is still controversy about the diagnostic advantage of employing ACE for pulmonary tuberculosis and inflammatory bowel disease[12-15]. The suggested mechanism of the increasing serum ACE level is that ACE is secreted into circulation by cells of the macrophage-phagocytic system (epithelioid cells) within granulomas[16]. There are only a few studies showing the role of ACE in the tissue of the granulomatous inflammation of Crohn's disease (CD) and intestinal tuberculosis (IT).

The aim of the present study is to analyze the ACE levels in tissue that was obtained by colonoscopy in CD patients and IT patients, and to establish its clinical application in making the differential diagnosis between the two diseases.

MATERIALS AND METHODS

Subjects

We performed a retrospective analysis of 54 patients, who were diagnosed with Crohn's disease or intestinal tuberculosis by colonoscopic biopsy from May 1995 through June 2005. The diagnosis of CD or IT was established by considering all the factors of the clinical manifestations, and also the colonoscopic, radiologic and histopathologic findings. The diagnosis of CD was based on the clinical manifestations, the perianal lesions, the colonoscopic findings (longitudinal mucosal ulceration, fistula, cobblestone appearance, stricture and a normal area of mucosa between the inflammatory lesions), and the histopathologic findings (noncaseating granuloma or transmural inflammation). The diagnosis of IT was based on the clinical manifestations, the colonoscopic findings (circular ulceration at the ileocecal area and the absence of the typical CD findings), and the histopathologic findings (caseating granuloma, AFB (acid-fast bacilli) stain (+), culture (+) or PCR (+) without caseating granuloma). If any of these typical findings were not identified, then IT was diagnosed by the combined findings of pulmonary tuberculosis or if the patient displayed clinical improvement following a course of anti-tuberculosis medication. The exclusion criteria included incomplete medical records, an insufficient basis for diagnosis, damaged tissue slides and a failed differential diagnosis between the two diseases. Finally, after this exclusion was done, this study was performed on 19 patients with CD and 17 patients with IT.

Colonoscopic classification

The disease severity on the endoscopic examination was grouped into the following 4 grades by using the 'Simplified

Table 1. Simplified severity criteria for the colonoscopic findings of Crohn's disease

1: Quiescent – minor change in the vascular pattern
2: Mild – focal erythema
3: Moderate – aphthoid ulcer (<5 mm in size) within a 10 cm segment of colon
4: Severe – ulcers (>5 mm in size), stricture, fistula, extensive bleeding

Severity Criteria' (Table 1)[17]. The colonoscopic type of disease was categorized into 3 classes (1: small bowel, 2: ileocolic, 3: colic).

