Application of PCR and Adenosine deaminase assay test for the diagnosis of tuberculous meningitis infection

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Abstract

Introduction: Tuberculous meningitis is a medical emergency that low sensitivity of CSF smear staining and mycobacterial culture make its diagnosis difficult, so there is an urgent need for rapid, accurate and reliable laboratory test. The aim of this study was comparing the sensitivity and specificity of CSF adenosine deaminase (ADA) level with Polymerase Chain Reaction (PCR) test in the diagnosis of tuberculous meningitis (TBM).

Methods and Materials: In the period between January 2007 and January 2008 we had 49 patients with primary diagnosis of TBM in Bou-Ali University hospital. After committing and using the definite and probable TB as a golden standard, the final diagnosis for 29 of them was TBM.

Results: Our study showed that the sensitivity and specificity of CSF-ADA level in comparison with PCR results in diagnosis of TBM with a 6.5 IU/L cut-off were 100% and 85% respectively. Our study showed that selecting a 6.5 IU/L cut-off with respect to the PCR results of the patients makes a reasonable sensitivity and specificity for CSF-ADA test in TBM diagnosis.

Conclusion: Our study emphasized that CSF-ADA level measurement can be used as a good, rapid and reliable laboratory test for diagnosing tuberculous meningitis.

Key words: Tuberculous Meningitis, ADA, PCR

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Introduction

Five decades of tuberculosis control programs using potentially efficacious drugs and with the availability of BCG vaccine, have failed to reduce the prevalence of the infection in most parts of the world[1,2] and tuberculosis continues to kill young and middle-aged adults faster than any other disease apart from acquired immune deficiency syndrome (AIDS).

Some complicating factors such as: the emergence of multidrug-resistant TB,[3] HIV co-infection,[4] lack of patient compliance with chemotherapy, and variable efficacy of Bacillus-Calmette Guerin (BCG) vaccine has exacerbated the situation .Therefore, there is an urgent need for identifying new diagnostic laboratory tests for the identification of mycobacteria and eventually, developing new diagnostic tests .

Adenosin deaminase (ADA) is an enzyme that catabolizes adenosine to ionsine and ammonia by hydrolytic deamination.[5,6]ADA is produced by different cells of immunity system and mainly by immature T cells.[7,8]

ADA may be used as an indicator of activation of cell mediated immunity especially in circumstance in which T cells are involved.[9] However, increased level of ADA was observed in tuberculosis, bacterial infections, rheumatologic disease and lymphoproliferative disorders.[10]

So, in this study we compared the level of ADA in CSF samples of tuberculous meningitis patients with the results of microscopy, culture and PCR to evaluate the diagnostic value of these levels.

Methods and Materials

Between January 2007 and January 2008 we had 49 patients with presumptive diagnosis of tuberculous meningitis in Bou-Ali University hospital and among whom 29 had the final diagnosis of TBM after combining and using the definite and probable TB (on the basis of microscopy, PCR, and response to anti TB drugs) as a gold standard.

The British Medical Research Council (BMRC) system for grading meningitis further was used to describe neurological status of patients who were divided into three category. Category I: patients who were fully conscious and rational without neurological signs; Category II: patients who were confused but not comatose or had neurological signs; Category III: patients who were comatose or stuporose or had multiple cranial nerve palsies or complete hemiplegia or paraplegia.

One CSF sample from each patient underwent cell count, biochemistry and ADA measurement and the other was centrifuged and aliquoted into three 200 µl for microscopy, culturing and PCR respectively. All the CSFs underwent Auramin staining and culture on Lowenstein-Jensen media.

DNA extraction and PCR:DNA was extracted from 200 µl of centrifuged resuspended deposit of CSF sample

using DNA Extraction kit(Fermentas, Tehran, IRAN) and amplification was done with the Mycobacterium tuberculosis PCR kit (Cinnagen, Tehran, IRAN) in a Mini MJ Thermal Cycler apparatus (Bio Rad) according to the following program:93ºC for 60 sec and 72 ºC for 30 sec followed by 37 cycle 93 ºC for 20 sec,72 ºC for 30sec and ended by one cycle 93 ºC for 20sec and 72 ºC. Amplified DNA was detected by electrophoresis of 10 µl of amplified product on 1% agarose gel with 0.1% ethidium bromide. The amplified product at 160 bp region was detected. Gel Doc (UVP, USA) was used for documenting the gel picture. On the basis of the kit's instruction, positive control and DDW of the kit were used as the positive and negative control respectively.

