DOWN TO THE GROUND ZERO: PERSPECTIVE FROM TISSUE DIAGNOSIS IN NEUROLOGICAL TB

Maiti Sourav[1], Banerjee Moulima[2], Das Ruchira[3]

[1]Consultant Microbiologist& In-charge infection control department, Institute of Neurosciences, Kolkata.
[2]Consultant Pathologist, Institute of Neurosciences, Kolkata.
[3]MSc Microbiology, ST Xavier College, Kolkata, India.

ABSTRACT

Background: Central nervous system tuberculosis (CNS-TB), a severe form of extrapulmonary tuberculosis presents with nonspecific symptom leading to delayed diagnosis and increased morbidity. This study compares different modalities of diagnosis of CNS-TB [Multiplex real-time PCR/ GeneXpert, TB culture, Ziehl-Neelsen staining (Z-N staining) and histopathology]. **Method:** This is a cross sectional observational study conducted in the Department of Neuropathology. Total 128 brain and spinal cases which were clinically suggestive of TB and operated were evaluated. **Result:** Granuloma, giant cell and necrosis were less evident in spinal TB compared to brain. GeneXpert and culture positivity were higher (100%) in tissue having poorly formed granuloma compared to well-formed granuloma. Z-N stain has poor sensitivity (around 32%). Compared to culture GeneXpert was 100% sensitive and 96% specific in brain TB (detected from tissue) and 87% and 89% in spinal TB respectively. **Conclusion:** Combined all these modalities provide a wholistic approach so that not a single case of tuberculosis is missed.

Keywords: CNS-TB; Tissue Diagnosis; GeneXpert; Culture; Histopathology.

INTRODUCTION

Although many anti-tubercular drugs are now available in the market *Mycobacterium tuberculosis* in central nervous system (CNS) remains a difficulty in therapeutic terms as well as diagnostically. Relative rarity coupled with the non-specific symptoms puts CNS TB in a considerable diagnostic challenge. Cerebral tuberculomas are a rare and serious form of TB due to the haematogenous spread of *Mycobacterium tuberculosis* [1,2].

Spinal TB is one of the oldest diseases annoying mankind with resultant spinal deformity commonly known as Pott's spine. Compared to the pulmonary TB, extra-pulmonary TB cases face difficulty in establishing the diagnosis because of paucity of microorganisms which is probably the result of immune mediated reaction in majority [3,4]. Therefore, diagnosis of TB from these types of tissues becomes very difficult; also host response in tissue may decrease the bacillary load that ultimately results in false negative result.

On the contrary, histopathological diagnosis is based upon conceptualization of tissue reactions which might not be specific but suggestive of tuberculosis. Lots of look-a-like conditions might mimic chronic inflammation/ tuberculosis on histopathology because of similar pathological reaction. How much these correlate together when viewed from diverse angles of histopathology and microbiology is a subject under research. Also, conventional means like Ziehl-Neelsen staining and culture have poor yield in CNS-TB as literature reveals [1,2,5]. Whether a nested polymerase chain reaction in the form of GeneXpert could provide a better answer was our query.

DOI: 10.31878/ijcbr.2020.62.07

eISSN: 2395-0471
pISSN: 2521-0394

AIM: To study the correlation between histopathology and microbiological analysis of surgically resected brain and spinal tissues from clinical cases.

MATERIAL AND METHODOLOGY

Study design: This is a cross sectional observational study

Ethical approval: Ethical committee of the institution approved the study.

Locus of study: Conducted in the Department of Neuropathology

Time frame: October 2016 to May 2019.

Sample size: Total 128 brain and spinal cases

Inclusion criteria: Brain and spinal cases which were clinically suggestive of TB and operated were included for evaluation.