Histopathologic diagnosis and immunohistochemical staining

The hematoxylin-eosin stained slides were obtained from the archives of the Department of Pathology, Bundang CHA General Hospital, and they were reviewed by a pathologist (H.K.), who was without knowledge of the previous diagnosis. Those cases without granulomas and evidence of either Crohn's disease or tuberculosis were excluded. New hematoxylin-eosin stained slides were made from a 4 μm -thick, 10% formalin fixed, paraffin embedded tissue section. The histologic features were then re-examined. For immuno-histochemical staining, 4 μm-thick serial sections were taken from the blocks that bore characteristic features of each case. They were fixed on special coated slides (Starfrost) for 90 seconds at 58℃. The tissue sections were immunohisto-chemically stained by the streptavidin-biotin protocol. After deparaffinization with xylene 3 times for 10 minutes each time, and then they were rehydrated in a graded series of alcohol solutions (100% alcohol for 3 times (10 seconds each), and then 95%-80%-70% alcohol for 10 seconds each time). To block the endogenous peroxidase, they were reacted in 3% hydrogen peroxide for 10 minutes. For antigen retrieval, they were incubated in citrate buffer in a microwave oven for 20 minutes. After washing in phosphate-buffered saline (PBS) buffer for 10 minutes, the tissue sections were incubated at room temperature for 60 minutes with the primary antibody, the ACE mouse monoclonal antibody (CD143, Novocastra Laboratories Ltd, United Kingdom, diluted 1:1000). All the slides were washed in PBS buffer for 10 minutes and the secondary antibody was applied with using a Dako Envision Kit (DAKO, Denmark). After washing in PBS buffer for 10 minutes, the peroxidase activity was stained by using the enzyme substrate 3,3'-diaminobenzidine tetrahydrochloride (DAB) for 1 minute, and then the slides were counterstained with Mayer's hematoxyin for 10 seconds. To reduce any bias or differences in the staining conditions, all the slides were randomly placed without knowledge of the diagnosis, and they were stained at the
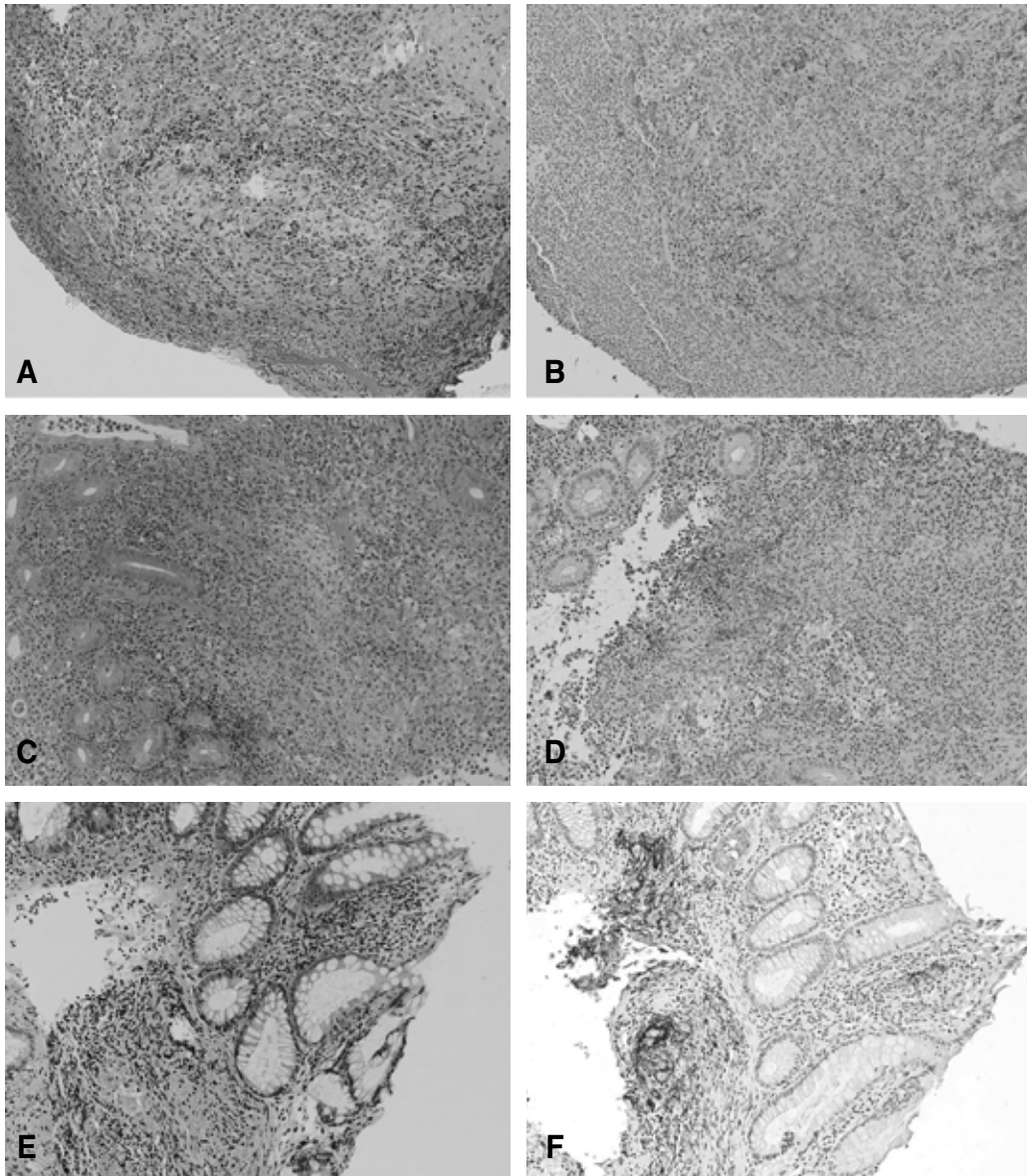
Table 2. Immunoreactive scoring (IRS) system for the grade of the ACE expression in the granuloma of the immunohistochemical stained tissue slide

IRS = staining intensity (SI) x percentage of positive cells (PP) in the granuloma
SI: 0 (negative), 1 (weak), 2 (moderate), 3 (strong)
PP: 0 (negative), 1 (<10%), 2 (10~50%), 3 (50~80%), 4 (>80%)
Maximum IRS = 3×4 = 12

same time by the same pathology technician.

