

Using Ascitic Fluid Adenosine Deaminase Assay in the Diagnosis of Tuberculous Ascites

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ABSTRACT

AIMS: This study aimed to analyze the diagnostic values of the Adenosine Deaminase (ADA) test for detecting Tuberculosis (TB) ascites in Vietnam.

MATERIALS AND METHODS: A cross-sectional study was conducted on 43 patients with ascites. ADA, acid-fast bacilli (AFB) smear microscopy and culture as well as histological tests were performed. Diagnostic values including sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were estimated.

RESULTS: The area under the curve (AUC) of ascitic fluid ADA in diagnosis of TB ascites was 0.937 (95% CI: 0.85 - 1.0) with $p <$

0.001. The optimal cut-off value was 30.2 U/L with high sensitivity (100%) and specificity (88.5%), 85% PPV and 100% NPV.

CONCLUSIONS: Ascitic fluid ADA assay is a useful diagnostic tool to detect TB ascites.

Key words: Adenosine Deaminase; Tuberculosis; Ascites; Vietnam

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INTRODUCTION

Tuberculosis (TB) is a global public health problem and among top ten causes of mortality in worldwide^[1,2]. According to WHO, in 2016, there were about 6.3 million new TB cases in the world, of which extra-pulmonary TB accounted for about 15%^[1]. In Vietnam, the incidence of extrapulmonary TB was 17.4-18.9% of all TB cases from 2005 to 2008. TB ascites is the sixth common type of extrapulmonary TB^[3]. Currently, some diagnostic methods are available to identify TB ascites, of which results from culture of ascitic fluid/peritoneum is considered a gold standard^[4]. However, it is a time-consuming approach when requiring several weeks for obtaining the accurate results. Meanwhile, other methods such as acid-fast-stained smears or histological detection has limitations such as insufficient sensitivity, invasive procedure, or not available in every hospital due to lack of resources or complicated techniques^[5,6]. Identifying other simple manners without invasion is important for improving the diagnosis of TB ascites.

Previously, Adenosine deaminase (ADA) has been demonstrated that is highly sensitive and specific in the diagnosis of extrapulmonary TB^[7-9]. Several studies found that ADA in ascites can be used to detect TB peritonitis with 100% sensitivity and 92%-100% specificity^[10-12]. A systematic review of Lin Tao et al. indicated that ADA has 93% sensitivity and 94% specificity in diagnosing TB ascites^[13]. However, other studies ADA has a limited diagnostic capacity in identifying TB peritonitis or among patients with other diseases such as liver cirrhosis^[14-16]. Moreover, the threshold for

diagnosis, sensitivity, and specificity of ADA depends on the age and prevalence of tuberculosis in each region. Given the diversity of findings across nations, we performed this study to test the diagnostic values of ADA in detecting TB ascites.

MATERIALS AND METHODS

Study design and sampling method

Cross-sectional data of 43 patients with ascites treated at Central Lung Hospital, Hanoi Lung Hospital and Bach Mai Hospital from January 2019 to August 2019 were used for analysis. They were included if they (1) were confirmedly diagnosed to have TB; (2) Age \geq 16 years old; and (3) Agreed and gave their written informed consents. Patients were excluded if they were (1) under 16 years old, and (2) had blood diseases or autoimmune diseases. Fifty patients were conveniently recruited, of which 43 patients agreed to participate (response rate 86%). The study protocol was approved by the Institutional Review Board of the Hanoi Department of Science and Technology (Code: 4528/QD-UBND).

Measurement

ADA activity measurement technique: The activity of the ADA enzyme in a patient’s blood is determined by an enzyme kinetic

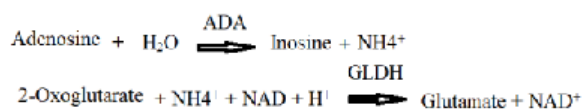


Figure 1 ADA reaction.

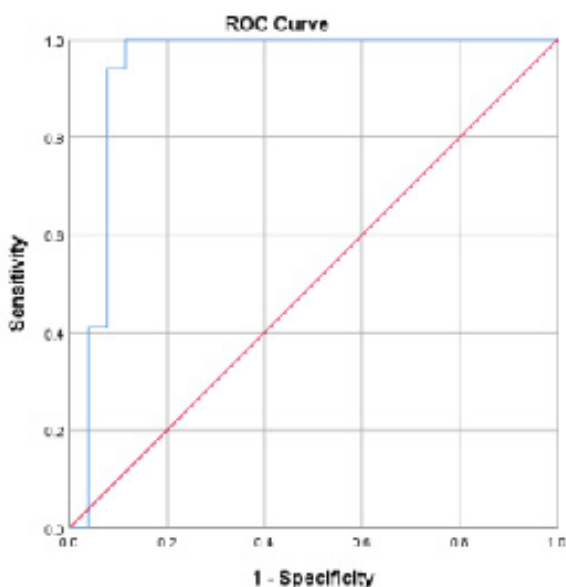


Figure 2 ROC curve – Diagnostic value of ADA in the diagnosis of TB ascites.

