

Research Article

**Comparison of Smear, Culture, PCR and Bactec test
on CSF in CNS Tuberculosis**

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ABSTRACT

Introduction: Tuberculosis is an ancient disease that is known to have existed in prehistoric times. Tuberculosis is one of the commonest communicable diseases in majority of the developing countries. It is caused by the Mycobacterium tuberculosis, which usually affects the lungs but may cause lesion in any organ or tissue of human body.

Objectives of the study: The basic aim of the study is the comparison of smear, Culture, PCR and Bactec test on CSF in CNS tuberculosis.

Methodology of the study: This study was conducted in the Paediatric Department of Aziz Bhatti Shaheed Teaching Hospital Gujrat. In this study the data of 53 children aged from 6 months to 13 years suspected of having tuberculous meningitis from 3 different teaching hospital, including Mayo Hospital Lahore, Jinnah hospital and Shaikh Zayed Hospital Lahore were included.

Results: A total 53 patients were included in the study. Bactec test was positive in 4 of 32 (12.5%) highly probable cases and 4 of 18 (22.22%) probable cases. Total 8 of 53 (15.09%) cases had Bactec positive. Most common X-ray chest finding was lymphadenitis total 10 of 53 (18.86%) case had positive X-ray chest findings.

Conclusion: It is concluded that Polymerase Chain Reaction is the method for Rapid diagnosis of Tuberculous Meningitis. PCR was done on CSF and it was positive in 50.94% compared to Bactec and conventional smear and culture which showed positivity of only 15.09% & 3.77% respectively.

Key words: Microbiology, TB, Diseases

INTRODUCTION

Tuberculosis is an ancient disease that is known to have existed in prehistoric times. Tuberculosis is

one of the commonest communicable diseases in majority of the developing countries. It is caused

by the *Mycobacterium tuberculosis*, which usually affects the lungs but may cause lesion in any organ or tissue of human body¹. In more advanced countries the incidence has declined rapidly since the end of Second World War, but disease is still present. Decline in the incidence came due to improvement in socioeconomic condition of people, improved sanitation and housing, BCG vaccination, early case detection and treating the affected persons. However, in developing countries tuberculosis is still a major health problem. In recent years there is resurgence of tuberculosis in Western countries due to AIDS, increasing number of immigrants from developing countries and increasing level of social deprivation in some inner city, areas of the developed world².

As children are only infected from infective adults, tuberculosis in children is a direct reflection of tuberculosis in adults.

Tuberculosis continues to be a constant threat to the child population wherever there is poverty, overcrowding and malnutrition³. In studies of tuberculosis, a differentiation has to be made between tuberculous infection evident by a positive tuberculin test and tuberculous disease in which there is clinical, radiological or bacteriological evidence of infection. The great majority of infected people remain asymptomatic⁴. No age is immune to tuberculosis. It may affect any age ranging from intrauterine to upper limit of paediatric age. Incidence of infection increases as the age advances.

Tuberculosis can affect any tissue or system of body⁶. Common presentations of tuberculosis are primary complex, tuberculous lymph adenitis and progressive primary disease.

Due to introduction of BCG vaccination just after birth the clinical pattern of tuberculosis has changed⁷.

Objectives of the study

The basic aim of the study is the comparison of Smear/ Culture, PCR and Bactec test on CSF in CNS tuberculosis.

Methodology of the study

This study was conducted in the Paediatric Department of Aziz Bhatti Shaheed Hospital Gujrat. In this study 53 children aged from 6 months to 13 years suspected of having tuberculous meningitis from 3 different teaching hospitals, including Mayo Hospital Lahore, Jinnah hospital and Shaikh Zayed Hospital Lahore were included.

Inclusion Criteria

1. History of fever, headache and neck stiffness of more than 2 weeks duration.
2. History of contact with active case of tuberculosis.
3. Abnormal neurological symptoms such as irritability, seizures, altered conscious level and positive signs of meningitis (nuchal rigidity, brudzinski signs, kernig sign), abnormal deep tendon reflexes, clonus, cranial nerve palsies, hemiparesis.
4. Poor response to antibacterial therapy.
5. Evidence of clinical improvement after anti-tuberculous therapy.

Collection of data

All suspected cases of tuberculous meningitis were evaluated by taking detailed history according to the designed proforma especially considering age, sex, socioeconomic condition of families, history of contact with tuberculous patients, BCG status and presenting complaints of fever, headache, neck stiffness of more than 2 weeks duration along with history of seizures and loss of consciousness. These children were also evaluated for the evidence of tuberculosis outside the central nervous system, and a clinical response to anti-tuberculous therapy. Supporting evidence was obtained by examination of cerebrospinal fluid, ESR, Montoux test, X-ray chest and CT scan as mentioned in the inclusion criteria.

The TBM cases were divided into three groups as mentioned above.

Apart from the, above mentioned investigations, all patients were subjected to specific tests. The

CSF of every patient was tested for the presence of mycobacterium by three different methods; The 6ml CSF was collected and divided into 3 portions.

