

FREQUENCY OF *M. TUBERCULOSIS* DETECTION IN CSF OF PATIENTS WITH SUSPECTED TUBERCULOUS MENINGITIS

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ABSTRACT;

Background; The annual incidence of tuberculous meningitis (TM) is unknown. TM is a disease that still often results in residual sequelae, and has a mortality rate ranging between 15 and 51%. Experience of countries such as Pakistan where drug-resistant tuberculosis and TM are prevalent is important. **Objective:** To determine the frequency of *M. Tuberculosis* detection in CSF of patients with suspected tuberculous meningitis at a tertiary care hospital. **Materials and Methods;** A total of 95 patients were included in this descriptive study. The demographic data like age, sex, height, weight, monthly family income and address of the patients was collected. Cases of TBM fulfilling inclusion and exclusion criteria were included in the study. CSF was collected after consent, through lumbar puncture (LP) done by the post graduate trainee (author or colleagues) under aseptic measures and was sent to the designated hospital laboratory for GeneXpert MTB/Rif assay and report was assessed for *M. tuberculosis* detection. Data was collected and entered in SPSS and analyzed. **Results;** total of 95 patients with tuberculous meningitis meeting inclusion criteria of our study were included in this study. Of these 95 study cases, 37 (38.9%) were male patients while 58 (61.1%) were female patients. Mean age of our study cases was 26.62 ± 5.94 years. Of these 95 study cases, 49 (51.6%) were from rural areas and 46 (48.4%) belonged to the urban areas while 62 (65.3%) were from poor families. Seventeen (17.9%) had diabetes and 25 (26.3%) were having history of hypertension. Mean weight of our study cases was 56.16 ± 10.27 kilograms and 66 (69.5%) had up to 60 kilograms of weight. *Mycobacterium tuberculosis* was noted in 49 (51.6%) of our study cases. **Conclusion;** High frequency of *Mycobacterium tuberculosis* was observed in our study in cerebrospinal fluid (CSF) of suspected patients of tuberculosis meningitis using genexpert MTB/RIF assay. *Mycobacterium tuberculosis* was significantly associated with age, socioeconomic status, diabetes and hypertension.

Keywords; Tuberculous meningitis, Mycobacterium TB, Genexpert.

INTRODUCTION:

Tuberculosis (TB) is a devastating illness caused by infection with *Mycobacterium tuberculosis* (MTB) and may involve any system of the body. It is estimated that about 1/3rd of the world's population is infected with tuberculosis (latent)¹. In 2014, an estimated 4 million new cases of TB appeared in South East Asia (SEA) WHO region which is about 41% of the estimated global TB cases². While in Pakistan, figures for new TB cases reached 3,08,417 in 2014³. About 10% of patients who have tuberculosis develop tuberculous meningitis (TBM)⁴, a severe and life threatening central nervous system (CNS) involvement. Approximately a third of patients with TBM die soon after presenting to the hospital and many of those surviving are left with severe neurological sequel⁵. Prompt diagnosis and treatment of TBM have been shown to be the best predictor of survival⁶. However, conventional confirmatory test available for TBM i.e. culture & sensitivity of cerebrospinal fluid (CSF) takes 1 to 4 weeks to return a positive test and time is one thing which a patient with TBM is very short of. Therefore the clinical value of culture technique is limited to diagnostic confirmation, drug susceptibility testing and molecular typing of *Mycobacterium tuberculosis*. Another most widely applied rapid diagnostic technique; Zeihl-Neelson (ZN) microscopic staining of CSF for acid fast bacilli (AFB) has a sensitivity rarely exceeding 20% for TBM⁷. With recent advances, more techniques have been evolved e.g. adenosine deaminase assay (ADA), nucleic acid amplification techniques (NAAT), Multiplex PCR, GeneXpert MTB/RIF etc., each of which has its own pros and cons⁸. Out of these GeneXpert has gained wide popularity.

The GeneXpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) is the only fully automated closed-cartridge real time DNA-based system that is easy to be operated by minimally trained staff and can detect TB in less than 2 hours and carries a smaller risk for biohazard and cross-contamination⁹. The Xpert MTB/RIF test was approved by the World Health Organization (WHO) in 2010 for the diagnosis of pulmonary TB following extensive evaluation projects in six countries led by the Foundation for Innovative New Diagnostics (FIND)¹⁰. The test has shown sensitivity above 90% for culture positive tuberculosis with high specificity in sputum samples, however Xpert MTB/RIF has also been used successfully for extra pulmonary samples including cerebrospinal fluid (CSF)¹¹. Since the introduction of the technology, cost per test has significantly dropped¹¹ and recently it is comparable to or even less than the conventional TB confirmatory test i.e. MTB culture & sensitivity. Availability has also increased rapidly in recent years. Currently 42 sites in Pakistan are offering GeneXpert³. To further betterment, under a project - known as TBXpert - by UNITAID, not only cartridge prices were dropped 40% leading to USD 9.98 per cartridge¹² in 145 countries but also 220 test machines and over a million cartridges were purchased in 2013 and with the co-ordination of WHO, installed free of any cost at various places in 21 countries of the developing world¹³ including Pakistan. As with the clinical practice, TBM cases in research are selected according to some clinical criteria known as composite reference standard (CRS) which in previous studies used to be author defined, causing diversity in definition and thus proving to be a major stumbling block in comparing various research findings and data. To address this problem, in 2010 at South Africa a committee was appointed by 41 international experts in research of TBM, to finalize a consensus criteria for tuberculous meningitis for use in future clinical research; i.e. Uniform Tuberculous Meningitis Research Case Definition¹⁴. This is kind of a scoring system (total score = 20) which distributes selected patients into three categories i.e. definite, probable and possible; where 'definite' being the ones with positive CSF *M. tuberculosis* culture or positive NAAT or AFB on smear microscopy in context of typical clinical findings and other two categories making up the suspected cases. Since the theme of this study is to use GeneXpert in place of other diagnostic tools and this that the use of assay in already confirmed cases of TBM can be a burden on resources and it is not a comparative study, segregation of certain number of patients into 'definite' category seems to reduce the yield of the study. Therefore confirmatory tests e.g. culture will not be included in this study and all the selected cases was termed 'suspected' only (score ≥ 6), irrespective of any category. When same approach was followed in part of a study at Vietnam in 2013 under the Oxford University Clinical Research Unit, researchers found 59.3% positive results in all the suspected cases¹⁵ (all categories combined) which were no more different than true positive results. According to a meta-analysis of 6 global studies, in an Expert group meeting report 2013 by WHO, pooled sensitivity of the assay in CSF was 55.5% when compared against composite reference standard (CRS)¹¹. The WHO Expert group recommends that Xpert MTB/RIF should be used in preference to conventional microscopy and culture as the initial diagnostic test when testing CSF from patients suspected of having TBM¹¹. Due to the urgency of diagnosis in suspected TBM cases, a rapid diagnostic test like Genexpert will have a great impact on survival. One published study in Pakistan in 2015, on Xpert MTB/RIF assay, included only one sample of CSF¹⁵.

Aim of this study was to determine frequency of *Mycobacterium tuberculosis* detection using GeneXpert MTB/RIF assay, in CSF of patients suspected to be suffering from TBM.

Materials and Methods

A total of 95 Patients fulfilling the diagnostic criteria in the Uniform Tuberculous Meningitis Research Case Definition (Score ≥ 6) aged less than 50 years of either sex were recruited in this cross-sectional study. Patients already on treatment for TBM, recurrent Tuberculous Meningitis, having contraindications to lumbar puncture i.e. local infection, bleeding tendencies, papilledema, etc; as evaluated on history and examination were excluded from our study. The patients were enrolled from the Inpatient department of medicine, Bahawal Victoria Hospital Bahawalpur. The demographic data like age, sex, height, weight, monthly family income and address of the patients was collected. Cases of TBM fulfilling inclusion and exclusion criteria were included in the study. CSF was collected after consent, through lumbar puncture (LP) done by the post graduate trainee (author or colleagues) under aseptic measures and was sent to the designated hospital laboratory for GeneXpert MTB/Rif assay and report will assessed for *M. tuberculosis* detection. Data was entered using software SPSS version 20.

