Journal of Microbiology and Infectious Diseases / JMID
2013; 3 (3): 104-108
doi: 10.5799/ahinjs.02.2013.03.0091

ORIGINAL ARTICLE

Diagnostic significance of ascites adenosine deaminase levels in suspected tuberculous peritonitis in adults

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ABSTRACT

Objective: There are contradictory reports about the use of adenosine deaminase (ADA) as a diagnostic marker in tuberculous peritonitis patients. Reports evaluating significance of ADA activity in the diagnosis of tuberculous peritonitis in adults are lacking in Nepal. We thus set out to investigate the ascitic fluid ADA levels in suspected tuberculous peritonitis patients and to determine the diagnostic significance of the test statistically.

Methods: This study population comprised of two different adult patients groups. Group I - 35 suspected cases of tuberculous peritonitis and Group II - 35 cases of transudative ascites - the control group (patients with biochemically proved transudates or hypoproteinaemia) and peritoneal tap was done. ADA estimation was carried out by spectrophotometry.

Results: ADA levels (Mean ± SD) in suspected tuberculous peritonitis and transudative ascites cases were 48.5±17.9 U/L and 19.8±7.7 U/L respectively (P<0.001). In the receiver operating characteristic (ROC) curve for ascites, ADA cut-off level of 41.5 U/L was found to yield the best results of differential diagnosis; sensitivity, specificity, positive predictive value, negative predictive value and accuracy of the test in tuberculous peritonitis cases were 80.0%, 97.2 %, 96.6%, 82.9%, 88.6% respectively.

Conclusion: ADA levels are elevated in suspected tuberculous peritonitis cases and it is a simple, rapid, inexpensive and the least invasive test. It is thus a useful biochemical marker for the early diagnosis of tuberculous peritonitis while waiting for the results of mycobacterial cultures or biopsies. *J Microbiol Infect Dis 2013; 3(3): 104-108*

Key words: adenosine deaminase, sensitivity, specificity, diagnostic significance, tuberculous peritonitis

Yetişkinlerde şüpheli tüberküloz peritonitte asit adenozin deaminaz düzeylerinin tanısal önemi

ÖZET

Amaç: Tüberküloz peritonit hastalarında adenozin deaminaz'ın (ADA) tanısal belirteç olarak kullanımı hakkında çelişkili raporlar vardır. Nepal'de erişkinlerde tüberküloz peritonit tanısında ADA aktivitesinin değerini araştıran bir çalışma bulunmamaktadır. Bu nedenle şüpheli tüberküloz peritonit hastalarında asit sıvıda ADA düzeylerini araştırmak ve istatistiksel olarak bu testin tanısal önemini belirlemek için yola çıktık.

Yöntemler: Çalışmaya alınan hastalar iki farklı erişkin hasta grubundan oluşmaktaydı. Grup I; 35 şüpheli tüberküloz peritonit olgusu, ve Grup II; transüdatif asitli 35 hastanın oluşturduğu kontrol grubu (transüda olduğu biyokimyasal olarak kanıtlanmış veya hipoproteinemisi olan hastalar) çalışmaya dahil edildi ve periton sıvıları alındı. ADA ölçümü spektrofotometri ile yapıldı.

Bulgular: ADA düzeyleri (ortalama ± SS)şüpheli tüberküloz peritonit ve transudatif asit olgularında, sırasıyla, 48,5 ± 17,9 U/L ve 19,8 ± 7,7 U/L idi (P<0,001). Asit değerleri için "alıcı işletim karakteristik" (ROC) eğrisinde, ADA kesme seviyesi 41,5 U/L alındığında en iyi ayırıcı tanı değerleri bulundu ve tüberküloz peritonit olgularında testin duyarlılık, özgüllük, pozitif prediktif değer, negatif prediktif değer ve doğruluk düzeyleri, sırasıyla, % 80,0,% 97,2,% 96,6,% 82,9,% 88,6 idi.

Sonuç: Şüpheli tüberküloz peritonit olgularında ADA düzeyleri yükselmektedir ve bu basit, hızlı, ucuz ve en az invaziv testtir. Mikobakteri kültürleri veya biyopsi sonuçlarını beklerken tüberküloz peritonit erken tanısı için bu en kullanışlı bir biyokimyasal belirteçtir.

