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Diagnosis of Pulmonary and Extra-pulmonary Tuberculosis: Role of Adenosine Deaminase Levels in Different Body Fluids

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ABSTRACT

Background: Tuberculosis (TB) is one of the major public health problems, particularly in developing countries. There are many tools to diagnose TB, one of them is the estimation of the level of adenosine deaminase (ADA) in various fluids in the body.
Objectives: To find the role of ADA levels in 4 different types of body fluids to diagnose TB and extra-pulmonary TB.
Materials and methods: This prospective study was conducted in the 3 main hospitals in Anbar governorate, Iraq for one year from July 2019 to June 2020. Four types of body fluids [serum samples, pleural fluid, ascetic fluid, and cerebrospinal fluid (CSF)] were collected from patients who were suspected of having TB. The levels of ADA were measured by ELISA. An accurate diagnosis of TB was done by Genexpert real-time polymerase reaction (PCR). The results were compared according to PCR findings.
Results: Out of 176 patients, there were 31 patients were proved to be positive, for TB by Genexpert real-time PCR. The highest mean level of ADA in positive TB cases by PCR was in the pleural fluid (58.258 ± 2.707), and the last was in the serum. There was a highly statistically significant difference between the ADA levels in all 4 body fluids between the positive and negative TB cases (P-value= 0.000). In the positive cases, the sensitivity of ADA levels in the serum, CSF, pleural fluid, and ascetic fluid were found to be 40%, 81%, 96%, and 94% respectively and the specificity was found to be 88%, 72%, 94%, and 90% respectively.
Conclusion: The ADA levels in all 4 body fluids were highly statistically significant difference between TB negative and positive cases diagnosed by PCR. However, they had different sensitivity and specificity levels.

Keywords: Adenosine Deaminase; Tuberculosis; Serum; Cerebrospinal fluid; Pleurisy; Ascites.

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INTRODUCTION

Tuberculosis (TB) is a significant disease induced by a group of mycobacterial bacteria. Tubercle bacilli Mycobacterium tuberculosis is one of the most serious causes of TB. TB considers one of the world's biggest health issues. The treatment and control of TB depend on a rapid and precise diagnosis [1]. There are several approaches available for diagnosing TB, including direct staining of Ziehl-Neelsen (Z-N), immunological methods, culture reaction, and polymerase chain reaction (PCR). Each of these approaches has a different range of sensitivity and specificity. Culture on selective culture media, which is considered the gold standard process, is the most definitive method of diagnosis, but this is a time-consuming method and takes several weeks to give results since Mycobacterium tubercu-

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losis is a slow-growing bacteria. Many types of body fluids are appropriate for the culture and other diagnostic methods, such as pleural fluids, cerebrospinal fluid (CSF), pericardial fluids, and ascitic fluid [2].

The Adenosine deaminase (ADA) is an enzyme that can catalyze and release ammonia by converting deoxyadenosine and adenosine to deoxyinosine and inosine. In the differentiation of lymphoid cells, this mechanism plays an important role and it is found in large quantities in active T-lymphocytes with an inversely proportional concentration to a degree of differentiation. The activity of the ADA enzyme is increasing during lymphocyte mitogenic and antigenic responses and ADA inhibitors might inhibit T-lymphocyte blastogenesis [3]. ADA is now known to be a good marker of cell-mediated immunity, especially the activation of T-lymphocytes [4]. When any form of body fluid was tested for clear evidence of a disease, the positivity rate of, acid .fast bacilli (AFB) may be very .low, this culture phase is time consuming, costly, and requires a long incubation period that might take more than 4 weeks and less than 25% of its positivity percentage [3, 4].

Any delay in diagnosis and treatment initiation might lead to poor prognosis and sequelae after infection might occur in up to 25% of cases and could promote the incidence of outbreaks [5], therefore the presence of rapid, inexpensive, and accurate diagnostic methods is required. There have been 2 prior studies from Iraq investigated the role of the ADA in myocardial infarction and thalassemia patients [6, 7], but up to our best knowledge, there is no study has been done in Iraq about the role of the ADA in the diagnosis of TB. Hence we are aimed to measure the diagnostic validity of the ADA levels in different types of body fluids for the diagnosis of pulmonary and extra-pulmonary TB which are proved to be positive by Genexpert-Cefede-USA Real-Time PCR.

MATERIALS AND METHODS

Patients and controls

The study was included patients of different genders and ages who clinically had TB manifestations and were found to be positive by gene-expert real-time PCR. The study was conducted in three hospitals in Anbar governorate, Iraq (Maternity and Children Teaching Hospital, Al-Ramadi Teaching Hospital in Ramadi city, and Al-Falluja Teaching Hospital, Falluja city). The study was covered the period from the 1st July 2019 to the end of June 2020. The controls were used for patients who were found to be PCR negative. Samples were taken from the patient's serum, pleural fluids, ascetic fluids and CSF. The levels of ADA were calculated by the ELISA technique in these 4 types of body fluids as defined by the central disease control, 2015 [2, 8]. The age of the patients were divided into 6 groups 0-10, 11-20, 21-30, 31-40, 41-50 and $>$ 50 years.

