



CORRELATION OF FINE NEEDLE ASPIRATION CYTOLOGY (FNAC) AND CARTRIDGE BASED NUCLEIC ACID AMPLIFICATION TEST (CBNAAT) IN THE DIAGNOSIS OF EXTRAPULMONARY TUBERCULOSIS

Pathology

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ABSTRACT

Introduction: Tuberculosis is a leading cause of morbidity and mortality in India which requires early diagnosis for treatment and control of infection. Fine needle aspiration cytology (FNAC) and Ziehl Neelson (ZN) staining of FNA samples have low specificity and sensitivity respectively in extrapulmonary tuberculosis due to paucicellular nature of the disease. Cartridge- Based Nucleic Acid Amplification Test (CBNAAT) has a well established role in detection of tuberculosis. Recently, WHO has recommended its use in extrapulmonary tuberculosis as an initial diagnostic tool.

Material and Methods: This is a prospective study performed in Department of pathology and included 80 patients of suspected symptoms of extrapulmonary tuberculosis. Fine needle aspiration was done and the material was sent for CBNAAT. The rest of the material was used for preparing H&E stained and Z-N stained smears.

Result: Out of the total 80 cases, 58 cases demonstrated cytomorphological features suggestive of tuberculosis, 38 cases were positive for AFB on ZN staining and 53 cases showed CBNAAT positivity, thus proving the important role of CBNAAT in diagnosing extrapulmonary tuberculosis. This difference was also statistically significant (p value <0.05).

Conclusion: Combined use of FNAC, ZN staining and CBNAAT gives maximum diagnostic yield, greatly improving diagnostic value specially in patients with low bacillary load, those are likely to be missed on FNAC & ZN staining by increasing the rate of detection of extrapulmonary tuberculosis.

KEYWORDS

Extrapulmonary tuberculosis, CBNAAT, Ziehl-Neelsen stain, Fine needle aspiration.

INTRODUCTION

India is the home of world's largest tuberculosis burden, accounting for around a quarter of the Tuberculosis incidence globally.¹ Although pulmonary involvement is the most common presentation, tuberculosis can potentially affect any organ or system of the body. Extra pulmonary tuberculosis, according to WHO classification criteria is defined as an infection by *Mycobacterium tuberculosis* which affects tissues and organs outside the pulmonary parenchyma. Lymph node tuberculosis is commonest form of extra pulmonary tuberculosis accounting for 58 % of extrapulmonary lesions. Superficial lymphadenopathy is the most common extra pulmonary manifestation of tuberculosis.²

Conventional methods of diagnosis like sputum examination and radiological examination, though accurate in diagnosing pulmonary tuberculosis, are not helpful in diagnosis of extrapulmonary tuberculosis. As the number of *Mycobacterium tuberculosis* bacilli in extra pulmonary sites is often low, diagnosis of extra pulmonary TB cases still remains challenging.³ Mainly cytology and conventional smear microscopy have been used as the initial diagnostic tool in the diagnosis of these cases. Hence, differential diagnosis of tuberculous lymphadenopathy is broad and laboratory confirmation is of paramount importance to guide appropriate therapy.^{4,5}

Final Needle Aspiration Cytology (FNAC) is an efficient primary diagnostic tool, which can be used in the diagnosis at the initial step as well as in the follow-up post treatment of tuberculosis.⁶ FNAC is a safe, simple, rapid, cost effective and minimally invasive procedure which is now acceptable and widely practiced. Ancillary test like ZN (Ziehl-Neelson) staining i.e. AFB (Acid Fast Bacilli) staining from FNAC aspirate material help in more definite diagnosis by increasing the sensitivity and specificity of FNAC by demonstrating presence of AFB in the aspirate material.⁷ Histopathology is time consuming and is

difficult to undertake and establish the diagnosis of tuberculosis with high specificity.⁸

The aspirated material after doing FNAC is stained and studied for presence of epithelioid granulomas and caseous necrosis to make provisional diagnosis of tubercular lymphadenitis. In some late cases of tubercular lymphadenitis, there is liquefaction and abscess formation, in such cases it becomes difficult to find usual diagnostic cytological findings. Here, we have to rely on other adjuvant techniques like CBNAAT, Acid Fast Bacilli Staining and Culture.⁹