Anahtar kelimeler: adenozin deaminaz, duyarlılık, özgüllük, tanısal değer; peritonit tüberküloz

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Received: 30.09.2013, Accepted: 26.06.2013

INTRODUCTION

Adenosine deaminase (adenosine amino hydrolase, EC 3.5.4.4. ADA, isoenzymes ADA1 and ADA2) an enzyme required for purine degradation is widely distributed in human tissues.[1] ADA helps in proliferation and differentiation of lymphocytes especially T lymphocytes. ADA is a significant indicator of active cellular immunity.[2] Thus, ADA has been proposed to be a useful surrogate marker for the diagnosis of tuberculosis (TB) because it can be detected in body fluids such as pleural, pericardial, cerebrospinal fluid and peritoneal fluid and elevated ADA levels have been reported in these cases.[3,4]

By estimation of ADA levels for the diagnosis of extra pulmonary tuberculosis,[5-11] sensitivities and specificities of greater than 90 percent have been reported. ADA predicts disease probability by 99% in countries with high prevalence of TB12 but there are other studies which do not show such good results.[13-15]

The prevalence of TB in Nepal is high. The annual incidence rate of TB in Nepal is quite high and is reported to be 163 per 100,000 population.[16] This has been attributed to difficulties in providing health care and treatment facilities in the remote mountainous terrain of the country. Literature survey revealed no studies being reported from Nepal that evaluates the significance of ADA levels in the diagnosis of suspected tuberculous peritonitis. Hence, this study was designed and conducted to assess the role of ascitic fluid ADA in the early laboratory diagnosis of tuberculous peritonitis in adults in Nepalese population.

METHODS

Setting

This prospective study was carried out on patients admitted in the medical ward of a centrally located tertiary care hospital in Kathmandu, Nepal from July 2008 to July 2010. Patients from all parts of the country and especially the poor sections of the society come for treatment in this hospital as most of the services provided are free, including all investigations. The hospital receives about 60 sputum specimens per day for performing Ziehl-Neelsen staining for diagnosis of TB and eight to ten peritoneal fluid specimens for various ascites investigations every month. The ethical review committee of the hospital permitted to carry out this study and informed consent was taken from the patients before inclusion in the study. Their results were dispatched immediately after the tests were performed, so that the patients get appropriate treatment.

Patients

Abdominal paracentesis was performed on 70 consecutive patients with ascites and these were divided into two different patients groups. Group I - clinically suspected cases of tuberculous peritonitis - 35 cases, on the ground of clinical findings and lymphocytic exudates (less than 3 g of protein per 100 ml of fluid) with no response to one week of broad spectrum antibiotics treatment and / or radiologic findings consistent with lung TB and sputum positive for acid fast bacilli (AFB). Group II - control group - transudative ascites cases (less than 2 g of protein per 100 ml of fluid, patients with biochemically proved transudates or malnutrition with hypoproteinaemia) and with no evidence of TB clinically and sputum smear negative for acid fast stain. Ascitic fluid samples (2-3 ml) were collected with aseptic precautions by abdominal paracentesis from both the study population groups. Patients with any malignancies (or hemorrhagic ascites) and less than 15 years of age were excluded in the study. All subjects selected in the study were tested for HIV and only those found negative for HIV were included in the study.

Laboratory tests

ADA estimation was carried out by spectrophotometry method based on the principle of Guisti and Galanti method of enzymatic analysis [17] which is based on indirectly measuring the formation of ammonia produced when adenosine deaminase acts in an excess of adenosine. ADA levels were calculated and expressed in unit per litre (U/L). ADA MTB diagnostic kit from Microexpress - a division of Tulip Diagnostics Pvt. Ltd., India was used according to the manufacturer's instructions.

Statistical analysis

The continuous variables were presented as range, mean ± SD or quartiles and the categorical variables were calculated by percentages and ratio (sex ratio). The continuous variables were compared by using the Student t test or Mann-Whitney U test. A receiver -operating characteristic (ROC) curve was plotted and the area under the curve (AUC) was calculated and an optimum cut-off value was established. For all analyses, a P value of <0.05 (two-tailed) was taken as statistically significant. Diagnostic test 2x2 contingency tables were used. Sensitivity, specificity, positive and negative predictive values and accuracy of the test were calculated All evaluations were performed with SPSS version 17.0.

