ORIGINAL ARTICLE

RAPID CONFIRMATION OF TUBERCULOUS MENINGITIS IN CHILDREN BY LIQUID CULTURE MEDIA

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Background: Tuberculous meningitis (TBM) is an important and serious complication of tuberculosis (TB) related to high morbidity and mortality. Childhood TBM is a diagnostic dilemma as the clinical features are non-specific and cerebrospinal fluid (CSF) mycobacterial count is low. The diagnosis is made mostly on clinical grounds, CSF analysis and radiological findings and treatment is initiated before the culture results arrive. These non-specific rapid diagnostic tools may be misleading resulting in over diagnosis or misdiagnosis. The conventional way of establishing a definitive diagnosis of TBM is by solid-medium culture that requires 4–7 weeks which may often lead to injudicious exposure to hazards associated with anti-tuberculous therapy (ATT). **Methods**: This study was carried out at the Microbiology department of University Of Health Sciences Lahore. The aim of this study was to assess the reliability of clinical features and CSF analysis in diagnosing childhood TBM by utilizing BACTEC Mycobacterial Growth Indicator Tube (MGIT) 960 system as a rapid confirmatory tool. It was an observational study that included 100 CSF samples from suspected paediatric TBM cases. After carrying out the biochemical and cytological analysis the samples were subjected to Ziehl-Neelsen (ZN) staining and inoculated onto MGIT 960 system. The culture growths were tested for sensitivity to first line ATT. **Results:** Of the 100 samples inoculated, only 14 yielded a growth, thus emphasizing unreliability of clinical criteria to diagnose TBM. CSF biochemistry was found insignificant for the diagnosis of TBM (p=2.260). The CSF cytology, however, was found significant for diagnosing TBM (p=0.001).**Conclusions:** The study showed that BACTEC MGIT 960 can be effectively used as a rapid diagnostic tool for the diagnosis of extra pulmonary tuberculosis.

Keywords: *Mycobacterium tuberculosis*, Cerebrospinal fluid, BACTEC MGIT 960, Tuberculous meningiti

INTRODUCTION

Paediatric TB is a global health problem especially in the developing countries.[1] TBM is the most serious form of extra-pulmonary TB (EPTB), and is associated with high morbidity and mortality in children. TBM occurs in 7–12% of tuberculous children, neurological sequelae occur in 20–25% cases of TBM.[1] TBM accounts for 20–45% of all types of TB among children, when compared with only 2.9–5.9% of adult TB.[2]

In developing countries, up to 90% of symptomatic cases of pediatric TB are diagnosed solely from clinical history and examination. Non-laboratory investigations have a limited role in the diagnosis of childhood tuberculosis.[3] Tuberculin skin testing (e.g., the Mantoux test) gives negative results in up to 40% of children with tuberculosis, and radiographic findings are often non-specific.[3] TBM is sometimes difficult to diagnosis with certainty, especially in its early phase (about 1–2 weeks after onset). During the early phases of infection typical clinical manifestations of TBM have not fully developed, furthermore, polymorphonuclear pleocytosis in CSF during early phase give the impression of pyogenic meningitis making the diagnosis difficult.[4] Pleocytosis with lymphocytosis, increased CSF protein and reduced glucose levels may support the diagnosis but still not reliable as other pathologies may have similar effects on the CNS.[5] Microscopic examination by ZN staining of specimens for AFB plays a key role in the in the initial diagnosis of TB. The overall sensitivity of the microscopy has been reported to range from 22–78%, however, the sensitivity appears to be lower in non-respiratory specimens due to the lower bacterial load and lower for some non tuberculous mycobacterial (NTM) species due to poor staining of the cell wall.[6] Isolation by using conventional solid media as shown LJ medium is associated with low sensitivity and is time consuming.[7] The nucleic acid assays, owing to low sensitivity and high costs, are only an adjunct and not the substitute for the conventional procedures.[8] Immunological and serological assays, until now, have not shown to be predictive enough to warrant their routine use as diagnostic tests for TB.[9] In view of these limitations, there is a need for less complicated and more accurate diagnostic tests. New tools are needed to radically transform the fight against TB and to eliminate the disease.[10] Liquid culture media such as that used in the MGIT (Becton Dickinson) system have been introduced and extensively evaluated.[11] Recently, the BACTEC MGIT 960, a newly developed nonradiometric, fully automated, continuously monitoring system, was introduced as an alternative to the radiometric BACTEC 460 for growth and detection of Mycobacteria. The performance of the MGIT 960 system for detection of *M. tuberculosis* strains has been

found to be excellent.[12–14] Indeed, the MGIT system provided a 20% or greater improvement in total recovery rate for all Mycobacteria (249vs.172 of 278), *M tuberculosis* complex (61 vs. 48 of 65), MAC (92 vs.69 of 105), and miscellaneous *Mycobacterium* species (96 vs. 55 of 108) over a traditional LJ slant.