For ADA evaluation, we used the results of ADA test which was performed in the laboratory of our University hospital on the basis of kit's manual . We took 6.5 IU/L of CSF-ADA level as cut-off as suggested by Baheti[11] and Merrikhi[12] previously. We used SPSS software for analyzing our results. We did Chai square test for qualitative variables and t-student test for quantitative variables.

Results

In this study we had 49 patients with primary diagnosis of tuberculous meningitis in Bou-Ali University hospital among whom, with combining and using the definite and probable TB as a gold standard, 29 had a final diagnosis of TBM.

Comparison of the characteristics and cerebrospinal fluid parameters in TBM patients in two PCR positive TBM and ADA positive TMB patients have been shown in Table 1.

Table 1:Comparison of patients characteristics and cerebrospinal fluid parameters in tuberculous meningitis

	PCR POSITIVE* TBM	**ADA POSITIVE *TBM*** **	
Age(years, mean[SD])	42.6, 19.0	46, 19.6	
Gender(male)	17	9	
**State of Disease BMRC I BMRC II	12 16	5 9	
CSF Parameters			
Total cell count(/µl, mean[SD]) Polymorphonuclear cell(% , mean[SD]) Percentage of Lymphocytes(%, mean[SD])	318.9, 436.2 28.9, 30.3 64.6, 33.0	381.0,521.4 29.0, 34.3 68.8, 32.9	
Glucose(mMol/L,mean[SD])	58.1, 25.1	63.9, 28.0	
Protein (g/L, mean[SD])	53.7, 82.7	77.1, 109.5	
Adenosine deaminase Level (IU/L, mean[SD])	12.3, 18.2	19.0, 20.4	
P<0.05 *TBM: On the basis of microscopy , culture, PCR , and response to anti TB drugs **The British Medical Research Council (BMRC) system *****6 missing data**			

Our data suggested that there is not any significant relation between ADA levels, protein, glucose and the total cell count content of CSF samples of TBM patients (P>0.05) but there is a significant relation between ADA level and severity of the clinical nervous symptoms of TBM patients (P=0.04,df=11). Microbiological findings of the microscopy, culture and PCR of TBM patients are shown in Table 2. PCR and the results of PCR positive patients were shown in Figure1.

Table 2-CSF-ADA level results in comparison with Microscopy, Culture and PCR

	ADA	
	+	-
Microscopy		
Positive	1	0
Negative	12	9
Culture		
Positive	5	2
Negative	7	8
PCR		
Positive	11	2
Negative	0	9

ADA cut-off value=6.5 IU/L

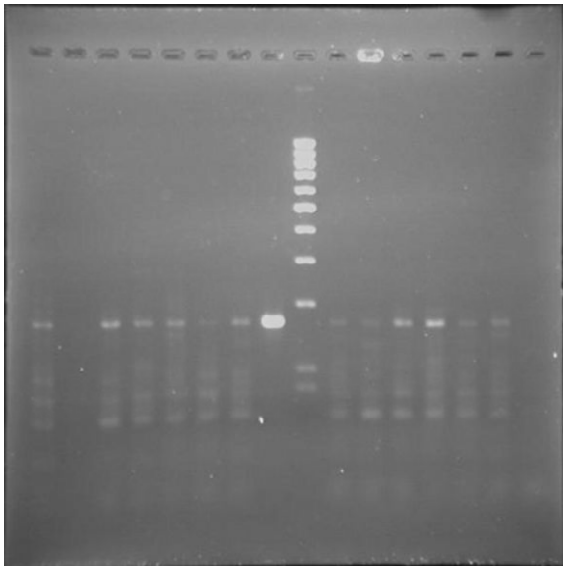

Fig.1_Polymerase Chain Reaction results of CSF samples of TBM patients; Line 1-7:TBM patients, Line 8:Control Positive(a 166bp band),Line 9:1 Kbp Ladder, Line 10-15:TBM patients, Line 16:Control Negative

In this study sensitivity and specificity of ADA in comparison with culture were 41.6% and 80% respectively. Our data also showed that sensitivity and specificity of ADA in comparison with PCR were 100% and 85% respectively.

Discussion

In the interpretation of CSF-ADA levels for diagnosis of TBM two important point must be considered.1) the definition of TBM patients which may have a direct impact on the results of this interpretation and 2) the value of Cut-off which has a great importance in the evaluation of the sensitivity and specificity of the CSF-ADA test. The amount of this cut-off is controversial at the present time.