Analysis of the ACE expression

The grade of the ACE expression in the granulomas of the tissue slides was assessed by the immunoreactive scoring (IRS) system (Table 2)[18]. All the tissue slides were randomly analyzedby a certain pathologist at one time to minimize measurement errors, and then the slides were re−examined for verification.

Figure 1. The expression of tissue ACE was detected by immunohistochemical staining with mouse monoclonal antibody in paraffin wax embedded tissue slides, and the grade of expression was assessed by immunoreactive scoring (IRS) system.
(A)&(B) presented 4 (1 (staining intensity) x 4 (% of positive cells in granuloma)) point of immunoreactive scoring (IRS), (C)&(D) presented 8 (2x4) point, and (E)&(F) presented 9 (3×3) point. (Hematoxylin and eosin in a, c, e, x 200, CD 143 stain in b, d, f, ×200)

Table 3. Characteristics fo the Patients with Crohn's Disease and Intestinal Tuberculosis

| No. | Diagnosis | Sex | Age | Immunoreactive scoring (IRS) system[1] | | | Granuloma | AFB | Colonoscopic finding | |
				Staining intensity[2]	% of positive cell[3]	IRS			Severity[4]	Type[5]
1	Tuberculosis	F	42	0	0	0	+	+	3	3
2	Tuberculosis	M	75	1	2	2	+	−	3	2
3	Tuberculosis	F	13	1	2	2	+	−	4	3
4	Tuberculosis	F	27	1	3	3	+	−	3	2
5	Tuberculosis	M	47	1	4	4	+	−	4	2
6	Tuberculosis	M	28	2	2	4	+	+	4	3
7	Tuberculosis	M	28	2	3	6	+	−	3	2
8	Tuberculosis	F	25	2	2	4	+	−	4	2
9	Tuberculosis	F	20	2	3	6	+	−	4	2
10	Tuberculosis	F	35	3	3	9	+	+	3	2
11	Tuberculosis	F	28	3	2	6	+	−	2	2
12	Tuberculosis	M	42	3	2	6	+	−	4	1
13	Tuberculosis	F	36	3	2	6	+	+	4	2
14	Tuberculosis	M	43	0	0	0	+	−	4	2
15	Tuberculosis	F	53	2	2	4	+	+	4	2
16	Crohn	F	42	1	1	1	+	.	4	2
17	Crohn	M	38	1	2	2	+	.	4	2
18	Crohn	F	19	2	2	4	+	.	4	3
19	Crohn	F	47	3	4	12	+	.	2	2
20	Crohn	M	34	3	3	9	+	.	4	2
21	Crohn	M	25	3	4	12	+	.	3	3
22	Crohn	F	27	3	3	9	+	.	3	3
23	Crohn	M	16	3	3	9	+	.	4	2
24	Crohn	F	35	3	2	6	+	.	4	2
25	Crohn	F	22	3	4	12	+	−	3	2
26	Crohn	F	26	3	4	12	+	.	3	2
27	Crohn	F	18	3	3	9	+	.	4	2
28	Crohn	M	17	3	4	12	+	.	3	2
29	Crohn	M	24	3	4	12	+	.	4	2
30	Crohn	M	18	0	0	0	+	−	4	2

[1]RS = staining intensity (SI) x percentage of positive cells (PP) in granuloma;
[2]SI : 0 (negative), 1 (weak), 2 (moderate), 3 (strong);
[3]PP : 0 (negative), 1 (<10%), 2 (10~50%), 3 (50~80%), 4 (>80%);
[4]Colonoscopic severity : 1 (quiescent), 2 (mild), 3 (moderate), 4 (severe);
[5]Colonoscopic type : 1 (small bowel), 2 (ileocolic), 3 (colic)

Table 4. Comparison of Crohn's disease and Intestinal Tuberculosis with Granuloma