A. The first 2ml CSF collected was used for Bactec test which was done at Gulab Devi Hospital Lahore. There all the CSF samples were initially processed through the following steps:

1. CSF sample was mixed with equal quantity of NaOH-N-acetyl-L cysteine for 5 minutes in a 50 ml centrifuge tube.
2. Then phosphate buffer (Ph 6.8) was added up to 50 ml ring and mixed.
3. Then samples were centrifuged at 2000g for 20 minutes.
4. Supernatant was discarded.
5. Resuspended the sediment with 1-2 ml of phosphate buffer and after mixing this concentrate was used to make smear and to inoculate 12 B medium. All vials of 12 B medium were tested on the Bactec 460 instrument to purge with 5% CO₂ before inoculation and 12B medium was supplemented with antimicrobial PANTA PLUS supplement prior to inoculation. PANTA supplement is lyophilized mixture of five antimicrobial drugs: polymyxin B,

amphotericin B, Nalidixic acid, trimethoprin and azlocillin. This supplement, when added to 12B medium it suppresses growth of contaminating micro-organisms during primary is isolation. PANTA reconstituting fluid (PRF), used to rehydrate lyophilized PANTA supplement. It also contains a growth promoting substance.

Statistical analysis

The collected data were analyzed using SPSS software (version 17). The results are presented as a mean with 95% confidence interval limits or standard deviations. The significant value for P <.05 was accepted as statistically significant.

RESULTS

A total 53 patients were included in the study. Out of these 53 patients 27(50.94%) patients were male and 26 (49.05%) were females with male to female ratio of 1.03:1. This study was conducted in age groups 2 month to 14 years. Maximum number of cases 32(60.37%) were between 1-5 years. Next common age group was 6-10 years, 11(20.75%). There were 3(5.66%) and 7(13.20%) patients of age <1 year and >10 years respectively. The median age is 3 years.

Table 01: Results of Smear, Culture, PCR and Bactec Test

	Smear (Z-N staining)		AFB culture (L-J Medium)		Bactec Test 12 B Medium		PCR	
	No.	%age	No.	%age	No.	%age	No.	%age
I	2	6.25%	2	6.25%	4	12.50%	18	56.25%
II	0	0	0	0	4	22.22%	8	44.44%
II	0	0	0	0	0	0	1	33.33%
Total	2	3.77%	2	3.77%	8	15.09%	27	50.94%

Smear Vs Culture

p > 0.05

Smear Vs Bactec

P < 0.05

AFB culture Vs Bactec

P <0.05

PCR Vs Smear, Culture, Bactec. P <0.05(using Z score for proportion)