RESULTS;

A total of 95 patients with tuberculous meningitis meeting inclusion criteria of our study were included in this study. Of these 95 study cases, 37 (38.9%) were male patients while 58 (61.1%) were female patients. Mean age of our study cases was 26.62 ± 5.94 years (with minimum age was 19 years while maximum age of our study cases was 41 years). Mean of the male patients was 27.35 ± 6.34 years while mean age of the female patients was 26.16 ± 5.68 years ($p=0.342$). Our study results have indicated that majority of our study cases i.e. 78 (82.1%) were aged up to 30 years. Of these 95 study cases, 49 (51.6%) were from rural areas and 46 (48.4%) belonged to the urban areas. Mean monthly family income was 21455.78 ± 5443.23 rupees and 62 (65.3%) were from poor families. Seventeen (17.9%) had diabetes and 25 (26.3%) were having history of hypertension. Mean weight of our study cases was 56.16 ± 10.27 kilograms and 66 (69.5%) had up to 60 kilograms of weight. *Mycobacterium tuberculosis* was noted in 49 (51.6%) of our study cases.

DISCUSSION;

Meningitis is the inflammation of meninges that results in many signs and symptoms. 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. 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.

A total of 95 patients with tuberculous meningitis meeting inclusion criteria of our study were included in this study. Of these 95 study cases, 37 (38.9%) were male patients while 58 (61.1%) were female patients. A study conducted by Nabi et al ¹⁶ from Islamabad reported 55 % female gender preponderance which is in compliance with our study results. A study conducted by Saleem et al from Lahore ¹⁷ also reported 52 % female gender predominance which is in compliance with our study results. A study conducted in Turkey by Yasar et al ¹⁸ reported equal distribution of male and female patients. A study conducted by Sher et al ¹⁹ from Karachi has also reported 57 % female gender predominance which is close to our study results.

Mean age of our study cases was 26.62 ± 5.94 years (with minimum age was 19 years while maximum age of our study cases was 41 years). Mean of the male patients was 27.35 ± 6.34 years while mean age of the female patients was 26.16 ± 5.68 years ($p=0.342$). Our study results have indicated that majority of our study cases i.e. 78 (82.1%) were aged up to 30 years. A study conducted by Nabi et al ¹⁶ from Islamabad reported 34.86 ± 17.54 years mean age of the patients with tuberculous meningitis which is close to our study results. A study conducted by Saleem et al ¹⁷ from Lahore also reported mean age for male patients were 34 ± 17 years and for female patients it was 29.81 ± 17.72 years ($p\text{-value}=0.398$) which is in compliance with that of our study results. Fazel et al ²⁰ from Karachi reported 29.65 years mean age of the patients with tuberculous meningitis which is close to our study results. A study conducted by Saleem et al from Lahore ²¹ also reported 34 ± 17 years mean age which is similar to our study results. A study conducted in Turkey by Yasar et al ¹⁸ reported 32.18 ± 13.62 years mean age of the patients with tuberculous meningitis which is close to our study results.

Of these 95 study cases, 49 (51.6%) were from rural areas and 46 (48.4%) belonged to the urban areas. Mean monthly family income was 21455.78 ± 5443.23 rupees and 62 (65.3%) were from poor families. Seventeen (17.9%) had diabetes and 25 (26.3%) were having history of hypertension. A study conducted by Shore et al²² from Lahore reported 22 % diabetes which is close to our study results.

Mycobacterium tuberculosis was noted in 49 (51.6%) of our study cases. a study at Vietnam in 2013 under the Oxford University Clinical Research Unit, researchers found 59.3% positive results in all the suspected cases¹⁵ which is in compliance with our study results. According to a meta-analysis of 6 global studies, in an Expert group meeting report 2013 by WHO, pooled sensitivity of the assay in CSF was 55.5%¹¹ which is close to our study results. Similar results have been reported by an Indian study conducted by Anuradha et al²³.

CONCLUSION;

High frequency of *Mycobacterium tuberculosis* was observed in our study in cerebrospinal fluid (CSF) of suspected patients of tuberculosis meningitis using genexpert MTB/RIF assay. *Mycobacterium tuberculosis* was significantly associated with age, socioeconomic status, diabetes and hypertension.

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