The present study was approved by the department of training of staff personals in the Anbar Health Directorate. Informed consent was taken from all patients.

Methods

Human ADA ELISA Kit (Catalog Number. MBS700350, MyBioSource, com) was used for the quantitative determination of ADA concentrations in serum, pleural fluids, ascetic fluid, and CSF. Serum samples were obtained from all patients, while other types of body fluids were collected, including samples of pleural fluid, ascetic fluid, and CSF depending on the type of disease and doctors' decisions. To find out the diagnostic utility of the level of this enzyme in TB patients, levels of ADA in the 4 types of body fluids were tested using the ELISA technique.

Principle of the Assay

The enzyme-linked immunosorbent assay (ELISA) employs the immunoassay technique of the enzyme of the quantitative sandwich. Specific antibody for ADA was pre-coated into a microplate. Samples and standards were pipetted into wells and any ADA present attaches to the antibody. After removing any unbound materials, a biotin-conjugated specific antibody to ADA was added into wells. To removing any unbound avidin-enzyme reagent, a substrate was added to the wells and then formed a color consistency with the ratio of ADA concentration which bound in the first step after the wash. The color growth was discontinued and the strength of color was measured by an ELISA reader.

Exclusion Criteria

Serum, pleural fluids, ascitic fluids, and CSF samples that were found to be hemorrhagic and mixed with a high number of red blood cells (RBCs), diabetic patients, and all cases that were under treatment were excluded from the study.

Statistical Analysis

The data were analyzed using IBM-SPSS version 24. Comparison between the PCR positive and negative results according to the age groups were presented in a table. The mean of ADA levels in both negative and positive PCR findings and the sensitivity and specificity of ADA levels in 4 types of body fluids were compared. An independent sample T-test was used in the comparison between the means. The sensitivity was measured by the following formula: TP/TP+FN (TP, true-positive results; FN, false-negative results); and specificity was measured using the following equation TN/TN+FP (TN, true negative results; FP, false-positive results). A P-value of less than 0.05 is considered a statistically significant difference.

RESULTS

The ages of our patients were ranged from 5 to 63 years with a mean age of 25 ± 5. Out of 176, there were 31 patients were proved to be positive by Genexpert real-time PCR. There was no statistically significant difference between the 2 groups

Table 1. Distribution of the PCR results for patients with the suspected TB according to age groups*.

Age groups in years	Total Number(%)	PCR results	
		Positive Number(%)	Negative Number(%)
0−10	34 (100)	7 (20.5)	27 (79.5)
11−20	38 (100)	5 (13.2)	33 (86.8)
21−30	30 (100)	6 (20)	24 (80)
31−40	26 (100)	5 (19.3)	21 (80.7)
41−50	22 (100)	4 (18.2)	18 (81.8)
>50	26 (100)	4 (15.4)	22 (84.6)
Total	176 (100)	31 (17.6)	145 (82.4)

* P-value =0.933

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Table 2. Comparison of mean ADA levels in patients and controls for various types of body fluids by PCR in 176 patients.

Type of sample	Total number	ADA Level (U/ml)		P-value
		Positive cases(n=31) mean ± SD	Negative cases(n=145) mean ± SD	
Serum	176	28.419 ± 1.803	22.283 ± 2.0197	0.000
CSF	45	15.419 ± 1.336	7.083 ± 0.9538	0.000
Pleural fluid	86	58.258 ± 2.707	22.648 ± 1.5658	0.000
Ascetic fluid	45	55.097 ± 2.947	25.435 ± 1.8364	0.000

(PCR negative and positive) according to the age groups P-value =0.933, as shown in Table 1.

The highest mean level of ADA in positive TB cases by PCR was in the pleural fluid (58.258 ± 2.707), and the last was in the serum. There was a highly statistically significant difference between the ADA levels in all 4 body fluids between the positive and negative TB cases (P-value= 0.000) as shown in Table 2.

The sensitivity/specificity of the ADA test in the 4 types of body fluids were found to be 40% / 88% , 81% / 72%, 96% / 94%, and 94% / 90% for serum, CSF, pleural fluid and ascitic fluid respectively as shown in Table 3.

