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The Comparison of Adenosine Deaminase Activity Values With Polymerase Chain Reaction Results in Patients With Tuberculosis

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Three methods in the diagnosis and treatment of tuberculosis have been compared in this study. Serum adenosine deaminase activities of patients with tuberculosis was compared with those of control groups with (+) and (–) PPD (purified protein derivative) results and were found to be higher than the controls. Within the controls the PPD (+) group displayed higher adenosine deaminase activities in comparison to the PPD (–) group. All patients had growth of B. Tuberculosis in the culture medium and all but one had positive polymerase chain reaction (PCR) results. Control patients were negative for culture and PCR. The sensitivity of ADA (adenosine deaminase) assay was 91.7% and specificity was 94.5%, whereas PCR had a sensitivity of 95.8% and a specificity of 100%. The ADA assay may be used in adjunction with other methods in the follow-up of tuberculosis with high sensitivity, specificity, and ease in applicability and specimen collection. J. Clin. Lab. Anal. 13:209–212, 1999.

Key words: tuberculosis; serum; adenosine deaminase; acid-fast stain; PCR; human; PPD

INTRODUCTION

Adenosine deaminase (ADA, EC3.5.4.4) is the enzyme that catalyzes irreversible hydrolytic deamination of adenosine and deoxyadenosine to inosine and deoxyinosine (1). Studies have demonstrated the existence of ADA in almost all mammalian tissues. It has also been shown that there is a relation between cellular immune response, lymphoreticular cell activity, and ADA activity (2,3). ADA activity has been shown to be high in rapidly prolpherating cells. Especially in T lymphocytes, ADA activity has been determined to be approximately ten times higher than in erythyrocytes (4). ADA activity values have importance in T-lymphocyte differentiation and prolipheration (5). There is evidence that ADA activity increases in pleural effusions of patients with tuberculosis with a sensitivity of 100% and a specificity within the range of 85–93% (6,7). The increase of ADA activity in patients with tuberculosis may indicate the cellular immune response and T-lymphocyte activation in this disease (6).

Tuberculosis is still an important public health issue. Timely diagnosis, treatment, and follow-up deserves attention of the medical community. For this purpose, several laboratory tests are employed in practice. Two of the widely accepted methods in the diagnosis of tuberculosis are cultural growth of the bacteria and amplification of microbial DNA via polymerized chain reaction (PCR). Cultural growth of the bacteria is accepted as the "gold standard" and is used as a reference to assess the value of new methods suggested.

The aim of this study was to compare ADA activities and PCR results with culture studies in patient and control groups and elucidate the sensitivity and specificity of the methods for the diagnosis of tuberculosis.

MATERIALS AND METHODS

The control group consisted of 35 individuals: 15 PPD (–) and 20 PPD (+) people, and 24 patients with pulmonary tuberculosis were assayed for ADA activity in serum via the Galanti and Guisti method (8). This method is based on the monitorization of the blue-colored indophenol derived from ammonium, sodium hypochloride, and phenol solution in alkali medium catalyzed by ADA. All patients were diagnosed with tuberculosis. Patients selected to be included in the study were diagnosed by physical examination and radiological inspection (PA lung X-ray and lung tomography). The differential diagnosis was made by culture studies. Afterwards, serum ADA and PCR analysis in sputum were done.

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In addition DNA amplification product was detected on agarose gel electrophoresis in the patient group. The control group was selected from those who neither smoked nor drank alcohol.

For the PCR analysis the method described by Kocagöz et al. was used (9). Briefly, the sputum samples from patients were first decontaminated by 4% NaOH and homogenized followed by acid neutralization and microscopy (acid-fast staining, Ziehl–Neelsen method). The remnant was fractionated into two parts. The first fraction was inoculated on Loewenstein-Jensen medium in a total volume of 0.2 ml for cultural studies and incubated for 6 weeks at 37°C. The second fraction was used for PCR. The fraction to be used for PCR was first purified from inactivator chemical (e.g., stain), then bacterial DNA was isolated by simple boiling as described before. The *Mycobacterium tuberculosis* (*M. tuberculosis* complex) -specific 245-bps DNA described as 15986 (insertion sequence 986) was amplified by the commercial primers pp 2000 mTUB (Kreatech Diagnostics, Netherlands).

The 15 PPD (–) individuals, 9 men and 6 women, had an age distribution of 37.8 7.4 years. The 20 PPD (+) individuals, 9 men and 11 women, had an age distribution of 40 8.7. Among the patient group consisting of 10 men and 14 women the age distribution was 37.5 9.4.

RESULTS

Within the PPD (–) group, serum ADA activity had a distribution of 13.3 4.2 U/L with a maximum of 22 U/L and a minimum of 5.38 U/L. The PPD (+) group had a mean serum ADA activity of 22.2 5.4 U/L with a minimum of 10.8 U/L and a maximum of 31.8 U/L. All control group individuals, including PPD (+) and (–), had negative acid-fast stain and PCR results. The serum ADA activity in tuberculosis patients was 47.9 2.0 U/L with a minimum of 27.3 and a maximum of 93.2 U/L. All patients were positive for acid-fast stain and PPD results except for one with a negative PPD. The ADA activity values for the control and the patient groups are demonstrated in Table 1. The difference between means were tested by student's *t*-test and, using acid-fast stain as the standard, the chi square test results showed that ADA assay had a sensitivity of 91.7% and a specificity of 94.5%, whereas PCR had a sensitivity of 95.8% and a specificity of 100%.