Exclusion criteria: Insufficient sample and improper sampling/ non representative samples were rejected

Methodology: Relevant patient particulars and clinical history and consent were collected. Tissues were processed for both microbiological and histopathological investigations. Tissue impression smears were made for Gram stain, ZN stain and/or special stain. Tissues were cultured for bacteria and *Mycobacterium tuberculosis*. For TB culture Mycobacterial Growth Indicator Tube (MGIT) (a liquid mycobacterial culture system using Middlebrook 7H9 broth that monitors the consumption of oxygen by

Correspondence: Dr. Moulima Banerjee, Department of Pathology, Institute of Neurosciences, Kolkata, 185/1, A J C Bose road, Kolkata. Email: moulimabanerjee16@gmail.com

fluorescence and detects the growth) was used. Automated detection of Mycobacterial complex and Rifampicin resistance was done by *GeneXpert* (a molecular test based on nested real-time polymerase chain reaction which gives result in less than 2 hours). After the sample was run, we obtained negative or positive results accordingly along with rifampicin resistance or sensitivity. Very low, low, medium and high indicates the load of the organism.

Histopathological methods for tissue processing were carried out as per standard protocol and slides were stained with hematoxylin and eosin. Relevant immunohistochemical / special stains were done. Histopathology characteristics were noted with reference to type of infiltration, caseation necrosis, and nature of granuloma and giant cells.

Tissues having GeneXpert positivity and/or culture positivity were included as confirmed TB cases. Tissues having chronic inflammatory reactions with/without granuloma formation were possible TB cases. Tissues revealing histopathological nature otherwise were included as non-TB cases.

RESULTS

Out of 128 cases (59 brain tissue and 69 spine tissue samples) 25 were confirmed TB (brain:4; spine:21) and 17 possible TB cases (brain:7; spine:10). 86 non-TB cases comprised mainly of glioblastoma, lymphoma and metastatic deposits (Fig1). Confirmed brain TB cases had chronic inflammatory reaction in 75% with one case having acute inflammatory reaction. Granuloma formation was noted in 50% with caseation necrosis and giant cells in all. Despite GeneXpert positivity (100%), TB culture was positive in 50% only. Compared with culture, GeneXpert was 100% sensitive and 96.49% specific but ZN positivity was rare. Compared with GeneXpert, ZN was 25% sensitive. ZN was found to be specific (98-100%) (Fig 2). Frontal lobe lesions in 50% cases turned out to be tuberculoma. 1 case of TB was found in cerebellar region. Among the three cases with very low mycobacterial DNA load ZN and culture positivity were 33% and 67% respectively. ZN was negative in brain tissue with low mycobacterial DNA load. Tissues having ill-defined granuloma had 'very low' mycobacterial DNA load with 50% culture-positivity. In well-formed granulomatous lesions GeneXpert as well as cultures were totally non-contributory (Fig 3).

Majority (67%) of confirmed spinal TB cases had chronic inflammatory reaction. Well-defined granuloma was evident in 24%, giant cells in 29% and necrosis in 48%. Culture and GeneXpert positivity were 71% and 90% respectively. Two culture-positive cases had negative GeneXpert result. This translated into 86.67% sensitivity of GeneXpert with 88.89% specificity in case of spinal TB. Upper thoracic, lower thoracic and lumbar vertebral involvement was noted in case of spinal TB. ZN positivity was better in spinal TB compared to brain TB with 33% sensitivity. In spinal tissues with very low mycobacterial load, ZN and culture positivity were better (38% and 69% respectively) compared to low load (17% and 67% respectively). When assessed Mycobacterial load in comparison with host immune response, both GeneXpert and culture positivity were 100% in ill-defined granuloma. In well-formed granuloma, culture positivity and GeneXpert positivity dropped down to 60% only (Fig3).

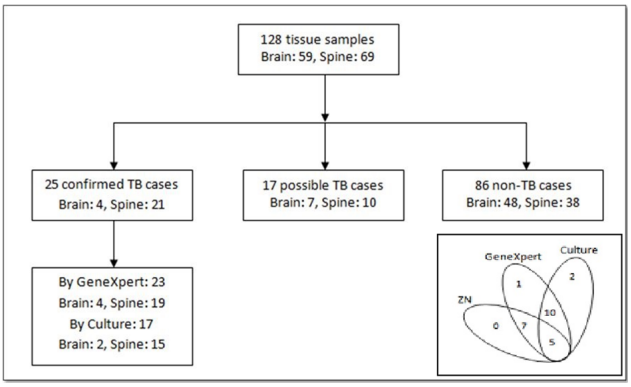

Fig 1: Schematic distribution of the cases.

