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A Five Years Study of Tuberculous Meningitis in Iran

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ABSTRACT

Background: Tuberculous meningitis (TBM) is a severe form of extra pulmonary tuberculosis with high mortality and morbidity rate in all age group patients specific in adults and children. The incidence and prevalence are not exactly known in Iran. In this study, we tried to evaluate the role of rapid diagnosis and to find out the highest risk group patients.

Methods: Totally, 1783-suspected patients with tuberculous meningitis whose CSF specimens were admitted at Noor Pathobiology Laboratory, Tehran, Iran were enrolled in this study from January 2009 until December 2013. All specimens were checked for MTB by direct examination, culture and PCR tests, and for the adenosine deaminase (ADA).

Results: Confirmed positive cases were aged from 13 to 82 yr old with mean age 46.63 yr (SD±18.84). The number of diagnosed positive MTB was different by the 3 applied protocol, 64 by PCR, 28 by culture and 33 by direct examination. Considering the result of PCR protocol the TBM was approved in 64 patients with rate of 3.59%. Two patients had other infection as well, one 56 years old with VZV and the other patient who was HIV positive was 27 years old. Increased ADA titer higher than cutoff was relevant with other results of positive samples except in two cases.

Conclusion: Analysis of the results proved adults are more at risk for tuberculous meningitis than children in Iran are. It is also confirmed PCR method provide the most efficient, rapid and reliable results for these patients who are at the critical situations.

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Introduction

Tuberculous meningitis (TBM) is the most severe complication of TB that frequently occurs and reports in childhood. The patient history and clinical manifestations of tuberculosis are different in children that those observed in adults (1). Neonates have the highest risk of progression to severe forms of tuberculosis. Mortality is believed to be highest in early childhood because of a high incidence of disseminated forms of tuberculosis in this population (2, 3). It is reported 20–60% of the children dying of tuberculosis are known to have brain/meningeal involvement, in

contrast to 5% or less in adults especially in the developing countries (4). The incidence TBM was indicated 31.5 per 100 000 in neonates as compared with 0.7 per 100 000 among older children (10–14 years) (5).

Early identification of tuberculous meningitis is important and crucial for the success of the treatment. Diagnosis often remains problematic despite many significant advances in diagnostic techniques. Therefore, tuberculous meningitis has remained a serious problem for the physician because of the difficulty in making an early diagnosis and the severe consequences of delaying treatment. The detection of Mycobacterium tuberculosis DNA is widely used in cerebrospinal fluid samples using polymerase chain reaction diagnostic method (6, 7). In several studies results of PCR method was compared with the results of smear and culture with successful outcome (8-11). However, the total number of tuberculosis cases in the world is increasing (12).

This study was planned to evaluate the frequency rate of tuberculous meningitis positivity among all received CSF specimens.

Materials and Methods

Specimens

Those suspected patients to tuberculous meningitis whose specimens were accepted at laboratory were entered in the study from January 2009 till December 2013. Totally, 1783 CSF specimens were analyzed by PCR technique.

Ethic Consideration

We have not done any new experiment on the patients sample except those that requested. However, all patients' information including personal and laboratory results keep secure as a routine procedure by indicating a specific code to maintain confidentiality their records.

These specimens collected from various clinical laboratories to investigate for tuberculosis infections in Tehran and other provinces of Iran. Positive culture and PCR results were considered as criteria for positive TBM. All of the CSF specimens were obtained prior to the commencement of anti-tuberculosis chemotherapy by physicians at the hospitals and set straight to the laboratory. Specimens with unsuitable volume or received with improper conditions were all excluded from study.

Data Analysis

Clinical information about the patients was analyzed by Microsoft Excel (Version 2007).

Conventional Method

Smears were directly prepared to stain by Zeihl-Nelsen method for each specimen (13, 14). In addition, all specimens were inoculated onto 2 slops of Lowenstein–Jensen to incubate at 35ºC and examined for growth of M. tuberculosis, it was then confirmed by biochemical tests. Raising adenosine deaminase activity was also evaluated on CSF specimens by ADA kit (Diazyme Laboratories, US). According to the kit values in above the 9U/L was considered meaningful.

DNA extraction and PCR protocols

DNA extraction was performed in an identical manner for all patients' samples using High Pure PCR Template preparation kit (Roche Co.). The kit is designed to purify nucleic acids from different requested specimens for PCR test. It contains a primary step as a prelysis for some specific specimens such as tissue or even embedded tissue. The main steps are started of applying proteinase K and binding buffer (containing 6M guanidium-HCl,10 mM urea, 20% Triton X-100 and Tris-HCl) on samples, then use of inhibitor removal, washing and elution buffers respectively. At each step, reagents will be added

to the filter tube. Supernatant will be passed through collection tube after centrifugation. This procedure will highly reduce the contaminations and increase the efficiency of the recovery rate of the nucleic acids as much as possible.

