ORIGINAL ARTICLE

Diagnosed tuberculous meningitis using cerebrospinal fluid polymerase chain reaction in patients hospitalized with the diagnosis of meningitis in referral hospitals in Isfahan

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Background: Tuberculosis (TB) remains one of the leading infectious diseases throughout the world. Among various forms of extrapulmonary TB, tuberculous meningitis (TBM) is the most severe form and remains a major global health problem with a high mortality rate. Our study was designed to evaluate tuberculous polymerase chain reaction (PCR) positive rate in patients who present with fairly long symptoms of meningitis. **Materials and Methods:** The 162 Patients with an indolent onset of symptoms compatible with central nervous system infection were admitted. Sample of cerebrospinal fluid (CSF) was evaluated for biochemistry and tuberculous real-time PCR. Data analyzed by Student's *t*-test and Fisher's test. **Results:** Patients were mostly male (69.8%), with a median age of 43.69 ± 22.67 years. CSF real-time PCR results in 6 patients (3.7%) were positive for tuberculous DNA. Of these 6 patients, 4 of whom were men and two of whom were women. In other words, the frequency of positive tuberculous DNA was in male 5.3% and female 1.4%, respectively. **Conclusion:** Given that we live in Iran and in the vicinity of the tuberculous endemic countries, if we face a meningitis case with lasting symptoms and tendency to be chronic, TBM should be considered.

Key words: Meningitis, real-time polymerase chain reaction, tuberculosis

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INTRODUCTION

Meningitis is the inflammation of meninges that results in many signs and symptoms.[1] A wide variety of causes include infectious and noninfectious are important. Among the infectious causes of meningitis, some result in chronic meningitis that defines as the signs of meningitis last for weeks to months. In this group, fungal and tuberculosis (TB) are important causes; and TB meningitis is the commonest cause.[2] Central nervous system (CNS) infection due to TB includes three clinical categories: Meningitis, intracranial tuberculoma, and spinal involvement. All these forms of CNS infection are encountered frequently in regions where the incidence of TB is high.[1]

Central nervous system TB accounts for about 5% of all cases of TB.[3] Among various forms of extrapulmonary TB, tuberculous meningitis (TBM) is the most severe form and remains a major global health problem with a high mortality rate.[4]

Early recognition of TB meningitis is of paramount importance because the clinical outcome depends greatly upon the stage at which therapy is initiated.[1] According to the serious complications and sequels of TBM, it is important to be aware of the prevalence of these entities in any region and also to diagnose rapidly and properly and avoid from inappropriate treatment. One of the new methods that could provide important aids for the diagnosis of TB meningitis is cerebrospinal fluid (CSF) polymerase chain reaction (PCR).[1] PCR as a diagnostic test for TBM has a sensitivity of 50% and a specificity close to 100%.[5] Use of real-time PCR for TB results in early diagnosis and treatment, therefore, results in decreasing mortality and morbidity.

Our country is in the neighborhood of the area with endemic TB, and we have a lot of immigrants from those countries repeatedly. Because of the PCR is so specific and considerably better than TB culture and smear our study was designed to evaluate TB PCR positive rate in patients who present with fairly long symptoms of meningitis. To answer this question: Is there any TB PCR positive in cases with nontypical clinical picture of meningitis?

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MATERIALS AND METHODS

Patients were recruited at Alzahra Hospital and Kashani Hospital, top referral hospitals in Isfahan, Iran. Patients with clinical symptoms of meningitis were admitted. They had symptoms compatible with CNS infection (for example fever, headache, loss of consciousness, nausea, etc.) with or without meningeal sign(s) in physical examination (neck stiffness, Kernig's sign, Brudzinski's sign). The patients with more than 1-week of symptoms initiation were evaluated; in the other hand the patients were in the subacute phase. If there was no contraindication for performing a lumbar puncture (LP), LP was done. Patients with pleocytosis in the CSF (CSF white blood cell >5) entered the study; also these patients were received no more than one dose of antibiotics. Although in typical TB meningitis there is significant pleocytosis with lymphocyte predominance and high CSF protein levels and significant decrease of CSF glucose levels (hypoglycorrhachia), but in the first 2-3 weeks of symptom initiation, typical CSF pattern of TB meningitis may be absent.[6] So not only typical or suggestive TB meningitis CSF patterns entered, but also patients with normal or mildly increased levels of CSF protein, normal or mildly decreased CSF glucose levels, neutrophil predominance were entered the study. In the other hand, all patients with CSF pleocytosis were included. Patients with mass-like lesion in imaging, brain abscess, HIV positive, posttraumatic, abrupt onset of symptoms, postsurgery and children were excluded. Our Sample consisted of 162 individuals. Written informed consent was obtained from the patient or a close relative for those who are unstable or unconscious at the time of presentation.