method based on the reaction as following (Figure 1):

ADA tests used chemicals from Biosystem, ADA calibrator and control. AU680 Backman Coulter automatic biochemical system was used to test the ADA. There were two types of reagents for testing. The reagent A included 4 × 8 milliliters (mL) Tris 125 mmol/L; 2-cetoglutarate 1.1 mmol / L; adenosine 6.5 mmol / L; glutamate dehydrogenase >100 U/L; sodium azide 0.95 g/L; with pH 6.8. Meanwhile, the reagent B consisted of 1 × 10 mL NADH 1.5 mmol/L and sodium azide 9.5 g/L. Two reagents were mixed in the ratio 4:1: 4 mL of reagent A + 1 mL of reagent B. The reagent was kept

being stable for 30 days at 2-8°C. After opening the reagent, it was kept in the cooler of the analyzer for 12 days. The standardized ADA was from cattle with Tris 50 mmol/L.

Other tests: acid-fast bacilli (AFB) smear microscopy and culture (BATEC MGIT) as well as histological tests were performed according to the standard procedures at Central Lung Hospital, Hanoi Lung Hospital and Bach Mai Hospital. Moreover, ascitic fluid and blood tests were performed to measure lactate dehydrogenase-LDH, protein, total cell, % lymphocytes (in ascitic fluid), and white blood cell and C-reactive protein (in blood). Other diseases such as pneumonia, malignancy, or cirrhosis was diagnosed according to the standards of the Ministry of Health.

Statistical analysis

Data were analyzed using SPSS software version 20.0. Chi-squared and Mann-Whitney tests were used to compare demographic and clinical characteristics between TB ascites and non-TB ascites groups. Sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), receiver operating characteristic (ROC) and area under the curve of ADA in diagnosing TB ascites were estimated. P-value of less than 0.05 was statistical significance.

RESULTS

Among 43 TB patients, 17 patients (39.5%) had TB ascites. There was no difference in age and gender between TB ascites and non-TB ascites patients ($p > 0.05$) (Table 1).

Table 2 indicated that the ADA activity level, LDH, protein, the number of cells and % lymphocytes were significantly higher in TB ascites group compared to non-TB ascites group ($p < 0.05$).

The area under the curve (AUC) of ascitic fluid ADA in diagnosis of TB ascites was 0.937 with $p < 0.001$. The optimal cut-off value was 30.2 U/L with high sensitivity (100%) and specificity (88.5%) (Figure 2).

Table 3 indicated that that the cut-off point of 30.2 U/L of ADA provided a high diagnostic value of TB ascites with 100% sensitivity, 88.5% specificity, 85% PPV and 100% NPV. There were 3 cases of false positives which were peritoneal metastases. Meanwhile, AFB method had only 8.3% sensitivity, MGIT method had 41.2% sensitivity with ascitic fluid, and 47.0% with all specimens. Histological procedure had 88.9% sensitivity.

DISCUSSION

This study contributes to the current literature that ascitic fluid ADA assay was a good tool for diagnosing TB ascites. The diagnostic values of this indicator were also better than AFB or MGIT approaches.

In our study, the mean ADA concentration in patients with TB ascites was significantly higher than that of other diseases. The AUC of ascitic fluid ADA assay was 0.937 (95%CI: 0,851-1,000), suggesting that ADA was very valuable in the diagnosis of TB ascites. Our result differed from findings from other previous studies^[17,18-20], which might be due to the heterogeneity in patient selection, time of testing, or methods to measure ADA. A prior literature indicated that in coun-

Table 1 Age and gender characteristics.

Characteristics	TB ascites n (%)	Non-TB ascites n (%)	p
Total	17 (39.5%)	26 (60.5%)	
Male	12 (70.6%)	17 (65.4%)	0.7
Female	5 (29.4%)	9 (34.6%)	
Age (years), Median (IQR)	54 (33.5-61)	55 (45.5-61.25)	0.4

Table 2 Results of Pleural perfusion fluid tests and Blood tests.

Tests		TB ascites		Non-TB ascites		P
		n	Median (IQR)	n	Median (IQR)	
Ascitic fluid	ADA	17	65.07 (53.84-83.4)	26	16.6 (13.18-26.26)	<0.001
	LDH (U/L)	11	214 (121-373)	16	60.5 (46.3-160.8)	0.044
	Protein (g/L)	17	55.2 (43.5-65.4)	26	21.4 (7.8-36.7)	<0.001
	Cells (cells/mm ³)	17	2080 (1165-4560)	26	370 (200-870)	<0.001
	% lymphocytes	17	70 (64-80)	26	55 (30-72)	0.021
Blood tests	WBC (G/L)	17	6.24 (5.68-8.23)	26	7.87 (4.88-13.3)	0.371
	CRP (mg/L)	15	36.8 (12.9-84.4)	17	13.2 (7.1-60.3)	0.146

IQR: interquartile range; LDH: lactate dehydrogenase; ADA: adenosine deaminase; WBC: white blood cell; CRP: C-reactive protein

Table 3 Values of some tests in the diagnosis of TB ascites.