Parsad and his colleague[15] reported a sensitivity and specificity of 100% and 97.87% respectively with a 3.30IU/L cut-off value for ADA in the diagnosis of tuberculous meningitis. On the other hand, Chotmongkol etal in their study[16] reported 75% sensitivity and 93% specificity for CSF-ADA level in diagnosis of TBM with a 15.5IU/L cut-off and Kashyap et al[17] in their study reported that with a 11.39 IU/L cut-off , the sensitivity and specificity of ADA measurement in diagnosis of TBM in CSF samples of their patients were 82% and 83% respectively.

Corral et al.[18] reported a 57% sensitivity and 87% specificity with a 8.5IU/L cut-off for CSF-ADA level in the diagnosis of TBM in HIV infected patients and Gautam etal.[19] in their study reported the sensitivity and specificity of 85% and 88.0% respectively for CSF-ADA levels in diagnosis of TBM with a 6.97IU/L cut-off value. Behati and his colleagues[11] reported 95.85% sensitivity and 92.85% specificity for CSF-ADA test in differentiating tuberculous meningitis from non tuberculous meningitis with a 6.5IU/L cut-off.

PCR studies in TBM patients revealed very different sensitivities and specificities .Some showed a good and acceptable sensitivity and specificity for this test in TBM diagnosis[21,22] while some other studies showed poor sensitivity and specificity for PCR in TBM diagnosis.[23]

In this study, sensitivity and specificity of CSF-ADA level in comparison with PCR results in TBM diagnosis in our 29 TBM patients with a 6.5 IU/L cut-off were 100% and 85% respectively. Our study also showed that CSF-ADA level has a significant relation with the results of PCR in the diagnosis of TBM (Pearson Chi-Square=0.018, df=11) and it may be used instead of PCR in less developed areas. Our finding showed that there is a significant relation between CSF-ADA level and the severity of the nervous symptoms ;the result which is in accordance with Jakka et al. study(20). In these two studies there is a direct association between the level of CSF-ADA and the adverse neurological outcome in studied population.

This study along with Gautam et al.[19] and kashyap et al. studies,[17] reemphasized that CSF-ADA level measurement can be used as a good, rapid and reliable laboratory test for

diagnosing tuberculous meningitis at least in high prevalence and in cases of the incidence of tuberculosis in low income regions.

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کاربرد PCR و تست اندازه گیری سطح آدنوزین دآمیناز مایع نخاع در تشخیص مننژیت سلی

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چکیده

سابقه و هدف: مننژیت سلی یک اورژانس پزشکی است که حساسیت کم رنگ آمیزی و کشت مایکوباکتریومی تشخیص آنرا مشکل می سازد و بنابراین نیاز فوری برای گسترش و ابداع آزمایشات بالینی صحیح ، سریع ، و قابل اعتماد برای آن وجود دارد.هدف از این مطالعه مقایسه حساسیت و ویژگی سطح آدنوزین دآمیناز مایع نخاع با تست واکنش پلی منراز زنجیره ای (PCR) در تشخیص مننژیت سلی (TBM) بوده است.

روش بررسی: در طی دی ماه ۱۳۸۵ تا دی ماه ۱۳۸۶ ، ۴۹ بیمار با تشخیص اولیه مننژیت سلی در بیمارستان بوعلی شهر زاهدان پذیرش شدند و از میان آنها با تلفیق تعاریف سل احتمالی و سل قطعی بعنوان استاندارد طلائی، برای ۲۹ نفر تشخیص نهائی TBM داده شد.

یافته‌ها: مطالعه ما موید آن است که می توان از تست اندازه گیری سطح ADA مایع نخاع بعنوان یک تست آزمایشگاهی خوب ، سریع و قابل اعتماد در تشخیص مننژیت سلی در نواحی که دسترسی به روش های مولکولی وجود ندارد ، استفاده کرد.

نتیجه‌گیری: مطالعه ما نشان داد که حساسیت و ویژگی اندازه گیری سطح ADA مایع نخاع با در نظر گرفتن سطح تشخیصی ۶/۵IU/L به ترتیب ٪۱۰۰ و ٪۸۵ بوده است. همچنین این مطالعه موید آن است که انتخاب سطح تشخیصی ۶/۵IU/L با توجه به نتایج PCR بیماران ، حساسیت و ویژگی قابل قبولی برای تست ADA در تشخیص TBM بوجود می آورد.

واژگان کلیدی: مننژیت سلی ، **ADA** ، **PCR**