Inset showing the venn-diagram of different diagnostic modalities in confirmed TB cases.

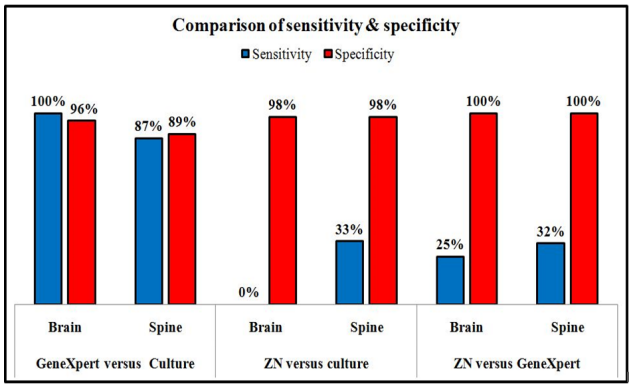

Fig2: Bar chart showing the calculated sensitivity and specificity of GeneXpert and ZN staining among brain and spinal tissues.

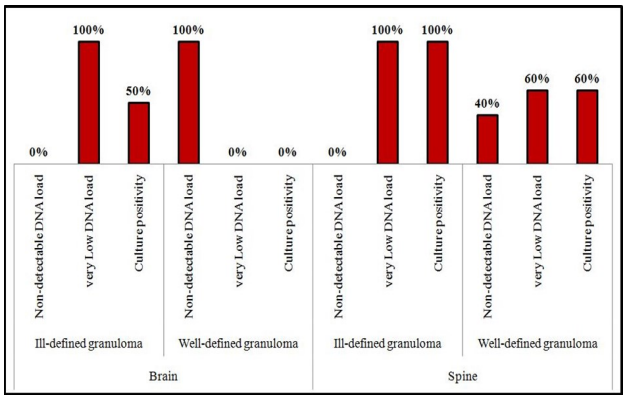

Fig 3: Bar chart showing Mycobacterial load and culture positivity rates among brain and spine tissues having scanty and well-formed granuloma respectively.

When we analyzed all the chronic inflammatory lesions of brain and spine, well-formed granuloma was found in few (18% and 17% respectively) but with abundance of caseation necrosis (82% in both). Giant cells were less commonly seen in spinal lesions (47% vs 64%). GeneXpert was positive in only 36% of brain lesions, compared to 94% of spinal lesions. The same trend was noted in case of ZN positivity (9% and 29%) and culture positivity (18% and 65%) (Fig No. 4). Among the brain lesions, frontal lobe lesion was frequent (55%), whereas

in case of spinal lesions, the distribution increased from craniovertebral junction (3%) to lumbar region (38%).

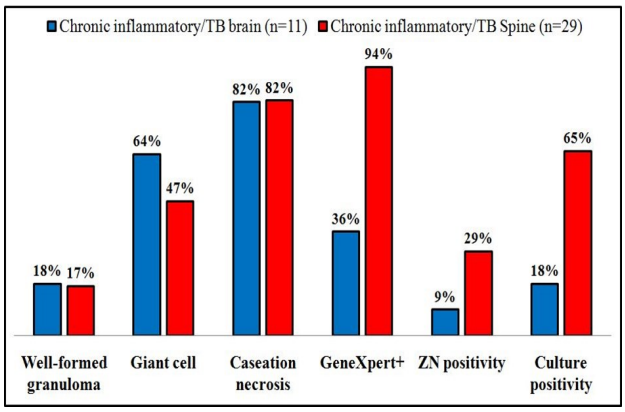

Fig 4: Bar chart showing histopathological evidence of host immune response and microbiological detection rates among chronic inflammatory lesions of brain and spine.