Besides making a rapid diagnosis of EPTB in order to avoid its sequels, correct diagnosis is also very important in order to avoid overtreatment and its associated side-effects, social and financial aspects. The large proportion of bacteriologically unconfirmed cases could result in clinical misdiagnosis of EPTB, which may include underdiagnosis as well as overdiagnosis.[15] It is thus necessary to support the initial assessment by culture confirmation.[16] The microbiological diagnosis of TBM remains a true challenge, mainly because of low counts of Mycobacteria in CSF. Rapid bacteriological confirmation by liquid-media culture seems to be an appropriate and feasible solution of this very issue.

The objectives of the study were to establish a rapid and confirmatory diagnosis of TBM using MGIT 960 system. And also to assess the reliability of clinical judgment and chemical and cytological CSF analysis as rapid diagnostic tools, taking the MGIT system as a confirmatory test.

MATERIAL AND METHODS

This Analytical cross sectional study was conducted in Department of Microbiology of the University of Health Sciences (UHS) Lahore, Pakistan. The study was completed in six months, from January 2008 to June 2008.

100 CSF samples from suspected tuberculous meningitis patients were taken who fulfilled the following inclusion criteria:

Patients fulfilling the clinical criteria including:

- Fever of more than 2 weeks duration
- Headache
- Signs of meningeal irritation
- Altered conscious level
- Focal neurological deficits

Patients already on antituberculous drugs and having co-morbid disease were excluded from the study.

All CSF samples were received from Pediatric Neurology ward of Children Hospital that largely caters low socioeconomic population. All the procedures, such as processing of specimens, smear preparation, inoculum preparation, making dilutions, inoculation of media and subculturing were done in a biological safety cabinet level II with protective gloves and respirator masks. All the specimens were processed as soon as they reached the laboratory. Each specimen was divided into three parts to be used for the procedures enumerated below:

1. Total leucocyte count and differential leucocyte count.
2. Biochemical analysis; CSF glucose and protein concentration
3. Staining for AFB and Culture

Total leukocyte count was done by using Improved Neubaur Chamber. For differential leucocyte count, smears were made taking sediment from centrifuged CSF specimens and Giemsa staining was done. Biochemical analysis was done at the chemical pathology lab of UHS. CSF protein was measured by calorimetric endpoint method using a kit by 'Linear Chemiacals'. Glucose measurement was done by GOD–PAP method using the kit 'Ecoline 1000'. Z-N staining and culture procedures were carried out according to the 'MGIT Procedure Manual' by 'Find diagnostics' issued in 2006.

Due to turbid appearance, 11 specimens needed decontamination, 2 of which were blood-stained. Before inoculating into MGIT medium, the specimens were subcultured on blood and chocolate agar to check for contamination that turned negative for all samples.

The enrichment with reconstituted PANTA was added to the MGIT medium prior to inoculation of specimen in MGIT tube. All inoculated MGIT (7 ml) tubes were entered in the BACTEC MGIT 960 instrument after scanning each tube. The instrument maintains 37±1 °C temperature, since the optimum temperature for growth of *M. tuberculosis* is 37 ºC. MGIT tubes were incubated until the instrument flagged them positive. After a maximum of six weeks, the instrument flagged the tubes negative if there was no growth. Once a MGIT tube was positive by fluorescence or by visual observation, the tube was vortexed and smear was made from the positive tube and Z-N staining was done. Specific identification of *M. tuberculosis* was done by incorporating Paranitrobenzoic (PNB) acid 500 µg/ml of MGIT tubes. The results were obtained in 3–11 days.

For drug susceptibility testing 5 MGIT tubes were labeled for each test culture, namely, Growth control (GC), Streptomycin (STR), Isoniazid (INH), Rifampicin (RIF), and Ethambutol (EMB). 0.8 ml of BACTEC 960 SIRE Supplement was added to each of the MGIT tubes aseptically. 0.1 ml (100 microliter) of properly reconstituted drugs were added in the respective labeled tubes aseptically. The instrument interpreted results at the time when the growth unit (GU) in growth control reached 400 (within 4–13 days). At this point, the GU values of the drug vial were evaluated. Susceptibility testing against Pyrazinamide (PZN) was carried out at a lower *p*H of the medium, since PZN is active only at the low *p*H in vitro.