DISCUSSION

The results of Genexpert real-time PCR which is a fully automated molecular technique were considered to be a highly responsive and highly specific tool for the diagnosis of TB [9]. Seventeen percent of our suspected patients of having various forms of TB were found to be positive for TB. Those cases that were proven to be positive by Genexpert were used to measure the diagnostic validity of using ADA levels in 4 types of body fluids. In 2015, Salmanzadeh et al. [5], the cut-off level was 26, this was supported the benefit of levels of ADA in serum in the diagnosis of TB. The mean ADA level in serum was stated to be 26 IU/L in patients with pulmonary TB, 19.48 IU/L in pneumonia-related patients, 15.8 IU/L in patients with pulmonary carcinoma, and 10.7 IU/L in a group of control (P-value<0.05). In Pulmonary TB patients, they have used a value of 26 IU/L as a cut-off for ADA and mentioned a degree of sensitivity and specificity of 35% and 91%, respectively. These results were supported our findings with some variations, but the above research on pulmonary TB included, while, pulmonary and extrapulmonary forms of TB in our study. The above study has concluded that the operation of ADA in serum is not a helpful method for TB diagnosis [5].

Gupta et al. have concluded that ADA measurement is characterized by specific and sensitive tests (> 90%), useful in differentiating tubercular from non-tubercular causes, and both in pulmonary and extra-pulmonary disease. The properties of this test are simple, cheap, and quick. Therefore it might help in early diagnosis, improve the prognosis, and reduce the spread of disease and sequelae [10]. Almost similar results have been obtained by many other studies [11–13]. The mean ADA levels in serum in patients with TB and non-TB were 20.8 (±5.97) and 10.6 (±2.9) U/L, respectively, with a P-value < 0.05 which means that the 20.8 mean level of ADA in serum might act as a proper indicator for TB. They have concluded that the amount of serum ADA might be suggested as an acceptable index for the diagnosis of TB, but the sensitivity is poor [14].

Table 3. The sensitivity and specificity of ADA levels in 4 types of body fluids

Type	Total number	Sensitivity	Specificity
Serum	176	40%	88%
CSF	45	81%	72%
Pleural fluid	76	96%	94%
Ascetic fluid	60	94%	90%

It is well understood that TB remains a big cause of global mortality and morbidity. TB diagnosis relies on the clinical presentation, the radiological findings, and the tuberculin test results. However, clinical and radiographic characteristics are variable and false-negative results could be given by the tuberculin test. In these circumstances, empirical anti-tuberculous therapy could be started. Therefore, finding some simple, accurate, and useful diagnostic methods for TB is very critical [4, 15, 16].

ADA is an enzyme that catalyzes adenosine to inosine for hydrolysis and deamination of deoxyadenosine to deoxyinosine. ADA concentration test in pleural fluid was used to diagnose pleurisy of TB [2, 4, 14]. The role of ADA in pulmonary TB diagnosis has been studied by Hassanein et al. The study has included 20 sputum-positive acid-fast bacilli (AFB) patients, 10 bronchogenic carcinoma patients, 10 pneumonia patients, and 10 regular healthy subjects. They have concluded that it is possible to use ADA levels in pleural fluid and sputum in the diagnosis of TB but the sputum level is very poor [17]. A similar finding has been reported by another study [18].

In lung TB and other common respiratory illnesses, the function of ADA activity has been studied. The research has been performed on 53 cases of active pulmonary TB; ADA levels were ranged from 31.42 to 45.80 in this population, with a mean of 39.97 ± 2.24 μ /l. The ADA amount has ranged from 37.28 to 43.28 in 14 cases of TB pleural effusion, with a mean of 40.19 ± 1.57. The ADA level has ranged from 22.42 to 32.12 in 33 cases of non-TB lung diseases, with a mean of 25.80 ± 1.41, and the ADA level has ranged from 12.84 to 19.46 in 35 healthy individuals, with a mean of 16.2 ± 2.85. Their research has shown that in patients with pulmonary and pleural TB diseases and non-pulmonary TB diseases, the activity of serum ADA was higher than in controls. ADA activity was the highest [19], which was confirmed our results.

Atta et al. from Egypt have evaluated the diagnostic benefit of ADA levels in serum and sputum samples for the diagnosis of pulmonary TB. They have reported a significant increase in sputum ADA in the TB group. The mean level of the ADA in serum samples and sputum samples was 31.9 ± 8 and 14.8 ± 2.4 U/L respectively in the TB group. There has a statistically significant increase in serum ADA in the TB

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group concerning the other groups (P-value= 0.001). According to their results, they have concluded that using sputum ADA levels for the diagnosis of pulmonary TB is possible [20]. The levels of ADA in serum samples were almost similar to our results, however, we didn't evaluate the sputum levels of ADA.

The present study was concluded that ADA levels were showed a highly significant difference in the 4 fluid types (serum, pleural fluid, ascetic fluid, and CSF) between the positive and negative TB cases. However, their sensitivity and specificity were different. The limitations of the study include small sample size and the ADA level was not measured in the sputum. Hence, a further study in the evaluation of the diagnostic validity of ADA levels in sputum samples is recommended.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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