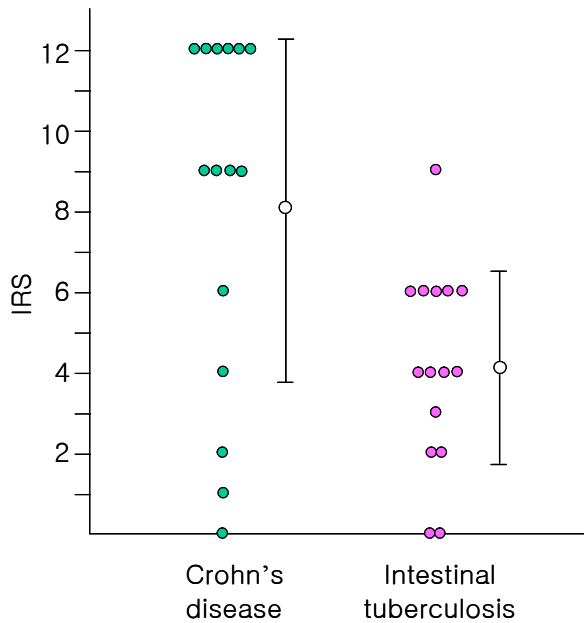|	Crohn's disease (n=15)	Intestinal tuberculosis (n=15)	P-value[1]
Age (mean±S.D.)	27.2±9.8	36.1±15.2	0.044
Sex (Male : Female)	7 : 8	6 : 9	1.000
Immunoreactive scoring (mean±S.D.)	8.07±4.38	4.13±2.47	0.006

S.D., Standard deviation
[1]Mann-Whitney test, Fisher's exact test and Student t-test

Statistical analysis

All the data was analyzed by using SPSS ver. 11.0 for Windows (SPSS Inc, Chicago, IL, USA). The results were represented by means±S.D. (*standard deviation*). The Mann-Whitney test, Fisher's exact test and Student's t-test were used for analysis of age, gender and IRS, respectively. p-values less than 0.05 were considered statistically significant. To determine the optimal cut-off value of the IRS for the differential diagnosis between CD and IT, the Receiver Operating Characteristic (ROC) curve and the area under the

Figure 2. Distribution of immunoreactive scoring (IRS) for ACE in the Crohn's disease group and the intestinal tuberculosis group (means±SD).

Table 5. The Optimal Cut off Value for Immunoreactive Scoring (IRS) in the Differerential Diagnosis between Crohn's Disease and Intestinal Tuberculosis.

| IRS value	Sensitivity	1-Specificity
−1.00	1.000	1.000
0.50	0.933	0.867
1.50	0.867	0.867
2.50	0.800	0.733
3.50	0.800	0.667
5.00	0.733	0.400
7.50	0.667	0.067
10.50	0.400	0.000
13.00	0.000	0.000

IRS, Immunoreactive scoring

ROC (AUROC) curves were applied.

RESULTS

Clinical characteristics

Granulomas were presented in 15 of 19 patients with CD (78.9%) and in 15 of 17 patients with IT (88.2%) (Table 3). The mean age of the granuloma-positive CD group was younger than that of the granuloma-positive IT group (27.20±9.80 vs. 36.13±15.19, respectively, p=0.044), but there was no statistical significant difference in the gender ratio (p=1.000) (Table 4).
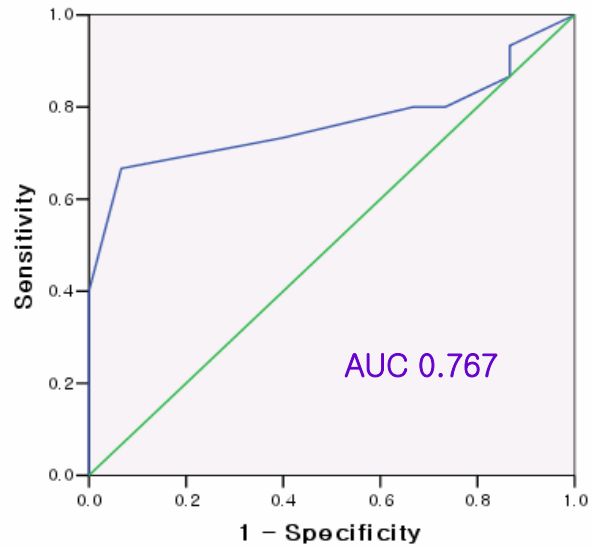
Figure 3. The ROC curve and the AUROC were analyzed for immunoreactive scoring (IRS) in making the differerential diagnosis between Crohn's disease and intestinal tuberculosis.

Immunoreactive scoring (IRS) system

ACE was presented in the cytoplasm of the epithelioid cells in the granulomas from 13 of the 15 clinical CD patients (93.3%) and from 14 of the 15 clinical IT patients (86.7%)(Figure 1). The IRS score of ACE was greater in the patients with CD than that of the patients with IT (8.07±4.38 vs. 4.13±2.47, respectively, p=0.006) (Figure 2). There was no correlation between IRS and the colonoscopic determined disease severity or the colonoscopic determined disease type.