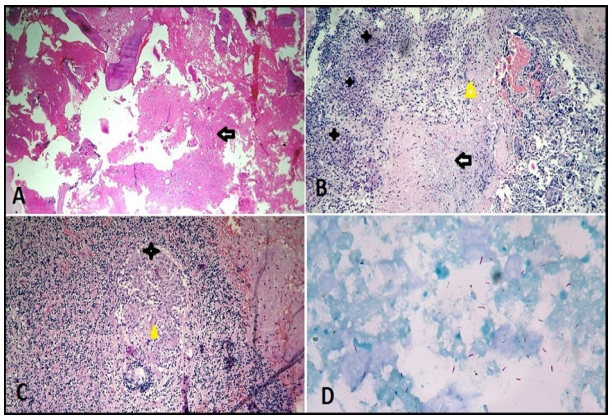

Fig 5: Histopathological photomicrograph of spectrum of tuberculosis in brain and spine.

A. Widespread caseating necrosis (arrow) with fragmented bone in spinal TB [H&E, X 40]. **B.** Ill-defined granuloma (star), langhans giant cells (arrowhead) and necrosis (arrow)[H&E X 100]. **C.** Well defined granuloma (arrowhead) with Langhans giant cells (star) and intense inflammatory reaction in brain TB [H&E, X 100]. **D.** Acid fast bacilli in background necrosis in a brain TB case [Z-N stain, X1000]

Among the confirmed TB cases, ill-defined granuloma with necrosis was the predominant pattern in brain (50%), whereas well-defined granuloma with some necrosis was most common pattern in case of spinal TB. Among the necrotic spinal granuloma, 60% were GeneXpert positive whereas all non-necrotic granuloma yielded very low mycobacterial load.

We observed more frequent association of Langhans type of giant cells with culture and GeneXpert positivity compared to the other types of giant cells in general. In our study, among GeneXpert negative cases Langhans giant cells were observed in only 2% compared to other giant cells (11%). In GeneXpert positive cases, they comprised of the sole majority giant cells (from 33% in very low load to 100% in low load).

Among the confirmed TB cases, ill-defined granuloma with necrosis was the predominant pattern in brain (50%), whereas well-defined granuloma with some necrosis was most common pattern in case of spinal TB. Among the necrotic spinal granuloma, 60% were GeneXpert positive whereas all non-necrotic granuloma yielded very low mycobacterial load.

We observed more frequent association of Langhans type of giant cells with culture and GeneXpert positivity compared to the other types of giant cells in general. In our study, among GeneXpert negative cases Langhans giant cells were observed in only 2% compared to other giant cells (11%). In GeneXpert positive cases, they comprised of the sole majority giant cells (from 33% in very low load to 100% in low load).

DISCUSSION

Tuberculosis in brain and spine usually results from hematogenous spread from a primary focus – lungs and in minority gastrointestinal tract (cranial TB) and genitourinary tract (spinal TB). Subpial and subependymal 'Rich foci' form a reservoir giving rise to intracranial manifestations in the form of tuberculous meningitis and intracranial tuberculoma formation [3].

In spinal TB hematogenous spread to the dense vasculature of the vertebral cancellous tissue facilitates the dissemination to the paradiscal region [3]. Central vertebral body lesions are thought to result from involvement of intraosseous venous system. Caseous granulomas are hallmark of tuberculosis in prevalent areas.[2] It comprises a cellular necrotic region rimmed by epithelioid macrophages and lymphocytes and multinucleate giant cells (Langhans giant cells) (fig no 5).

In early part of the disease bacterial load is more which is depicted histopathologically by increased proportion of necrotic tissue. However as host immune response takes upper hand more inflammatory reaction, well-defined granulomas are seen, and bacterial load gets decreased [6]. In-vitro studies of human tuberculous granulomas have revealed that *Mycobacterium tuberculosis* induces large multinucleated giant cells with >15 nuclei per cell [7]. Also, the presence of foreign body type giant cells is noted due to destructed sub-cellular material of *Mycobacterium tuberculosis*. However, histopathological characteristics are non-specific because other granulomatous diseases like syphilis, actinomycosis, histoplasmosis, toxoplasmosis, sarcoidosis etc. may also be characterized by the presence of granuloma.

In our study, confirmed brain TB cases demonstrated granuloma formation in 50% only. However, for the spinal tissues this was further less (24%). Giant cells were seen in all the confirmed brain TB cases along with caseation necrosis but, only 29% in spinal TB cases. Langhans giant cells were noted in specific association with brain TB. (100% in brain TB compared to culture and in spine TB the association with culture was low - 20%.)