Test	Specimen	n	Group	TB ascites (n)	Non-TB ascites (n)	Sens (%)	Spec (%)	PPV (%)	NPV (%)	Acc (%)
ADA (U/L)	Ascitic fluid	43	≥ 30.2	17	3	100	88.5	85	100	86.9
			< 30.2	0	23					
AFB	Sputum	25	(+)	1	1	8.3	91.7	50	47.8	48
			(-)	12	11					
MGIT	Ascitic fluid	43	(+)	7	0	41.2	100	100	72.2	76.7
			(-)	10	26					
	All specimens	43	(+)	8	1	47	96.2	88.9	73.5	76.7
			(-)	9	25					
Histological procedure	Biopsy tissue	17	(+)	8	0	88.9	100	100	88.9	94.1
			(-)	1	8					

ADA: adenosine deaminase; AFB: acid-fast bacilli; MGIT: smear microscopy and culture; Se: sensitivity; Sp: specificity; PPV: positive predictive value; NPV: negative predictive value; Acc: Accuracy of of diagnostic method). * 1 case of tuberculosis pulmonary with AFB (+) and MGIT (+) in sputum. However, this patient was classified cirrhosis due to ascites by cirrhosis, protein in fluid was 7.71 g / L, this ADA was 7.6 U / L.

tries with high TB prevalence, ADA could replace pleural biopsy for diagnostic purposes, while in areas with low tuberculosis burden, ADA had low PPV (7%) but substantially high NPV (99.9%)^[18].

The optimal cut-off point for ascitic fluid ADA assay was 30.2 U/L with 100% sensitivity, 88.5% specificity, 85% PPV and 100% NPV for diagnosing TB ascites. However, if a higher cut-off threshold was used, the sensitivity would decrease while the specificity would increase. For example, with a cut-off threshold of 38.4 U/L, the sensitivity was 94.1% and the specificity was 92.3%. A study in South African study used a cut-off threshold of 30 U/L for a 94% sensitivity 92% specificity, 57% PPV, and 99% NPV^[17]. A meta-analysis based on 16 published studies related to ADA in the diagnosis of TB ascites showed that a cut-off threshold of ≥ 30 (30-40) U/L was used in 14 studies and <30 U/L was used in 2 studies. The pooled sensitivity and specificity were calculated to be 93% (95% CI: 89-95%) and 96% (95% CI: 94-97%), respectively^[22].

Identifying the presence of tuberculosis bacteria is the gold standard for TB diagnosis. Our results revealed that all methods had high specificity, but each diagnostic method had certain limitations. AFB had a positive rate <10% because it required at least 10,000 bacteria/1mL of fluid. Moreover, culture of TB bacteria had a low PPV from 12-70%, and the results could only be informed after 2-6 weeks, which affected significantly the treatment as well as increase the risk of spreading TB to the community. The histological results of pleural and peritoneal biopsies may help to guide TB diagnosis with TB images characterized by tuberculous lesions, gangrene necrosis, the presence of epithelioid cells and giant cells. However, this requires an invasive procedure and cannot be performed on some patients such as exhaustion, or had coagulopathy. Today, many molecular biology techniques have been developed for detection of the presence of tuberculosis bacteria with high sensitivity and specificity; but the cost is high and requires expensive equipment. In patients

with peritoneal effusion, diagnostic bacteria tests also have a high specificity and PPV (100%). However, the positive rate of these tests is still low. AFB could detect 1/13 positive cases, with 8.3% sensitivity. Histological biopsy could identify 8/9 positive cases with typical tuberculous lesions, indicating 88.9% sensitivity. These findings were somewhat similar to previous studies in South Africa and Egypt^[17,20].

The diagnostic value of ascitic fluid ADA assay is further confirmed in patients who have not found bacteriological evidence. The cut-off threshold of 30.2 U/L had 100% NPV, or in other word, this threshold could indicate that risk of TB ascites was not existed. A study in South Africa showed that there were 13 cases of false positive (ADA ≥ 30 U / L) including: cancer, systemic lupus erythematosus, heart failure, nephrotic syndrome, renal failure, and cirrhosis. There is only 1 case of false negative (ADA = 18 U / L). This patient was diagnosed with tuberculosis by histopathological findings of peritoneal biopsy^[17].

CONCLUSION

Ascitic fluid ADA assay is a useful diagnostic tool to detect TB ascites. As a low-cost test compared to other diagnostic tools, ascitic fluid ADA assay should be selected as a preferred choice in resource-constrained settings.

Future Perspective: Ascitic fluid ADA assay is not used as a standard procedure to detect TB ascites in clinical settings. It is believed that this tool can be applied widely in the future for TB ascites prognosis and diagnosis.

Summary Points: (1) Adenosine deaminase (ADA) has been demonstrated that is highly sensitive and specific in the diagnosis of extrapulmonary TB. (2) Diagnostic values of Ascitic fluid ADA assay vary across settings. (3) This study found Ascitic fluid ADA assay is a useful diagnostic tool to detect TB ascites.

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