RESULTS

All patients in the suspected tuberculous peritonitis group were sputum smear positive for AFB, had some abnormality on CXRs (upper lobe lesion, lateral or bilateral or with cavities) and had lymphocytic exudates with total protein > 3 g/dl. In contrast, in the transudative ascites (control) group, all patients were sputum smear negative for AFB, had no abnormality on CXRs and had ascites total protein < 2 g/dl. The age (mean ± SD) and the ratio of male / female in suspected tuberculous peritonitis was 43.80 ± 16.31 and 3/1 and in transudative ascites, the control group it was 44.03 ± 14.64 and 2 / 1 respectively. The mean ADA levels (mean ± SD) in suspected tuberculous peritonitis and in transudative ascites cases, were 48.51 ± 17.91 U/L and 19.28 ± 7.69 U/L, respectively. The difference between the ADA values in the two groups was found to be highly significant (P< 0.001). Box plots of the ADA activity in the suspected tuberculous peritonitis (case) and transudative ascites (control) groups are shown in Figure 1, together with the 90th percentile range, 75th and 25th percentiles. The usefulness of ascites ADA level as a biomarker for diagnosis of tuberculous peritonitis was evaluated using ROC curve analysis and the optimal cut-off value was determined to be 41.5 U/L (Figure 2). The area under the curve (AUC) for suspected tuberculous peritonitis group was 0.928 and standard error (SE) was 0.032 (95% confidence interval (CI) =86.4% - 99.1%, P <0.001). Seven patients in suspected tuberculous peritonitis group showed ADA values of less than 41.5 U/L and one patient in transudative ascites , the control group showed ADA value of greater than 41.5 U/L. Based on the cut-off value of 41.5 U/L, the ascites ADA sensitivity and specificity were 80.00% and 97.14% respectively. Positive predictive value was 96.55% and negative predictive value was 82.6%. The accuracy of the test in suspected tuberculous peritonitis cases was 88.6% (Table 1).

Table 1. Validity of Ascites ADA as a diagnostic test in suspected cases of tuberculous peritonitis

Study Group	Sensitivity % (CI)	Specificity % (CI)	PPV % (CI)	NPV % (CI)	Accuracy %
Tuberculous peritonitis	80.00 (62.5-90.9)	97.14 (83.5-99.9)	96.55 (80.4-99.8)	82.92 (67.4-92.3)	88.57

PPV= positive predictive value, NPV= negative predictive value, CI=95% confidence interval

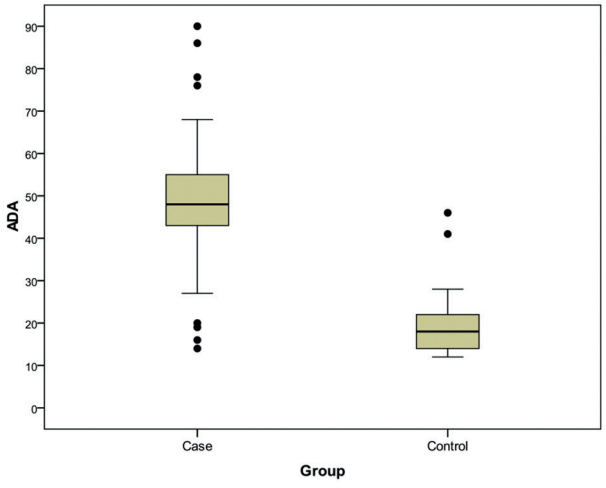

Figure 1. Box plots for ADA (U/L) levels in tuberculous peritonitis (case) and transudative ascites (control) groups. The plots show the 90th percentile (bars), 75th and 25th percentile (box) and median (bar in box).

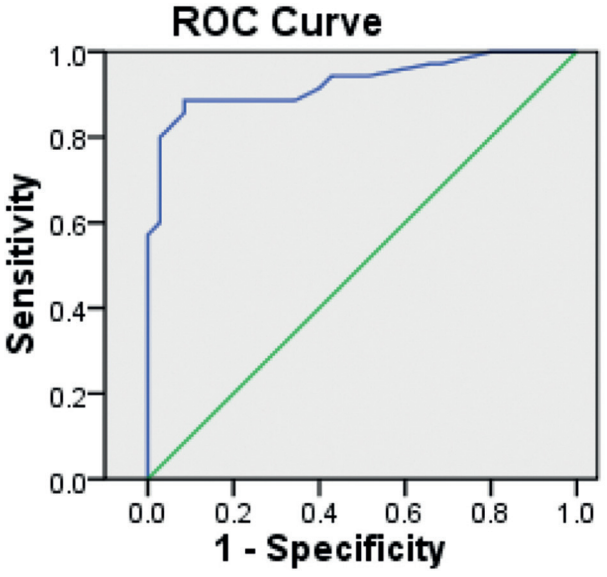

Figure 2. ROC curve of ascitic fluid ADA activity. Diagonal line indicates the line of no discrimination. AUC of this ROC curve is 0.928. SE = 0.032, 95% CI = 86.4% - 99.1%, P < 0.001. AUC: Area Under the Curve, SE: Standard Error, CI: Confidence Interval.

