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Diagnostic value of serum adenosine deaminase levels in sputum smear negative pulmonary tuberculosis patients in Nepalese population

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ABSTRACT

Objective: To assess the levels of adenosine deaminase (ADA) in serum in patients with sputum smear negative pulmonary tuberculosis (SNPTB) and to compare it with serum ADA levels in patients with non-tuberculous pulmonary disease - chronic obstructive pulmonary disease (COPD) and with healthy control group and to explore its validity as a diagnostic marker in serum in SNPTB patients. **Methods:** Three groups of study populations were made. Group I: SNPTB - 142 cases, Group II: non-tubercular pulmonary disease - COPD - 40 cases, Group III: healthy controls - 80 cases. Serum samples were collected and ADA assay was done by the method of Guisti and Galanti. **Results:** ADA levels (Mean±SD, U/L) in the three groups were as follows: Group I: 42.26±21.22, Group II: 23.31±8.22, Group III: 18.88±6.67. Difference between Group I and Group III was statistically significant ($P < 0.0001$). The test showed a high specificity 91.25% (95% confidence interval - CI 83.00 - 95.7) and a sensitivity of 83.10% (95% CI 76.08-88.37) in Group I. Positive predictive value, negative predictive value, positive likelihood ratio, negative likelihood ratio and accuracy in Group I were 94.00%, 69.52%, 9.49, 0.18 and 82.43% respectively. **Conclusions:** Overall assessment of the use of serum ADA levels as a diagnostic biochemical marker in smear-negative pulmonary tuberculosis patients showed promising results. Studies with a larger population group are required to validate its use as a routine diagnostic test in these cases.

1. Introduction

Definitive laboratory diagnosis and confirmation of sputum smear-negative pulmonary tuberculosis (SNPTB) poses a major challenge in the management and control of active pulmonary tuberculosis (TB) in clinical practice. It is estimated that there are 1.22 cases of SNPTB and extra-pulmonary TB for every case of smear-positive TB in developing countries[1]. In resource-poor settings, smear negative TB is difficult to diagnose and also difficult to exclude, especially in HIV infected patients[2]. The sensitivity of acid-fast bacilli (AFB) staining result is known to be poor varying between 30% and 70% depending on a number of factors relating to how the test is implemented[3,4]. Thus, nearly half of all cases of pulmonary TB are smear - negative, which means that the overall disease burden is substantial and is associated with treatment delay and hospitalisation[5]. Other tests for tubercle bacilli such as the culture, serology and the newer nucleic acid amplification are either time-consuming, lack sensitivity and specificity or are technology - intensive and expensive as well, thus limiting their usefulness and access to practical applicability especially in developing countries with scarce resources. Thus, the existing pipeline is limited for SNPTB, childhood TB and accurate prediction of reactivation of latent TB[6]. Finding a laboratory test for SNPTB cases, that is simple, easy-to-perform, rapid, reliable and inexpensive is an urgency and efforts to improve the quality of existing diagnostic methods are necessary[7].

Human adenosine deaminase (ADA ; EC 3.5.4.4; an enzyme of purine catabolism) activity has been found to be increased in various diseases such as tuberculosis[8,9], HIV, typhoid, infectious mononucleosis and certain malignancies especially those of hemopoietic origin[10-12]. ADA assay in various body fluids had established its usefulness in the laboratory diagnosis of extrapulmonary TB (such as meningeal[13], pleural[14], peritoneal and pericardial TB)[15-17], smear-positive TB and SNPTB[18-21]. Studies also showed a decrease of serum ADA activity after treatment

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in TB patients and could be of significant help to evaluate the response to the therapy and drug resistance levels[22,23]. Reports evaluating ADA role in laboratory diagnosis of SNPTB are lacking in Nepal. This study was designed to evaluate the role of serum ADA levels especially in the laboratory diagnosis of SNPTB patients in Nepalese population.

2. Materials and Methods

2.1. Setting

The prospective study was conducted at Department of Pathology, Bir hospital (National Academy of Medical Sciences, NAMS) and Sankata Pathology Laboratory, New Road, Kathmandu, Nepal between January 2008 and December 2008. Bir hospital (NAMS) is the oldest medical institute and tertiary referral center in the country. Informed consent was obtained prior to inclusion in the study. The research and ethical review committee of the Bir hospital (NAMS) approved the study.