Cartridge based nucleic acid amplification test (CBNAAT) is a new diagnostic test revolutionizing the diagnostic scenario by contributing in rapid diagnosis of tuberculosis and drug resistance.¹⁰ CBNAAT assay consists of a closed system that is based on real-time polymerase chain reaction (PCR), which requires minimal technical expertise in the diagnosis of tuberculosis and also drug (Rifampicin) resistance within 2 hours.¹¹ In 2014, WHO recommended CBNAAT over the conventional tests for patients suspected of having extra pulmonary tuberculosis. However, it was a conditional recommendation provided the low quality evidence available.¹²

MATERIAL AND METHODS

The present study was carried out at Pathology department of Maharaja Agrasen Medical College, Agroha (Hisar) on patient's presenting with suspected clinical features of extrapulmonary tuberculosis. Sample size of the study was 80 cases and duration of study was 1 year. FNAC of all cases was done under aseptic conditions and a part of the aspirate was used for preparing smears for cytological examination using H & E Staining and AFB demonstration by Z N staining while the remaining aspirate was sent out for CBNAAT in a sterile cartridge container and a printable test result was obtained in

about 2 hours. CBNAAT results were reported as negative or positive.

RESULTS

The study included 80 suspected patients with suspected clinical features of extrapulmonary tuberculosis. The aspirate was purulent in 70 cases, blood mixed in 9 cases and a yellowish fluid was aspirated in 1 case. Among 80 cases, the age of patients ranged from 8 months to 75 years with maximum number of cases in 21-40 age group, of which 36 were males and 44 were females. On fine needle aspiration cytology, various cytological features and patterns suggesting diagnosis of necrotizing granulomatous inflammatory pathology were demonstrated in 57.5% cases, followed by features of acute inflammatory pathology in 15% cases, granulomatous inflammatory pathology in 11.2% cases, features suggestive of chronic non specific inflammatory pathology in 5% cases, necrotizing inflammatory findings in 3.8% cases and features of metastatic lymph node in 2.5% cases. The positivity for *Mycobacterium tuberculosis* was 66.3% by CBNAAT and 47.5% by conventional ZN staining method. 15 cases which were negative on ZN Staining were detected by CBNAAT assay. This difference was statistically significant with p value <0.05. This comparison illustrated that CBNAAT is a better diagnostic tool to diagnose recurrent tubercular lymphadenopathy than the conventional ZN staining and microscopy.

Table I Comparison Of Fnac Diagnosis With Afb And Cbnaat Positivity

Diagnosis	Total no. of cases diagnosed on FNAC	Positive cases on AFB (ZN Staining)	Positive cases on CBNAAT
Low cellularity	1	0	0
Acute suppurative inflammatory pathology	12	0	1
Chronic non specific inflammatory pathology	4	0	1
Necrotizing inflammatory pathology	2	0	2
Necrotizing granulomatous inflammatory pathology	47	38	45
Non necrotizing granulomatous inflammatory pathology	9	0	4
Infected epidermal cyst	2	0	0
Metastasis/Malignancy	3	0	0
Total	80	38 (47.5%)	53 (66.2%)

DISCUSSION

Out of 80 cases in our study, twelve cases were diagnosed as Acute suppurative inflammatory pathology on FNAC, which were all negative on ZN staining, but 1 case was found to be positive on CBNAAT. Similar findings were present in a study by Patil SB et al¹³. Out of 79 cases of acute suppurative inflammatory pathology, four were found positive by ZN staining, and 32 showed CBNAAT positivity.

In four cases of Chronic non specific lymphadenitis in our study, which all showed up negative on ZN staining for AFB, one case again was picked up only by CBNAAT. Thus, here CBNAAT proved to be superior than both FNAC and ZN staining in correctly diagnosing false negative cases of extrapulmonary tuberculosis. Study by Patil SB et al¹³ was in concordance to our study, they found 4 cases to be ZN positive, 32 cases to be CBNAAT positive out of 167 cases diagnosed as chronic non specific lymphadenitis.

Among two cases of Necrotizing inflammatory pathology, both of which were negative on AFB (ZN staining), CBNAAT was able to detect them both, thus demonstrating its use as prime diagnostic tool. Similar outcome was seen in a study by Patil SB et al¹³. Out of 39 cases of necrotizing inflammatory pathology, 20 (51.28%) were found positive by ZN staining, and 22 (56.41%) showed CBNAAT positivity. Study by R SA et al¹⁴ was also found supporting the outcome in our study. They found 6 cases of necrotizing inflammatory pathology, among them 5 cases showed AFB positivity and 4 showed CBNAAT positivity.