PCR carried out on the prepared purified nucleic acid use of M. tuberculosis PCR kit (DNA Technology). It contained specific primers to target transposable element (IS6110) for amplification 330 base pair of template. 5 µl of template, 10 µl PCR buffer, 10 µl mixture (containing specific primers and dNTP, 2.5 U taq polymerase) were mixed and amplified with the recommended program (Table 1). The applied PCR kit was constructed in a format of competitive PCR with internal control. Provided specific primers could also amplify a product from fragment encoding 900 base pair as internal control to ensure of proper extraction and removal of any expected inhibitors. This fragment was added before commencing extraction procedure. The kit were also contained specific labeled probes for specific and internal products to enable us for detection the amplified products by the fluorescence detection, called Fluorescent

Table 1
Frequency of received specimens and positive cases during five years study

Year	Received Specimens n(%)	Positive Specimens n(%)
2013	285 (15.98)	13 (20.31)
2012	408 (22.88)	14 (21.88)
2011	443 (24.85)	13 (20.31)
2010	342 (19.18)	12 (18.75)
2009	305 (17.11)	12 (18.75)
Total	1783 (100)	64 (100)

Amplification-based Specific Hybridization method (DNA Technology).

Results

Totally, PCR were positive in 64 cases (Table 1). The frequency of TBM positive rate was 3.59% in 1783 tested CSF specimens. The patients ages were from 13 to 82 years old with

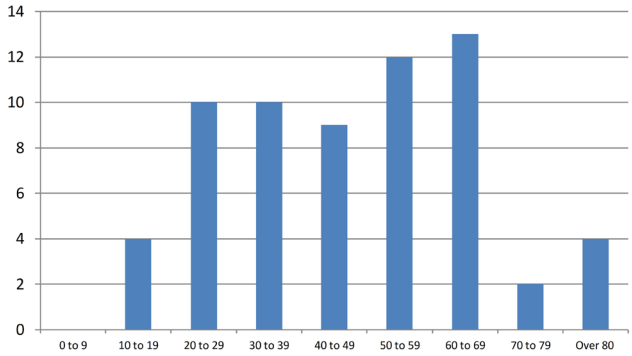

Fig. 1
Frequency the No. of tuberculous meningitis in various age groups

mean age 46.63 (SD±18.84) (Fig. 1). Female and male patients were 34 and 30 respectively.

Fourteen positive cases were of those specimens received from other provinces (Ahvaz, Tabriz, Gorgan, Zahedan, Shahrood, Qazvin, and Boushehr, and the rest 50 specimens were received from Tehran`s hospitals.

Number of positive specimens was 28, 33 and 64 by culture, smear and PCR in 64 approved positive cases respectively. Twelve specimens were smear negative- culture positive and 17 samples were smear positive – culture negative. Two patients had mixed infection, one with VZV who was 56 years old, and the other HIV with 27 years old. Analysis of the ADA results was also proved just 50% of the patients had meaningful results in agreement with Tuberculosis infection, 33.35% and 16.65% had the lower activity than 9 U/L and equal with it. ADA in one patients was repeated after 3 weeks and raised from 9 U/L to 21 U/L

Discussion

Extrapulmonary tuberculosis is a risk factor for TBM. There are a few prospective studies describing tuberculous meningitis in the world and no reports from Iran as far as we looked for it

in various medical databases. We therefore based this study just on laboratory findings in received patient's specimens who were suspected to have TBM.

We had no positive specimens in infants and children up to 10 years old. It confirms that TBM mainly affects adult patients in the studied suspected patients, since BCG vaccination program covers nearly all infants throughout the country. A meta-analysis of the published trials on the efficacy of BCG vaccination suggested a protective effect of 64% against TBM, although BCG vaccination is generally accepted to provide protective against TBM. However, this result can be a confirmation for reduction the effectiveness of vaccination in adults or might be the meaning of improper treatment. We had no positive specimens in infants and children up to 10 years old that may be due to highly covered vaccination program in our country. On the contrary, these results might be the meaning of improper treatment that needs to design separate study in this regards.

Although, the demonstration of acid-fast bacilli (AFB) in the cerebrospinal fluid (CSF) remains most favorable diagnostic and the most widely available method, but as the analyzed results revealed its sensitivity is remarkable lower than PCR. Smears were positive in 33 specimens (51.56%) of all 64 PCR positive cases, in agreement with other reports (15). On the other hand, we observed culture results were negative in 17 of smear positive cases. It means those patients were most probable under antibiotics treatment. Therefore, culture although is a time-consuming method its efficacy is also significantly lower than PCR that makes it nearly to be inefficient for TBM.

Association of HIV with TBM was low in this study. It more probably is due to low rate of HIV infection. It is frequently reported the rate of HIV is increasing in young adults (16). Therefore, it can be expected the frequency of TBM to be increased by increasing of HIV rate in adolescents. On the other hand, ADA results were in agreement with the TBM in 50%. ADA activity may need more time to be increased. The role of ADA in diagnosis of TBM has been investigated in other reports. Choi et al. has reported 58% sensitivity (17), while Kashyab et al. confirmed 82% for the sensitivity of ADA in TBM patients (18). Therefore, increased ADA is helpful but should not be a crucial criterion in TBM. No positive results in children less than 10 years and on the contrary observing the TBM in adults suggests design a separated study on the efficacy of tuberculosis treatment.

Conclusion

Analysis of the results proved adults are more at risk than children in Iran for tuberculous meningitis are. It is also confirmed that PCR method provide the most efficient, rapid and reliable results for these patients who are at the critical situations.

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