2.2. Study population

The study population comprised of three groups. Group I and Group II study populations were recruited from patients attending the medicine out-patient department of the Bir hospital (NAMS), Kathmandu.

Group I: 142 sputum smear negative pulmonary TB at least three sputum specimens, (early morning) negative for acid-fast bacilli, clinically and radiologic abnormalities (postero-anterior chest x-ray) consistent with active pulmonary TB . Also the patients showed a lack of clinical response to a trial of one week of broad spectrum antibiotic agents and a decision by clinician to treat with a full course of anti-TB chemotherapy and a positive response by the patient upon treatment.

Group II: 40 patients of non-tubercular chest disease – Chronic obstructive pulmonary disease (COPD) -selected based on gross clinical observations with obstructive signs and symptoms, confirmed by spirometry. These cases were negative for pulmonary TB as evidenced clinically and a negative acid -fast staining findings.

Group III: 80 healthy controls cases taken from volunteers -medical students, nurses and paramedical staffs – who had no respiratory signs and symptoms and also lacking any other infectious disease, malignancy or auto-immune disease.

2.3. Laboratory investigations

2.3.1. Sample collection

A blood sample (3 ml) was collected in a plain vial from each subject in the three study groups. In Group I patients, the blood sample was collected before the start of anti-tubercular treatment. Serum was separated by centrifugation at 2,500 rpm for 15 minutes at room temperature in a centrifuge and kept in a sterile vials and stored at 20℃.

2.3.2. Diagnostic tests

ADA estimation was done by the sensitive colorimetric method described by Guisti and Galanti[10], using ADA MTB diagnostic kit from Microexpress – a division of Tulip Diagnostics (P) Ltd., Goa, India.

2.4. Statistical analysis

The results were expressed as mean±SD. Statistical comparison was carried out by using the Student's t test. A two-tailed P value of < 0.05 was taken as statistically significant. Diagnostic test 2 – 2 contingency tables were made. Sensitivity, specificity, positive and negative predictive value, positive and negative likelihood ratio and diagnostic accuracy were calculated. All parameters were estimated with 95% confidence interval using the Stata 10.1 statistical software package (Stata Corp. College Station, Tx).

3. Results

Age and sex ratio of the three different study population group is shown in Table 1. The serum ADA levels (U/L) in Group I patient was high (42.26±21.22) compared to the Group III – healthy control cases (18.88±6.67) and was also statistically highly significant ($P < 0.0001$). The serum ADA values (U/L) in Group II was 23.35±8.22 (Table 2). The serum ADA test in Group I patients showed a sensitivity, specificity, positive predictive value and negative predictive value of 83.10% (95% CI 76.08-88.37), 91.25% (95% CI 83.00-95.7), 94.00% (95% CI 88.16-97.07) and 69.52% (95% CI 60.15-77.50). The positive likelihood and negative likelihood ratio in Group I patients were 9.49 (95% CI 4.66-19.34) and 0.18 (95% CI 0.128-0,268). The accuracy of the test in Group I study population was 82.43%. These parameters of validity of ADA as a diagnostic test are shown in Table 3.

Table 1.
Age and Sex ratio of the different study population groups (Mean ± SD)

Study Group	No. of patients	Age (years)	Sex Ratio(M:F)
Group I SNPTB cases	142	40.10 ±17.89	1.84:1
Group II COPD cases	40	36.95±15.10	2.33:1
Group III Healthy Controls	80	38.66±15.74	1.75:1

Table 2.
Serum ADA levels in different study populations groups

Study Group	No. of patients	Mean±S.D (U/L)	P value comparison
Group I	142	42.26±21.22	I & II P<0.0001
Group II	40	23.35± 8.22	I & III P<0.0001
Group III	80	18.88± 6.67	II & IIIP<0.0031

Table 3.
Validity of ADA as a diagnostic test

Study Group	Sensitivity % (CI)	Specificity % (CI)	PPV % (CI)	NPV % (CI)	LR + (CI)	LR − (CI)	Accuracy(%)
Group I	83.10 (76.08 − 88.37)	91.25 (83.00−95.7)	94 (88.16− 97.07)	69.52(60.15− 77.50)	9.49 (4.66−19.34)	0.18 (0.128 − 0.268)	82.43
Group II	40.00 (21.38− 61.34)	91.25 (83.00 − 95.7)	69.56 (49.13 − 84.39)	75.25 (65.81− 82.77)	4.57(1.83− 11.11)	0.65 (0.45−0.94)	74.16