Required information including age, gender, main complaint, other symptoms of the disease, duration of the symptoms onset before hospitalization, records of close contact, were collected using standardized forms. Enrolled patients underwent a detailed workup for meningitis including blood investigations, imaging (Chest X-ray, computed tomography [CT] scan and/or magnetic resonance imaging as and when indicated).

Following a lumbar puncture with standard and sterile method, about 10 ml CSF was obtained, transported to the laboratory within 1 h, and divided into two tubes: 1 (1-2 ml) for CSF cells, protein and glucose, bacterial smear and culture and 1 (8-10 ml) for TB PCR testing.

DNA was purified from 200 μl of centrifuged deposit of CSF sample using DNA Extraction kit (Roche, Germany) with standard DNA extraction protocol. Three sets of primers targeting different regions of the *Mycobacterium tuberculosis* genome were: PRIMER 1(5-GGCTGTGGGTAGCAGACC-3), PRIMER 2(5-CGGGTCCAGATGGCTTGC-3), PROBE(5-FAM-TGTCGACCTGGGCAGGGTTCG-TAMRA-3). *Mycobacterium tuberculosis* primers were synthesized by METABION (METABION, Germany). Then PCR was performed in real time PCR (QIAGEN-Rotor gene-Q) system with the thermal cycling according to the following program : 95°C for 6 min 1 time then ended by temperature cycling 95°C for 15 s and 58°C for 20 s and 72°C for 10 s in 40 times. In each run, positive and negative controls were used to control. Then the results were analyzed using the software program (SPSS Inc., Chicago, IL, USA). All statistical analyses were performed by Student's *t*-test and Fisher's test. Research project number of this study is: 289256.

RESULTS

A total of 162 consecutive patients with suspected meningitis were included. The mean age of patients was 45.63 ± 21.9 years old. Patients were mostly male (69.8%), with a mean age of 43.69 ± 22.67 years. The mean age of women was 49.86 ± 20.46. No statistically significant difference was detected between age of men and women by Student's *t*-test ($P = 0.2$).

In Figure 1, frequency of patient's age is shown. The most common age group of patients hospitalized with the diagnosis of meningitis was 20-29 years (33 patients-20/4%).

Cerebrospinal fluid reverse transcription-PCR results in 6 patients (3.7%) were positive for TB DNA. Of these six patients, four were men, and two were women. In other words, the frequency of positive TB DNA was in male 5.3% and female 1.4%, respectively. According to Fisher's test, there was no significant difference between the sexes ($P = 0.99$) [Table 1].

The mean age of patients with TB PCR positive was 43.6 ± 26.39 years, and TB PCR negative was 45.31 ± 22.15 years. Using *t*-test, no significant difference was observed between the two groups ($P = 0.87$). In Figure 2, the prevalence of TB DNA-positive cases by age group is shown.

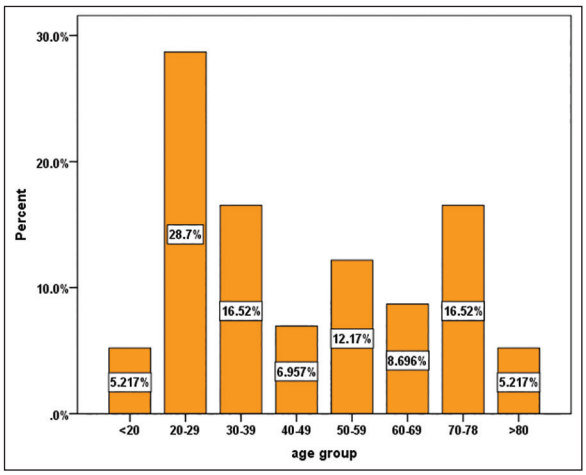

Figure 1: Frequency of age group in this study

Table 1: Distribution of TB DNA by sex

Sex TB DNA	Male		Female		Total	
	Numbers	Percent	Numbers	Percent	Numbers	Percent
Negative	109	96.5	47	95.9	156	96.3
Positive	4	3.5	2	4.1	6	3.7
Total	113	100	49	100	162	100

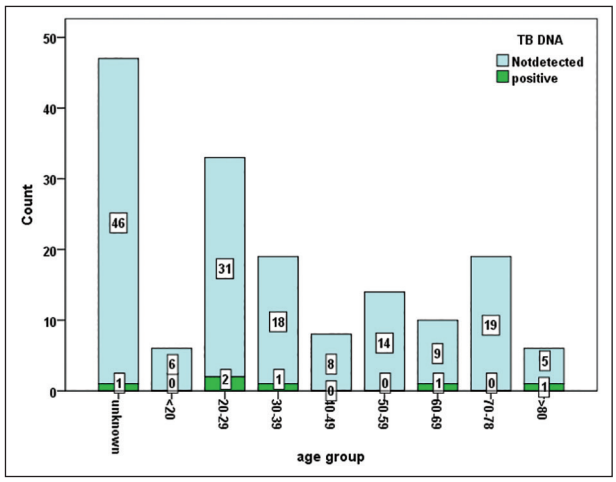

Figure 2: Frequency of TB DNA-positive cases by age group

DISCUSSION

Tuberculosis remained one of the leading infectious diseases throughout the world.[7,8]

Globally, there were an estimated 8.8 million incident cases of TB (range, 8.5 million-9.2 million) in 2010, 1.1 million deaths (range, 0.9 million-1.2 million) among HIV-negative cases of TB and an additional 0.35 million deaths (range, 0.32 million-0.39 million) among people who were HIV-positive. In the latest report, WHO determined 22 High TB Burden Countries (HBCs) that have been prioritized at a global level since 2000.[8,9] Afghanistan and Pakistan are in HBCs list, and we have a lot of immigrants from those countries repeatedly.