The data was analysed using SPSS-16. Mean±SD is given for quantitative variables. Frequencies, percentages and graphs are given for qualitative variables.

Pearson Chi-square and Fisher exact tests were applied to observe the associations between qualitative variables. A *p*-value of <0.05 was considered to be statistically significant.

RESULTS

The study analysed CSF specimens from 100 children ranging from 6 months–14 years in age with a mean age of 5.3±2.9 years including 44 male and 56 female patients.

No significant association was observed between MGIT results and CSF glucose levels (p=0.284) showing that from the total of 100 CSF samples, 49% had normal glucose level and 51% had low glucose level. Five (10%) cases with normal glucose were culture positive and 44 (89%) were culture negative and 9 (17%) cases with low glucose were culture positive and 42 (82%) were culture negative. (Table-1)

Taking into account the association between protein and culture, no significant association was observed between CSF protein level and culture result (p=0.093), showing that from the total of 100, 42% had normal protein in CSF and 58% had high protein. 3 (7%) cases with normal protein level were culture positive and 39 (92%) with normal protein were culture negative and 11(18%) cases with high protein were culture positive and 47 (81%) were culture negative. (Table-2)

No significant association was observed between the overall CSF biochemistry and culture results (*p*=0.260). The CSF biochemistry was found normal in 30%, suggestive of TBM in 39% and mixed/indeterminate in 31% of the specimens. The CSF cultures were found positive in 6% (n=2) of those with normal biochemistry, 20% (n=8) of those with biochemistry suggestive of TBM and 12% (n=4) of those with mixed/indeterminate CSF picture. (Table-3)

Significant association was observed between CSF cytology and culture results (*p*=0.001), showing that from the total of 100, 45% cases had normal CSF cytology, 53% had lyphocytosis and only 2% had neutrophilia. 2 (4%) cases with normal CSF cytology were culture positive and 43 (95%) were negative. Similarly, 10 (18%) cases with lymphocytosis were culture positive and 43 (81%) were negative and only 2 (2%) cases with neutophilia were culture positive (Table-4).

ZN-staining was negative for acid-fast bacilli for all cases. Growths of MTB were obtained in 14% of cases (n=14) on incubation into BACTEC MGIT-960 system with a mean culture time of 9.79±3.17 days. The sensitivity to first line ATT revealed resistance to INH, Streptomycin and Ethambutol in 2 cases (14.28%) (Figure-1).

Table-1: Association of CSF glucose concentration with culture

CULTURE	CSF GLUCOSE NORMAL n (%)	CSF GLUCOSE LOW n (%)	TOTAL
Positive	5 (10.2)	9 (17)	14
Negative	44 (89.7)	42 (82)	86
TOTAL	49 (49)	51 (51)	100

Pearson Chi-Square=1.15, *p*=0.284, Normal CSF Glucose=45–85 mg/dl; Low glucose<45 mg/dl

Table-2: Association of CSF protein concentration with culture

Culture	CSF protein Normal* [n (%)]	CSF protein High** [n (%)]
Positive	3 (7.1)	11 (18)
Negative	39 (92.8)	47 (81)
Total	42 (42)	58 (58)
Fisher exact test =2.828, p = 0.093		

*Normal CSF Protein=15–45 mg/dl; **High Protein=>45 mg/dl

Table-3: Association of CSF biochemistry with culture

MGIT	CSF BIOCHEMISTRY Normal* n (%)	TBM** n (%)	Mixed*** n (%)
Positive	2 (6.6)	8 (20)	4 (12)
Negative	28 (93)	31 (79)	27 (87)
Total	30 (30)	39 (39)	31 (31)

Fisher exact test =2.585, *p*=0.260
*Normal=Normal glucose and protein concentrations;
**TBM=Low glucose+High protein concentrations;
***Mixed=Low glucose+Normal protein or Normal glucose+High protein

Table-4: Association of CSF cytology with culture

MGIT	CSF CYTOLOGY Normal* n (%)	Lymphocytosis** n (%)	Neutrophilia*** n (%)
Positive	2 (4)	10 (18)	2 (100)
Negative	43 (95)	43 (81)	0 (0)
Total	45 (45)	53 (53)	2 (2)