Among all the confirmed TB cases, 5 tissues are found to be GeneXpert positive along with culture positivity and ZN positivity. However, 7 cases were noted with both ZN positive and GeneXpert positive but culture negative. 10 tissue samples showed culture positivity as well as GeneXpert positivity, whereas no tissue was found to be both culture and ZN positive. Only 2 samples had shown culture positivity without GeneXpert positivity and 1 tissue was detected to be positive for MTB by GeneXpert only.

In case of brain tissues, GeneXpert was found to be 100% sensitive and 96.49% specific in comparison to culture, whereas for spinal tissues, GeneXpert showed 87% sensitivity and 89% specificity. The sensitivity of ZN was found to be very low compared to the culture in both brain (0%) and spinal (33%) tissues. But ZN is very much specific compared to culture in brain (98%) as well as spinal (98%) tissues. In comparison with GeneXpert, 100% specific was noted in ZN. This varying data is evident due to different detection limits of ZN, culture and GeneXpert. There is no clear-cut study data with head-to-head comparison between these tests. However, in a study on pediatric sputum samples to conclude ZN detection limit as 10^4 bacilli per ml sensitivity was 20% [1]. In tuberculous meningitis in adults it was studied that ZN can detect 100 or more AFB per ml of CSF whereas culture can detect 10-100 bacilli per ml [5,8]. Considering these as detection limits for ZN and culture in case of brain and spine TB our situation can be explained.

GeneXpert is a new modality utilizing nested PCR technique which is yet to be evaluated extensively into CNS-TB cases. As compared to the culture, limit of detection for this assay has been found to be 131 cfu/ml (range 106.2-176.4 cfu/ml, 95% C.I.) for pulmonary samples [2]. Differences in limits of detection explain the different sensitivity and specificity we obtained. This can be appreciated from the Venn diagram (fig 1) of performance of different diagnostic modalities in confirmed TB cases.

In very low Mycobacterial load, brain tissues showed 33% ZN positivity and 67% culture positivity, whereas spinal tissues with 38% ZN positivity and 69% culture positivity were noted. In low Mycobacterial load, no significant positivity was seen in both ZN and culture in case of brain tissues but for spinal tissues ZN positivity and culture positivity was noted as 17% and 67% respectively.

Our study assessed the relationship between Mycobacterial load and host immune response in terms of histopathological features. In well-formed granuloma of brain tissues, GeneXpert failed to detect the Mycobacterial load, whereas in scanty granuloma, very low Mycobacterial load was detected. Culture positivity was noted to be 50% in case of scanty granuloma compared to nil in case of well-formed granuloma. For spinal tissues, well-formed granuloma showed 60% culture positivity as well as GeneXpert positivity, whereas in scanty granuloma 100% culture and GeneXpert positivity was noted. 2 cases showed despite GeneXpert negativity, cultures were positive which might be due to lack of homogenization of sample. Detection of very low or no Mycobacterial load may be due to presence of dead and degenerated mycobacteria with only low amount of DNA. Our study found that host immune response in terms of granuloma formation, giant cell formation and caseation necrosis is more pronounced in case of brain tissues than spinal tissues which correspond to the proportionate microbiological yield. In case of brain tissues, host response might have dampened the GeneXpert detection.

Since chronic inflammatory lesions in brain and spine commonly regarded as tubercular in nature, we tried to look into these lesions. We found 11 such brain tissues and 29 spinal tissues in our study. Interestingly, we found that only 36% of the brain tissues showed positivity for mycobacterial DNA. On the other hand, spinal tissues were almost positive for GeneXpert (94%). ZN positivity was poor in both the groups (9% and 29% respectively). Culture fared better than ZN by rendering 18% positivity in brain tissues and 65% in spinal tissues. Tissue reactions suggested towards possible TB by presence of caseation necrosis in 82% in both the categories. Along with this 64% and 47% giant cells in the respective categories support the suspicion. However, well-formed granuloma was seen in only about 18% cases. Literature suggests that caseation is a result of hypersensitivity reaction to Mycobacterial secreted antigens. Initially nascent granulomas are not protective later with recruitment of activated T cells a dynamic host pathogen response is established.[2] As of we know, in developing post-primary TB of lung, *Mycobacterium tuberculosis* do not survive and produce antigens except in the macrophages in sequestered sites [4,6]. So, the granuloma formation and caseation could be linked to these antigens and not by the intact bacilli whether viable or not [9].