PPV= positive predictive value, NPV= negative predictive value, LR_+ = positive likelihood ratio, LR− = negative likelihood ratio, CI = 95% confidence interval

4. Discussion

Identifying pulmonary tuberculosis in patients with negative sputum smear results is a diagnostic challenge. Considering the low yield of smear and culture in pulmonary tuberculosis, non−microbiological methods may provide new tools for diagnosis. The determination of adenosine deaminase levels is used as one of the tests to prove serosal tuberculosis. There is also some elevation in serum concentrations that could be used as a screening test[24]. This study focused on measuring the serum ADA levels in the study populations selected and evaluating its usefulness especially in SNPTB patients. Results of the study showed increased serum ADA levels in SNPTB patients (42.26± 21.22 U/L) compared to the healthy control cases (18.88±6.67 U/L, $P < 0.0001$ highly significant) and to non−tubercular chest disease − COPD cases (23.35±8.22U/L, $P < 0.0001$ highly significant). Previous studies had also shown elevated levels of serum ADA in SNPTB patients[20,21]. In a study done by Gupta etal [25], the mean serum ADA levels in smear negative pulmonary tuberculosis was found to be 43.5± 6.10 U/L which is comparable to the value obtained in this study. Clearly, the rise in serum ADA level was much higher in SNPTB cases in this study. With a cut−off value of 30 U/L, the serum ADA test showed a high specificity of 91.25% and a sensitivity of 83.10% in SNPTB patients as compared to either the non−tubercular chest diseases− COPD cases or the healthy control cases. Previous study had shown that taking 30 U/L as cut off value the specificity and sensitivity of ADA level as diagnostic test of pulmonary tuberculosis come to nearly 100%[25]. A high positive predictive value of 94.00% and a negative predictive value of 69.52% in SNPTB cases obtained in this study showed that ADA activity measurements as a promising diagnostic marker in these cases. A positive likelihood ratio and and a negative likelihood ratio of 9.49 and 0.18 shows that the ADA levels values can nearly provide an estimate to rule in or rule out the disease in SNPTB patients.

Increased serum ADA levels in pulmonary TB may be due to a stimulation of cell mediated immunity. A fully functioning cell mediated immune response is dependent on normal lymphocyte metabolism which is, in part regulated by the purine salvage enzyme, adenosine deaminase. Increased serum ADA activity, therefore, is also found in other diseases involving stimulation of cell−mediated immunity such as typhoid fever, infectious mononucleosis and bronchogenic carcinoma[20]. But, these non−tubercular infections can be ruled out on the basis of clinical presentations and criteria.

Due to non−availability of culture facilities for the tubercle bacilli at our hospital, culture confirmation of the SNPTB cases could not be performed. Additionally, a large cohort of patients , over a diverse geographic locations needs to be tested by this method to validate its use in routine use in clinical practice.

Patients with active TB are also capable of transmitting the infection. Existing diagnostic approaches have largely failed to interrupt TB transmission in populations with a high prevalence of HIV and drug−resistant TB[26]. Although, persons with SNPTB are less infectious than the smear−positive patients, their overall contribution to disease transmission is considerable because half of all patients with TB can present with negative sputum smear findings. Thus, accurate diagnosis of SNPTB patients is of utmost significance. Though newer rapid diagnostic tests for TB are being developed, but these are either not available in developing countries or are expensive, have poor sensitivity and specificity for smear−negative sputum samples and are not yet considered standard of practice. Field − based diagnosis of SNPTB is limited by the unavailability of diagnostic tests that are simple, rapid and accurate[27]. In the developing countries where TB is endemic, an ideal test for tuberculosis should be economic, minimally invasive, of high accuracy and quick to perform[28].

This study had shown that measuring ADA levels is a rapid, sensitive , inexpensive diagnostic marker in SNPTB patients, in whom otherwise the diagnosis is missed by sputum smear findings. The results of ADA assays should be interpreted with compatible clinical presentations and other laboratory examinations. Thus estimating ADA levels could be a valuable additional test, in the rapid diagnosis in cases of SNPTB patients.

Conflict of interest statement

We declare that we have no conflict of interest.

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