Fisher exact test=12.165, *p*=0.001, *Normal cytology: ≤ 5 mononuclear cells/µl; **Lymphocytosis: >5 cells/µl (predominantly lymphocytes); ***Neutrophilia: >5 cells/µl (predominantly neutrophils)

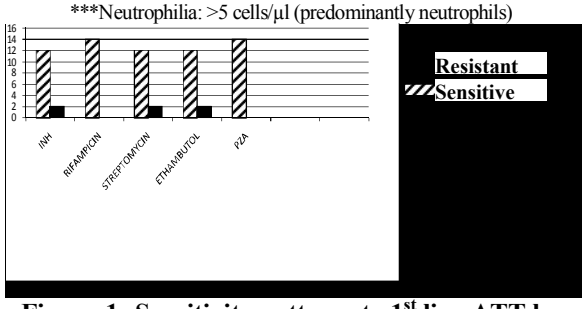

Figure-1: Sensitivity patterns to 1st line ATT by MGIT culture

DISCUSSION

Owing to its long culture time, the conventional confirmatory diagnosis with solid medium culture may delay the initiation of treatment in cases with borderline clinical features and inconclusive results of cytological

and biochemical analysis. On the other extreme, similar cases may face over-judicious exposure to ATT till the solid medium culture results are awaited.

Another case report was published in 1983 by Gill *et al*, in which six cases were wrongly diagnosed as TB and ATT was started without bacteriological confirmation and the diagnosis was delayed and none of them was found to have TB and one of them died due to unnecessary exposure to ATT or may be due to underlying disease which was not diagnosed.[17] In 1986, J. Dippenaar published a report of three cases in which pulmonary TB was incorrectly diagnosed because clinical and radiological findings were suggestive of TB.[18]

The sensitivity and specificity of BACTEC MGIT 960 for detecting mycobacteria was found to be 81.5 and 99.6% respectively in the meta analysis by Cruciani *et al*.[14] Artido *et al* evaluated MGIT 960 system for drug sensitivity to 1st line ATT in comparison with MGIT 460 system ATT where it showed good performance for susceptibility testing.[12] Similar results were found in the analysis carried out by Garrigo *et al*.[19] In a large comparative study carried out by Dongsi Lu *et al*, the overall sensitivities of the MGIT and LJ media were 86.5% and 59.7%, respectively, for the recovery of mycobacteria from clinical materials.[20]

In another study Hillemann *et al* tested 9,558 extrapulmonary specimens. The higher sensitivity of liquid system was achieved both with *Mycobacterium tuberculosis* complex and non tuberculous Mycobacteria.[14] In another multicenter study, Pfyffer *et al* cultivated a total of 1,500 clinical specimens including 70.9% respiratory and 29.1% non-respiratory specimens in liquid (MGIT and BACTEC 460) and on solid (egg and agar-based) medium. Overall, the mean times to detection for all mycobacterial isolates were 14, 13.5, and 23.1 days on MGIT, BACTEC, and solid media respectively. MGIT alone detected six additional isolates of *M. tuberculosis* (which were missed by BACTEC 460 and solid media).[21]

In a country where TB is prevalent, high degree of awareness is needed and suspected cases should always be confirmed by positive bacteriological findings in order to avoid over or under diagnosis. Hardly any work has been carried out locally so far to validate the reliability of conventional rapid diagnostic tools in order to guide the correct diagnosis. Furthermore, the diagnosis of TBM carries a special challenge in paediatric age-group owing to misleading clinical features, low bacterial cell counts and overlap from viral and other causes of meningoencephalitis.

The study was unique in the sense that MGIT system had so far never been used in Pakistan for the rapid diagnosis of tuberculous meningitis. In our study, growths of MTB were obtained in only 14% of cases (n=14) thus emphasizing the unreliability of clinical features for the diagnosis of TBM. The above mentioned non-specific analysis of CSF showed variable clinical as initial diagnostic tools in our study. The mean culture time on MGIT 960 system for MTB growths was 9.79±3.17 days that is much shorter than that of conventional solid media cultures. The confirmatory diagnosis by MGIT 960 system was not only rapid, it effectively ruled out the disease in 86% of the suspected cases, thus preventing the over-judicious exposure to ATT.

CONCLUSION

Clinical features, biochemical and cytological analysis of TBM only support the diagnosis and sometimes can be misleading, therefore, cannot be always relied on. Rather, BACTEC MGIT 960 can be used as rapid diagnostic tool for the diagnosis of TBM and guiding the correct management.

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