Considering the confirmed TB cases, proven by GeneXpert and/or culture, 50% necrotic scanty granuloma was the commonest finding among brain tissues. In contrast, spinal tissues showed varied distribution with necrotic well-formed granuloma being appreciable in 14% only. GeneXpert positivity was noted in 60% of necrotic spinal granuloma and in all of the non-necrotic spinal granuloma.

Another observation was noted in our study about Mycobacterial shape visualized in ZN. In few cases of spinal tuberculosis, intact bacilli were not seen but globular acid-fast structures were noted. In some studies, such morphological alterations have been mentioned [10, 11]. This strengthens our finding and our view that *Mycobacterium tuberculosis* gets altered in tissues causing difficulty in visualization by ZN but gets detected by GeneXpert and culture. On the other hand, it also supports the theory that Mycobacterial secreted antigens and not intact bacilli are present in tissue.

CONCLUSION

The take home message from this study can be summarized into the following points. Firstly, GeneXpert or PCR besides its proven role in pulmonary TB, is a very reliable and sensitive test to diagnose neurological TB as well. Culture and ZN staining though considered gold standard has limitations due to absence of intact live bacilli in some cases. This scenario was encountered in spinal TB cases in our study due to unknown reasons. Well-formed Granulomatous reactions in brain and spine can render both GeneXpert and culture as negative. Diagnosis in those cases becomes histopathology dependent and the cases cannot be stamped as confirmed TB cases. Those are considered possible TB cases and therapy started empirically.

Conflict of interest : Nil

Source of funding : Nil

REFERENCES

[1] Lighter J, Rigaud M. Diagnosing childhood tuberculosis: traditional and innovative modalities. CurrProbl PediatrAdolesc Health Care, 2009; 61.

[2] Helb D, Jones M. Rapid Detection of *Mycobacterium tuberculosis* and Rifampin Resistance by Use of On-Demand, Near-Patient Technology.Journal of Clinical Microbiology. 2010;48(1):229-37

[3] Garg RK, Somvanshi DS. Spinal tuberculosis: A review. The Academy for Spinal Cord Injury Professionals, Inc. 2011.

[4] Hunter RL. Tuberculosis as a three-act play: A new paradigm for the pathogenesis of pulmonary tuberculosis. Tuberculosis (Edinb).2016;97:8–17

[5] VerdonR,Chevret S, Laissy JP, Wolff M. Tuberculous meningitis in adults: review of 48 cases. Clin. Infect. Dis. 1996;22:982–8

[6] Ehlers S, Schaible UE. The granuloma in tuberculosis: dynamics of a host-pathogen collusion. Front Immunol. 2013;3:411

[7] Lay G, Poquet Y, Salek-Peyron P, Puissegur MP. Langhans giant cells from M tuberculosis induced human granulomas cannot mediate mycobacterial uptake. J Pathol. 2007;211:76–85

[8] Yeager H Jr, Lacy J, Smith LR, LeMaistre CA. Quantitative studies of mycobacterial populations in sputum and saliva. Am Rev Respir Dis 1967;95 (6):998-1004

[9] Logani S, Lucas DR, Cheng JD, Ioachim HL, Adsay NV. Spindle cell tumors associated with mycobacteria in lymph nodes of HIV-positive patients: Kaposi sarcoma with mycobacteria' and 'mycobacterial pseudotumor. American Journal of Surgical Pathology.1999;23(6):656–61

[10] Velayati AA, Farnia P, Masjedi MR, Konstantinovich G. Morphological modification by tubercle bacilli: no time for denial. Journal of Infection in Developing Countries 2012:6 (1):9799

[11] Karimi S, Shamaei M, Pourabdollah M, Sadr M, Karbasi M, Kiani A, Bahadori M. Histopathological Findings in Immunohistological Staining of the Granulomatous Tissue Reaction Associated with Tuberculosis. Tuberc Res Treat.2014;858396.