ROC curve and AUROC

The AUROC curve for IRS, which means a higher IRS score indicates the higher possibility of CD, was 0.767. If the IRS score is more than 7.5, then the sensitivity and the specificity are 66.7% and 93.3%, respectively (Figure 3, Table 5).

DISCUSSION

CD has traditionally been a very rare disease in Korea, and some cases were occasionally reported before the 90's. However, the number of reports has been increasing since the 90's[4].

As mentioned earlier, there are several problems for making the diagnosis of CD because of high incidence of IT in Korea. To find a solution for making the differential diagnosis, clinical, radiological, colonoscopic and histopathological methods or parameters have been studied, but no satisfactory method has yet been proposed. Under these circumstances, as a new

biomarker, the PCR method for detecting tuberculosis was introduced, and it has drawn much attention from clinicians and pathologists since it was applied in the clinical setting[8-11]. Contrary to diagnosing pulmonary tuberculosis, it has shown a different result for the diagnosis of IT; the specificity is very high (95~100%) but the sensitivity is very low (9.8~64.1%). Therefore, it is only recommended as an additional diagnostic tool.

There are several reports showing the elevated serum levels of ACE in granulomatous diseases. This can be explained by the fact that cells in the macrophage-phagocytic system (epithelioid cells) within the granulomas secret ACE into circulation[16]. Therefore, the serum ACE level reflects the total amount of granulomas, but the level may be influenced by the presence of specific or nonspecific ACE inhibitors such as albumin and its fragments, fibrinolytic fragments, insulin and the beta-chain of insulin[19-21].

Co-measurement of the serum ACE level and its activity can display significant results in a variety of granulomatous diseases, including sarcoidosis and military tuberculosis[12]. It has been generally accepted that the ACE level is particularly related with the disease activity of sarcoidosis. However, there is no consistent data that's been reported on the relation between ACE and pulmonary tuberculosis or inflammatory bowel disease[12-15]. The suggested explanation for this difference is that ACE polymorphism may influence the serum level of ACE, and these ACE levels may be one of the factors for the pathogenesis of inflammatory bowel disease[14].

With these results, we hypothesize that differences of the serum ACE level and polymorphism of the ACE gene may exist between CD and IT, and these difference can be utilized for making the differential diagnosis. Pertschuk LP, et al.[22] reported that detection of tissue ACE in sarcoid granulomas could be done via immunohistochemistry, and this might be used as a diagnostic method. Jaszewski R, et al.[23] measured the colonic mucosal levels of angiotensin I and II in the endoscopic biopsy samples obtained from patients with inflammatory bowel disease. He reported that the levels of the Crohn's colitis group were significantly increased compared to the ulcerative colitis group or normal subjects, and these levels were well correlated with the degree of macroscopic inflammation.

We designed our study's method by examining the previous reports. Immunohistochemical staining was performed to examine the expression of tissue ACE. Among the monoclonal antibodies used for the immunohistochemical staining for ACE, CD 143 (Novocastra Laboratories Ltd, United Kingdom) was chosen because it has the greatest value for signaling the ACE expression in granulomas. The grade of the ACE expression in the granulomas of the tissue slides was rated by the immunoreactive scoring (IRS) system[18]. As mentioned in the results, the degree of the ACE expression was different between CD and IT. A higher degree indicates a higher possibility of CD. We believe that this method can be a useful diagnostic tool to differentiate CD and IT.

The limitation of this method was that the expression of ACE totally depends on pathologist's judgment. The interpretation could be influenced by many factors. For example, there were some granulomas without any expression, and the range of the standard deviation of the IRS system was wide. In contrast to the tissue expression of ACE, the serum ACE level in the CD patients was lower than that of the healthy group in the previous published reports. We had a few problems that the serum level of ACE was inappropriately correlated with the tissue level, and the degree of the ACE expression didn't match with the colonoscopic determined severity.

To solve these problems, further prospective controlled studies with many patients are required, and the degree of the tissue ACE expression or the tissue ACE level should be compared with the serum ACE level. To determine the mechanism of the difference of ACE levels between CD and IT patients, the influence of ACE gene polymorphism[14], the possibility of a different macrophage pathway or a different activation pathway[24], and the possibility of involvement of delayed hypersensitivity or the complex immune effects of cytokines from many different immune cells[25] must be investigated. We believe that the usefulness of assessing ACE will depend on developing simpler clinical methods for making the differential diagnosis if these problems are to be solved.

In summary, the results of our study show that the degree of ACE expression in CD patients is significantly higher than that in the IT patients. We anticipate that the assessment of the tissue ACE expression will be helpful for making the differential diagnosis between CD and IT.

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