DISCUSSION

With the lack of specific clinical and laboratory markers, extrapulmonary manifestations of Mycobacterium tuberculosis in general and tuberculous peritonitis in particular have posed complex diagnostic challenges for centuries.18 Tuberculous peritonitis is usually paucibacillary and the classical method of Ziehl-Neelsen stain for TB bacilli have a low diagnostic yield with reported sensitivity ranging from 0 to 6% and culture positivity for TB bacilli varies from 20-83%.19,20 Analysis of ascitic fluid often shows lymphocytic predominance with a serum to ascites albumin gradient of <1.1 g/dL should alert the clinician to the possibility of tuberculous peritonitis and trigger more invasive diagnostic procedures.21 A peritoneal biopsy is usually done via laparoscopy or laparotomy to minimize any possible diagnostic delay. Computed tomography of the abdomen is the most useful radiographic study.22 Even though rapid diagnostic tests, such as polymerase chain reaction (PCR) for tuberculous peritonitis are promising, the role of ascitic fluid PCR is not firmly established.18 In areas with a high prevalence of TB, there is an urgent need for an alternate highly sensitive and a highly specific test for the early and accurate diagnosis of tuberculous peritonitis.

ADA is a helpful diagnostic tool in tuberculous ascites, specificity and sensitivity as high as 97 and 100% respectively, when the level is above 33 U/L.9 In this study, ADA level (mean ± SD) in suspected tuberculous peritonitis was 48.51 ± 17.91 U/L while in the transudative ascites, the control group it was 19.28 ± 7.69 U/L (highly significant, P < 0.001). The ascitic fluid ADA ROC analysis revealed the best cut-off value of 41.5 U/L yielding a good sensitivity of 80.0% and a high specificity of 97.14% to validate its use as a reliable diagnostic marker in these cases.

Kaur A et al.,[13] showed that ADA is not of sufficient discriminative value for diagnosing TB in peritoneal fluid with a sensitivity, specificity, positive and negative predictive values of 89%, 81%, 25% and 99% respectively (ADA >15 U/L). Dwivedi M et al.,[10] studied 49 patients with ascites of which [19] were of tuberculous etiology where at an ADA level of >33 U/L, the sensitivity, specificity, positive and negative predictive values were 100%, 96.6%, 95% and 100% respectively. Gupta V K etal.,[23] analysed [24] ascitic fluids samples, of which seven were due to tubercular etiology and with an ADA level of >30 U/L, the sensitivity and specificity were 100% and 94%. The sensitivity and specificity for tubercular ascites on the basis of ADA levels were 100% and 97% respectively, as per the study of Bhargawa etal.[11] Agarwal24 studied 30 cases of tuberculous ascites and using a cut-off value of 40 U/L reported sensitivity, specificity, positive and negative predictive values of 96%, 80%, 96% and 80% respectively. Thus ADA activity is a practical and useful approach to take therapeutic decisions in patients with suspected peritoneal TB. The beginning of empirical treatment when a patient has a high ADA value in ascitic fluid seems to be a good approach while waiting for the results of mycobacterial cultures and biopsies.[25] The results of the study clearly showed that ADA levels are significantly elevated in suspected tuberculous peritonitis as against non-tuberculous ascites causes. Estimating ascites ADA levels has a particularly significant role in areas where either the facilities for culture of TB bacilli or tissue biopsies are not available. A high ascites ADA value is strongly suggestive of it being of tubercular origin. A low ascites ADA value not necessarily eliminate it of not being of tubercular origin, but strongly suggestive of non-tubercular origin.

The study had a few limitations. The major one was that the investigation was carried out on the suspected cases of TBP and definite diagnostic tests such as culture or biopsies were not performed. Isoenzymes ADA1 and ADA2 levels were not determined individually in the ascitic fluid specimens. Further studies with a larger numbers of proven cases of tuberculous peritonitis are needed before definitive conclusions can be drawn. In addition to clinical findings and radiologic characteristics, ascites ADA estimation should also be considered in cases of tuberculous peritonitis especially in cases where the conventional methods like smear microscopy and culture often fail to establish an early diagnosis. Estimating ascites ADA levels in tuberculous peritonitis cases is a simple, rapid, inexpensive and the least invasive, highly specific and fairly sensitive method and significantly elevated ascites ADA levels are highly suggestive of tuberculous etiology and of diagnostic relevance.

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