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Value of Anti-Kp 90 Immunoglobulin A (IgA) Antibodies in Diagnosis of Active Tuberculosis: A Preliminary Report

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Abstract: The lack of a rapid and reliable method for diagnosis of active tuberculosis necessitated the development of new laboratory techniques. Diagnostic value of a recently developed ELISA for the detection of IgA antibodies to Kp 90 antigen of *Mycobacterium tuberculosis* was evaluated in this study and the results were compared with the conventional methods and polymerase chain reaction (PCR). Fifty six sera and body fluid (pleural, peritoneal, pericardial) samples from 23 patients with active tuberculosis and 26 control subjects were included in the study. Out of 28 samples obtained from the patients, 22 (79%) yielded positive result for IgA. Comparison of

all positive results implied high level of agreement between IgA measurement and other methods; 9/10 direct examination, 6/7 culture and 18/21 PCR positive samples were found to be IgA positive. Body fluids were as efficient as sera in terms of specimen selection; 14/18 (78%) body fluid samples yielded IgA positivity. IgA detection against Kp 90 antigen by ELISA appears as a rapid and reliable procedure for diagnosis of active tuberculosis. However, evaluation of the test in terms of sensitivity and specificity awaits further studies.

Key Words: Tuberculosis, Immunoglobulin A, ELISA, PCR

Introduction

The prevalence of *Mycobacterium tuberculosis* infections tends to increase in some countries as well as in Turkey in recent years. Diagnosis of tuberculosis, particularly in early stages, is difficult since the clinical features are nonspecific and the conventional diagnostic procedures have low sensitivity and/or are time consuming. The assessment of clinical, microbiological, radiological and pathological findings is required for diagnostic approach.

Since a fast, sensitive and specific method of diagnosis is still lacking, efforts are still being carried out to develop new laboratory techniques to overcome this limitation. Apart from the routine diagnostic procedures-tuberculosis culture, direct examinations of smears and skin test-, polymerase chain reaction (PCR) and serodiagnosis are recently applied techniques the reliability of which are not well established yet (1,2). The present study was undertaken to evaluate the value of detecting anti-Kp 90 immunoglobulin A (IgA) antibodies by a recently developed enzyme linked immunosorbent assay (ELISA) in diagnosis of active tuberculosis.

Materials and Methods

Patients. Twenty three patients (15 males and 8 females) with a mean age of 47.3 years (range, 17 to 72 years) who were diagnosed active tuberculosis in Hacettepe University Hospital, Ankara between January 1994 and March 1995 were included in the study.

The diagnosis of active tuberculosis was established if one or more of the following criteria exist; I) Isolation of *M. tuberculosis* on Middlebrook 7H10 medium and/or demonstration of acid fast bacilli on direct examination of the clinical specimens, II) Granuloma formation with caseification on pathological examination, III) Clinical and laboratory findings suggestive of tuberculosis and clinical response to anti-tuberculosis therapy during follow up of at least 3 months.

Control group. Twenty six subjects (15 males and 11 females) with a mean age of 46.8 years (range, 17 to 84 years) who had no mycobacterial disease were considered as the control group.

IgA measurement. Sera and body fluids obtained before initiation of antituberculosis chemotherapy were examined for Ig A antibodies to mycobacterial Kp 90 immunocrossreactive antigenic compound by ELISA. Di-

agnostic kits were supplied by Kreatech (Holland) and the test was performed according to the instructions of the manufacturer. Results were interpreted by measuring the optical density at 450 nm wave length spectrophotometrically. The number and origin of clinical specimens included in the test are shown in Table 1.

Table 1. Number and origin of the specimens collected for IgA measurement by ELISA.

Specimen	Patients (n)	Controls (n)
Serum	10	10
Pleural fluid	12	11
Peritoneal fluid	5	4
Pericardial fluid	1	3
TOTAL	28	28

Other laboratory investigations.

a) Direct examination of smears was performed for clinical specimens other than sera by using Ziehl-Neelsen (ZN) staining procedure.

b) Culture for *M. tuberculosis* was done on Middlebrook 7H10 medium.

c) PCR: After 24 hrs-55°C incubation of sputum, body fluids and bone marrow aspirate samples in digestion buffer (100 mM NaCl, 10 mM Tris-HCl pH 7.5, 25 mM EDTA, 0.5 mg/ml proteinase-K) phenol-chloroform extraction and ethanol precipitation were performed and prepared DNA was used for amplification. IS6110 specific primer set was used for amplification, 123 base pair products were analyzed by agarose gel electrophoresis and ethidium bromide staining (3).

The laboratory diagnosis of tuberculosis was accomplished if one or more of the aforementioned methods (direct examination, culture, PCR, IgA) yields a positive result.

Results

The number and ratio of direct examination, culture, PCR and IgA positive results obtained for the study population is indicated in Table 2.

Table 2. Microbiological test results interpreted as positive for *M. tuberculosis*.

Laboratory test (+)	Patients n=23 (%)	Controls n=26 (%)
Direct examination	10 (44)	0
Culture	7 (30)	0
PCR	21 (91)	1 (4)
IgA	18 (78)	4 (15)

IgA measurement yielded positive result for 22 (79%) of 28 clinical specimens obtained from tuberculosis patients. The comparison of all positive results implied high level of agreement between IgA measurement and other methods; 9/10 ZN, 6/7 culture and 18/21 PCR positive samples were found to be IgA positive. Out of 10 sera and 18 body fluids obtained from the patients, 8(80%) and 14 (78%) were found to be IgA positive respectively.

Discussion

The requirement for a reliable diagnostic test to detect *M. tuberculosis* resulted in the development of new laboratory procedures. Different antigens of *M. tuberculosis*, namely antigen 5, purified mycobacterial glycolipids, Calmette-Guerin bacillus (BCG) and antigen 60 (A60) were used to develop ELISA so far to confirm a tuberculosis etiology by antibody detection. The diagnostic value of anti A60 IgM, IgG and IgA antibodies was investigated in patients with pulmonary and extrapulmonary tuberculosis in previous studies (1,4-7).

Measurement of IgM antibodies facilitates the diagnosis of current infection or reactivation; IgG antibodies on the other hand cannot differentiate patients with active tuberculosis from those who had tuberculosis in the preceding 2 years (1). In one recent report, anti Kp 90 IgA antibodies were found to be positive in sera of 13 of 15 proven active tuberculosis patients (8). Because of its secretory function, detection of IgA in body fluids may be a valuable diagnostic clue for *M. tuberculosis* infections as well as monitorization of antituberculosis chemotherapy.

Our study was undertaken to evaluate the diagnostic value of a recently manufactured commercially available ELISA using Kp 90 antigen for detection of

IgA antibodies. The results indicated that IgA detection in sera and body fluids may be an appropriate alternative test in diagnosis of active tuberculosis. Although detection of IgA antibodies in 15% of control subjects suggests limited value of evaluation of the test, the positivity of IgA and other methods were found to be well correlated. The results of the study also implies that, in terms of specimen selection, body fluids are as efficient as sera for IgA detection. In con-

clusion, IgA detection against Kp 90 antigen by ELISA appears to be a rapid and promising procedure which can facilitate the diagnosis of active tuberculosis. Since the sample size of the present study is insufficient to evaluate the test in terms of sensitivity and specificity, further studies are in progress to establish the value of IgA detection in diagnosis of active tuberculosis and monitorization of therapy.

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