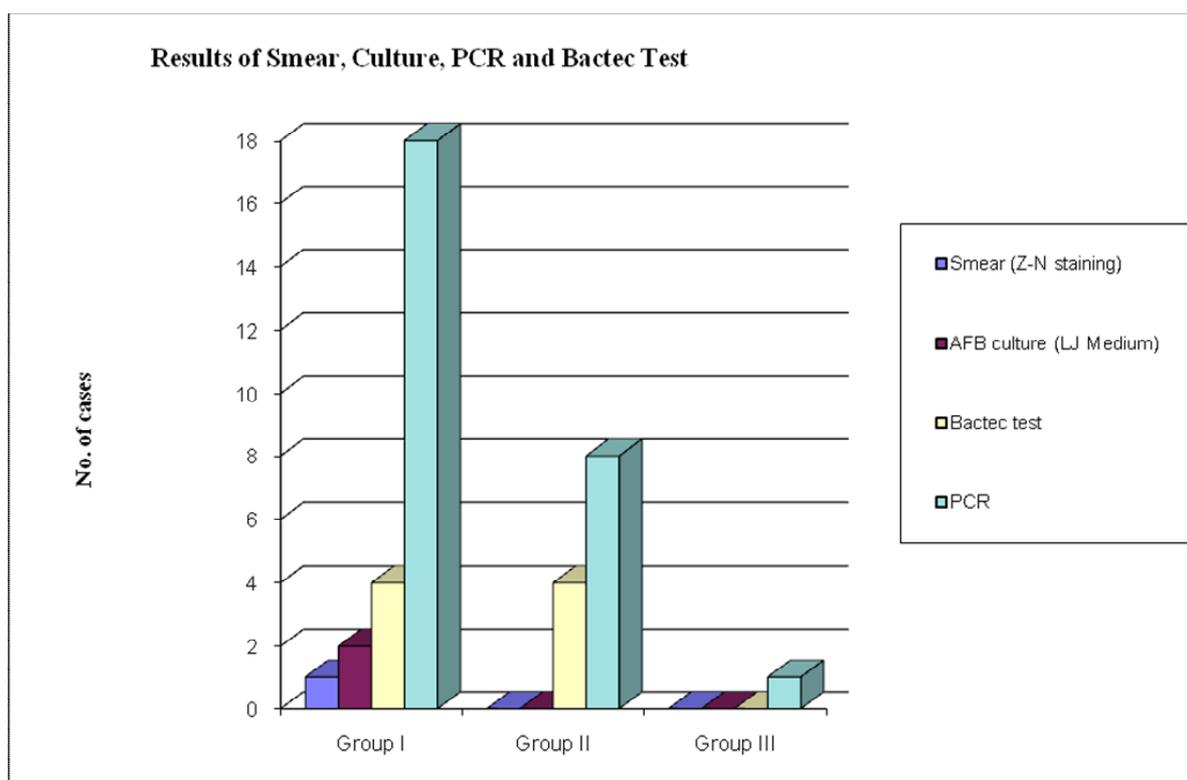


Figure 01: Result of Smear, Culture, PCR and Bectec test

Bactec test was positive in 4 of 32 (12.5%) highly probable cases and 4 of 18(22.22%) probable cases. Total 8 of 53 (15.09%) cases had Bactecpositive. Most common X-ray chest finding was lymphadenitis total 10 of 53 (18.86%) case had positive X-ray chest findings. Two patients had miliary shadows. All 53 patients had their CT scan done. 43out of 53 (81%) CT scan findings were suggestive of tuberculous meningitis. These findings were of hydrocephalus, periventricular leucencies and enhancement of basal cisterns and fissures. Five cases had cerebral infarcts as well.Fever was present in all patients and 50(94.33%) patients had seizures at the time of presentation. 10(18.86%) patients had irritability and 35(66.03%) were unconscious at the time of admission. 35(66.03%) patients had cranial nerve paresis at presentation. Out of 53 patients 40(75.47%) died during hospitalization and 13 patients (24.52%) were discharged from the hospital. 20 patients required surgical interventions and ventriculoperitoneal shunt was passed for hydrocephalus.

Table 02: CT scan findings in patients

	Total patients	Positive findings	No findings
Highly probable	32(60.3%)	31	1(7.14%)
Probable	18 (33.96%)	12	6
Possible	3(5.66%)	0	3(100%)

DISCUSSION

Tuberculosis is the world's leading cause of death from a single infectious agent. In developing countries like Pakistan tuberculosis continues to be the major health problem. The factors responsible for failure to control tuberculosis in Pakistan are low socioeconomic conditions, lack of health education, non-compliance and drug resistance. For the same reason mortality is high in developing countries. Tuberculosis affected an estimated 8.8 million people and caused 1.4 million deaths globally in 2010, including a half-million women and at least 64 000 children [13].The reported tuberculosis rates among children less than 15 years of age in the United

States increased by 40% from 1985 to 1993⁸. It is intricately linked with human immunodeficiency virus (HIV), as well as non-communicable diseases and ill-health determinants such as diabetes mellitus, smoking, alcoholism, and malnutrition [14]

It is estimated that children younger than 15 years contribute 15%–20% of the global burden [15]

The most severe complication of tuberculosis is infection of the central nervous system, which is invariably fatal if appropriate therapy is not administered promptly. Outcome of tuberculous meningitis is strongly associated with the stage of disease at presentation. In this study 66.03% children presented in Stage III and 32.07% in Stage II and 1.88% in Stage I and the mortality was 75.47% among them mainly those who presented in Stage III⁹. Our findings correspond to many other authors.

In this study the median age of tuberculous meningitis is 3 years which is higher than 12 months in USA. Male to female ratio was 1.03:1 in our study in comparison to 1.38:1 according to the data collected at Baylor College of Medicines Houston, Texas¹⁰. In this study 33.96% children were vaccinated with no difference in survival rate. The protective efficacy of BCG was found to be low in the moderately and severely malnourished group. The efficacy of BCG vaccine in preventing tuberculous meningitis was 77%. However, reported that BCG vaccine efficacy in preventing tuberculous meningitis in children was 52%. 66.03% children included in this study were not vaccinated¹¹.

It has been found that 2 patients had history of BCG vaccination but no BCG scar. It points out either the faulty administration or the decreased potency of vaccine due to improper storage. Our study 66.03% patients had positive history of contact with tuberculous patients.

Out of these 35.84% had adult cases of pulmonary tuberculosis with in the house. 33.96% did not disclose any history of contact¹².

CONCLUSION

It is concluded that Polymerase Chain Reaction is the method for Rapid diagnosis of Tuberculous Meningitis. PCR was done on CSF and it was positive in 50.94% compared to Bactec and conventional smear and culture which showed positivity of only 15.09% & 3.77% respectively. Although, many studies have shown higher sensitivity of PCR on sputum but the decrease sensitivity in case of CSF. The reason for this is poor yield of *T. Bacillus* in the specimen and secondly many patients who came to these tertiary care centers had already received anti tuberculous therapy which further reduced the chance of detecting myco-bacterium *Bacillus* in the CSF.

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