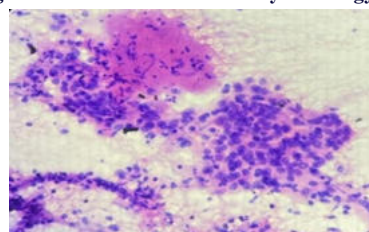
Out of 47 cases that were diagnosed as Necrotizing granulomatous inflammatory pathology in our study, ZN staining for AFB was able to

detect only 38 (80.85%) cases, while 45 (95.74%) cases were detected for tuberculosis by CBNAAT. Hence, in this category CBNAAT was able to detect 7 more cases than ZN staining. Study by Patil SB et al¹³ was in concordance to our study, they found 66 cases to be ZN positive, 57 cases to be CBNAAT positive out of 75 cases diagnosed as necrotizing granulomatous inflammatory pathology. R SA et al¹⁴ in their study found 14 cases of necrotizing granulomatous inflammatory pathology, among them 1 case showed AFB positivity and 3 showed CBNAAT positivity. This study had variation in observation made by our study in view of detecting less number of CBNAAT positive cases among cases of granuloma with necrosis.

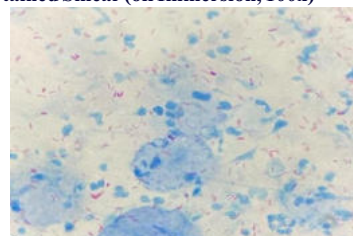
In nine cases that were diagnosed as Non-necrotizing granulomatous inflammatory pathology on FNAC, none of the case was found positive for AFB by ZN staining, while CBNAAT was able to detect 4 cases for tuberculosis. Here again, CBNAAT proved to be more effective than ZN staining by picking 4 more cases as positive for tuberculosis. Findings in favour of our study were present in a study by Patil SB et al¹³. Out of 78 cases non-necrotizing granulomatous inflammatory pathology, 4 were found positive by ZN staining, and 7 showed CBNAAT positivity. A similar study by R SA et al¹⁴ also found 21 cases of non-necrotizing granulomatous inflammatory pathology, among them 4 cases showed AFB positivity and 3 showed CBNAAT positivity.

Our study deduces that CBNAAT is adjunct to FNAC and ZN staining in diagnosing extrapulmonary tuberculosis. CBNAAT assay proved to have a clear advantage in detection of FNA missed cases. FNAC although is a simple procedure, but has a low specificity and has considerably lower edge than CBNAAT in correctly diagnosing extrapulmonary tubercular cases due to presence of some similar cytomorphological features in lesions other than those associated with tuberculosis. High immune response may also lead to altered cytomorphological findings and thus CBNAAT is more efficient in diagnosing these FNAC negative cases. AFB positivity on ZN staining is low in FNAC aspirates due to paucibacillary presence of AFB bacilli in extrapulmonary sites. Also previous history of taking ATT can also lead to fragmentation of the acid fast bacilli along with reduction in bacterial load further reducing the chances of demonstrating AFB on ZN stained smears. CBNAAT is found to be of great significance in these scenarios and can be integrated into a routine diagnostic protocol.

**Pictograph 1
Necrotizing Granulomatous Inflammatory Pathology (h&e, 40x)**



Afb On Zn Stained Smear (oil Immersion, 100x)



Cbnaat Machine



CONCLUSION

The diagnosis of extrapulmonary tuberculosis is a difficult task, given the paucibacillary nature of MTB in extra-pulmonary sites. As accepted worldwide, FNA is a safe, cost effective, non invasive and rapid method, that is ideal for use in resource limited settings to diagnose tuberculosis based upon cytomorphological features and ZN staining. Our study found FNAC, ZN staining and CBNAAT to be mutually complementary diagnostic tests. Additionally, our study deduced that CBNAAT was able to detect 2 cases which didn't show typical cytomorphological of tuberculosis on FNAC and 15 more cases of ZN staining negative cases, hence underlining the importance of CBNAAT over both FNA and ZN staining in diagnosing TB in extrapulmonary samples. In a developing country like India, incorporation of CBNAAT into routine diagnostic protocol will definitely improve the early diagnosis and treatment strategy, reducing morbidity and mortality related to TB and support WHO's goal of eradicating TB by 2025.

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