The exact incidence and prevalence of TBM in the most parts of the world are not precisely known. Some epidemiological details of extrapulmonary TB are available from developed countries. In developed countries, despite an overall decrease in numbers of TB cases, the proportion of extrapulmonary TB and TBM cases has increased.[10]

In 2005 in Iran, the incidence rates of TB had been 25-49/100000 population.[11] According to our study, 3.7% of cases were TB meningitis. And no statistically significant difference was detected between age of men and women. In accordance with previous studies, age and sex aren't significantly different.[12,13]

Among various forms of extrapulmonary TB, TBM is the most severe form and remains a major global health problem with a high mortality rate.[4]

Although national public health services follow a rigorous TB surveillance and treatment regimen to improve the impact of the disease, a better diagnostic test remains to be developed for the prescribed treatment regimen to be followed effectively.[13] The diagnosis of TBM has been a continuous challenge. Though the detection of mycobacterium by microscopy and culture remains the criterion standard for tubercular diagnosis, poor sensitivity in detection of low microorganism densities in CSF and mixed infections pose a significant challenge.[14] In addition, the time required for Lowenstein-Jensen cultures is about 8 weeks, which makes it unsuitable as a routine technique for rapid confirmatory diagnosis.[15] Thus, diagnosis basically remains presumptive and is based on clinical symptoms, neurologic signs, CSF findings, CT scans, and response to anti-TB drugs. Diagnosis often remains problematic despite many significant advances in diagnostic techniques. Early diagnosis is important for the success of the treatment. The CSF PCR assay represents a significant advance in the diagnosis of microbial diseases and TBM is no exception. The detection of *Mycobacterium tuberculosis* DNA in CSF samples using PCR is widely used diagnostic method because of its speed. A proper selection of *Mycobacterium tuberculosis*-specific DNA and a caution against contamination of CSF specimens are important for obtaining high specificity.[2]

Polymerase chain reaction studies in TBM patients revealed very different sensitivities and specificities. Entirely the most studies found low sensitivity for this test in TBM diagnosis (about 50%) however, the specificity was as high as 98%.[5,16-19]

The overall all of them believe in high specificity of PCR, so we designed this study to evaluate TB PCR positive rate in patients who present with fairly long symptoms of meningitis. Other studies such as in Thailand found that TBM was the second most important cause of chronic meningitis in their survey reflecting the importance of infectious diseases in tropical countries.[12] In a series of 83 cases of chronic meningitis from New Zealand, the most common diagnosed entity was TBM, which was found in 40% of the patients[20] that is higher than our study finding. Cases in our study were patients that their symptoms had not been started so acute or sudden, whereas they have investigated chronic meningitis that defines as suffering meningitis signs and symptoms for at least 4 weeks.[6]

Tuberculous meningitis is both an insidious disease, and it can have an atypical clinical picture so that the onset of TBM may manifest as acute or subacute meningitis. Identification of patients according to standard case definitions is important because early recognition and treatment of the disease is believed to be able to reduce the burden of this disease, lowering complication and mortality.

Limitations

There are some important limitations to our study. First, due to budget limitations and few numbers of cases with lasting symptoms of meningitis, we could only select 162 samples. Second, determining TB on acid fast bacilli (AFB) staining smear needs a large amount of CSF, but according to our patient's conditions, it was impossible to provide large CSF sample. Third, as TB culture lasts 6-8 weeks to be positive and has low sensitivity, we didn't perform this test. Further study with evaluating PCR, AFB smear And TB culture with larger sample size may be needed.

CONCLUSIONS

Given that we live in Iran and in the vicinity of the TB endemic countries, if we face a meningitis case with lasting symptoms and tendency to be chronic, TBM should be considered.

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AUTHOR'S CONTRIBUTION

KSh was contributed in concept, design, definition of intellectual content, clinical studies, manuscript preparation, manuscript editing, and manuscript review. ZT was contributed in literature search, clinical studies, data acquisition and manuscript preparation. MY was contributed in data acquisition and manuscript preparation. BA was contributed in definition of intellectual content and manuscript editing and review. AMK was contributed in, data analysis,and statistical analysis. FKh was contributed in design, definition of intellectual content and manuscript editing and manuscript review.

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