TABLE 1. ADA Activities in the Sera of Control and Patient Groups[a]

Cases	Mean ± S.D.
A (n = 15) U/L	13.4 ± 4.2
B (n = 20) U/L	22.3 ± 5.4
C (n = 24) U/L	47.9 ± 19.8
Student's *t*-test	
A–B	$P < 0.005$
A–C	$P < 0.0005$
B–C	$P < 0.0005$

[a]A, PPD (–) control group; B, PPD (+) control group; C, patient group.

DISCUSSION

Tuberculosis is an important health issue requiring diagnostic accuracy and development of better methodologies for follow-up and treatment. Microscopic studies sometimes are not satisfactory for diagnosing the bacterial strains. And, to obtain a positive culture, a certain concentration of bacteria is required and the growth of mycobaceria in culture is a time-consuming procedure. PCR methodologies that shorten the detection period and increase the sensitivity are also gaining wide acceptance (9–14); however due to a high number of cells in clinical specimens like blood and pleura, and the difficulties in purification of DNA, there is no consensus PCR technique that maximizes the sensitivity.

Typically the reported sensitivity ranges between 90.6 and 95.1 for PCR, 87.5 and 100 for culture, 53.7 and 99.8 for staining techniques and 91.7 and 94.7 for ADA (11,13,14). This value has been reported to vary between 8 and 69% for culture studies. In a study by Devallais et al., both sensitivity and specificity of PCR was reported as 100, 80, and 100%, respectively, for cultures (15).

Adenosine deaminase activities have also been investigated in different patient groups with tuberculosis for diagnosis and follow-up by several groups (16–19).

ADA enzyme catalyzes the deamination of adenosine and deoxyadenosine. The ADA_1 isoform has been found to be high in lymphocytes and monocytes and the ADA^2 isoform has higher activity in monocytes. There is a well-established relationship between Adenosine deaminase (ADA) activity and T-lymphocyte maturation, proliferation, and differentiation (2,3,5). Especially in situations where there is stimulation of cellular immune response detected as T-lymphocyte proliferation and macrophage activation, ADA activities have been found to be increased (20). On the contrary, immune system defects displaying with numerical and functional defects of peripheral T-lymphocytes have been found to be associated with decreased ADA activity (5,21).

Existing literature also supplies evidence of the increasing T-lymphocyte activity in patients with tuberculosis (22,23). Thus the simultaneous increase in ADA activity with increased production and activity of T-lymphocytes is important for marking out the cellular immune response in tuberculosis. Thus, the increase in T-lymphocytes may be used in conjunction with increased ADA activities as a better marker (24).

On the other hand, increased ADA activities have been observed in diseases like brucella, ricketsia, and infectious mononucleosis where there is increased ADA activity (8). ADA activities have been reported to be increased in a variety of malignant conditions, too (25–27). However none of these increases are specific. This fact may underestimate the diagnostic power of ADA. The better differentiation of pa-

tient groups in future studies will devour the question of disease sensitivity and specificity in terms of differential diagnosis. In our study, patients have been included and grouped with consideration of physical examination and radiological investigation (X-ray and CT of the lung). Patients were included in the study for ADA and PCR tests after detected positivity of culture studies for differential diagnosis. The aim of the study is to observe the efficacy of these two laboratory methods in the diagnosis.

Studies have provided evidence that patients with tuberculosis have increased ADA activity in pleural fluid, cerebrospinal fluid, plasma, and serum (6,22,24,28,29).

In this study, taking acid-fast stain positive and negative patients as the standard, serum ADA and PCR values were compared for the two control groups. ADA activity was found to be higher in the patient group when compared to the PPD positive and negative control group. In addition, the PPD positive control group was found to have higher ADA activities compared to the PPD negative control group. This may be attributed to the increased T-lymphocyte, monocyte activation. According to other studies (23,28), the activated T-lymphocytes and monocytes may have an increased ADA activity in PPD (+) patients than in PPD (–) patients. This study provides an explanation to the increased enzyme activities in PPD (+) patients observed. Our values for ADA in serum are lower than the previously reported values for ADA in tuberculosis patients' pleural and peritoneal effusions.

In this study, the specificity was 100% and sensitivity was 95.8% for PCR. These values are corroborated by the values obtained by Devallois et al. The specificity was 94% and the sensitivity was 91.7% for ADA. Voight et al. reported these values to be 100% and 96% for the ascites fluid in peritoneal tuberculosis. Serum ADA values have also been reported to be of diagnostic significance in the same study.

The demonstration of supportive results for pleural and peritoneal fluids in serum for ADA, suggests that serum is convenient for obtaining biological material for the estimation of ADA as a fast method for the monitoring of tuberculosis.

We believe that ADA activity is valuable to the diagnosis and follow-up of tuberculosis in conjunction with culture and PCR. The next step will be to determine the isoforms of ADA dominant in sera, pleural, peritoneal fluid, and cerebrospinal fluid of patients with tuberculosis.

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