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CORRELATION OF CYTOMORPHOLOGY, Z- N MICROSCOPY AND CARTRIDGE-BASED NUCLEIC ACID AMPLIFICATION TEST (CBNAAT) FOR DIAGNOSIS OF EXTRA -PULMONARY TUBERCULOSIS (EPTB) IN FINE NEEDLE ASPIRATION(FNA) SAMPLES.

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ABSTRACT

Introduction: Tuberculosis (TB) remains one of the world's deadliest communicable diseases. Extrapulmonary tuberculosis (EPTB) can occur in isolation or along with pulmonary focus with disseminated tuberculosis. Since clinical presentation of EPTB is an atypical and poor yield of the conventional diagnostic method due to its paucibacillary nature the diagnosis is often delayed and challenging. Though Z-N staining has low cost,high specificity and does not required high laboratory standards, the CBNAAT assay provides early diagnosis ,distinguishes MTB and mycobacteria other than tuberculosis and also provide rifampicin resistance simultaneously in a Fine needle aspirated sampling. Aims and objectives was to study and correlate the role of FNAC, Z-N staining and CBNAAT in diagnosing extra-pulmonary tuberculosis (EPTB). **Materials & Method:** It was retrospective study carried out in department of pathology, SVBCH, NAMO medical education and research institute ,silvassa, during period of October 2018 to march 2020(eighteen months) on patients having extra pulmonary tuberculosis (EPTB) were subjected to FNAC. In each case at least 3 smears for H & E, Giemsa stain and Z-N staining done from FNA aspirates. FNAC material sent for CBNAAT in studied cases . **Results:** Females are more commonly affected with male to female ratio 1:1.3.Second to third decade was commonly affected group. Most common site involved was cervical(63.76%) and cytological pattern was epithelioid granulomas without necrosis 68(49.27%) .Most common aspirated material were blood mixed pus (39.13%) and purulent(38.40%) observed. Overall AFB positivity seen in 76.08% compare to 95.65% positive CBNAAT finding. CBNAAT has shown higher levels of sensitivity and specificity for diagnosis of EPTB compare to cytology and Z-N microscopy. **Conclusion:** FNAC is simple, cost effective diagnostic tool with high degree of accuracy. FNAC coupled with AFB staining is the first line of investigation for EPTB. The present study highlights the utility of CBNAAT from FNAC material as one of the rapid and adjuvant diagnostic tool in EPTB. CBNAAT also used for diagnose Rifampicin resistance cases.

KEYWORDS : EPTB, FNAC, CBNAAT, AFB

INTRODUCTION

In India, the incidence of tuberculosis accounts for a quarter of the world's annual incidence of TB. Tuberculosis (TB) remains a big burden as well as an important health problem in developing and underdeveloped nations. It is a great challenge to eradicate TB country like India, where TB is endemic to due to lack of adequate diagnostic assays According to a WHO report around 2.15 million people notified for TB in India and a 17% cases fatality ratio was observed in 2018.[1]

The EPTB term has been used to describe the isolated occurrence of tuberculosis at body sites other than the lung. However when extra pulmonary focus is evident in a patient with pulmonary tuberculosis, such patient have been categorized under pulmonary tuberculosis as per guidelines the of WHO organization.Out of all cases of tuberculosis, EPTB constitutes about 15-20% of cases in immunocompetent patients and more than 50%of cases in HIV-positive individuals [2]. Extrapulmonary sites of infection commonly include lymph nodes, pleura, osteoarticular areas, and gastrointestinal & genitourinary systems, although any organ can be involved [3].The number of Mycobacterium Tuberculosis Bacilli (MTB) in extrapulmonary sites is often low, and diagnosis of EPTB still remains challenging. [4]

The conventional methods like sputum examination and chest X-ray are accurate in detecting the pulmonary disease but they are not helpful in the diagnosis of extrapulmonary tuberculosis and not able to detect Rifampicin-resistant cases. Fine needle aspiration cytology (FNAC) has emerged as the first line of investigation in the assessment of radiologically detected lesions. This is a safe, less traumatic, rapid, and easy method compared to the larger core or open biopsy but with low specificity. This procedure is cost-effective as well as easier to repeat, if necessary [5] .Positive Z-N smear requires more than 5000-10000 bacilli/ml, so of limited diagnostic value (0-40%) in the majority of paucibacillary EPTB samples [6],[7].

In line with these limitations more rapid and reliable methods are needed. In December 2010, WHO endorsed CBNAAT for use in TB laboratories. In 2012, CBNAAT was adopted in India by RNTCP and first started as a pilot project in Maharashtra. [8] The 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 TB and rifampicin resistance within 2hours.[9] [10]

CBNAAT purifies, concentrates, amplifies, and identifies the targeted rpoB nucleic acid sequences, and delivers the results in about 120 minutes. The sample reagent to clinical specimens' ratio was 3:1.. It is a rapid, fully automated test and based on the PCR technique that detects DNA directly from the clinical specimens along with Rifampicin resistance detection[16].

More recently a number of studies were done to evaluate this assay using non-respiratory clinical samples from patients suspected of having EPTB.[11,12] In 2014, WHO recommended CBNAAT over the conventional tests (including conventional microscopy, culture, or histopathology) for testing specific non-respiratory specimens (lymph nodes and other tissues) from patients suspected of having EPTB.[13]

Thus, we want to evaluate the role of CBNAAT in the diagnosis of EPTB using routinely collected FNA samples and compared it against cytology and Z-N stain.

AIMS & OBJECTIVES

This study was conducted to correlate the cytomorphology of fine needle aspirates in patients of Extra -Pulmonary TB (EPTB) with Z-N stain and CBNAAT and also to assess the role of CBNAAT in the early identification of MDR-TB (rifampicin resistant) suspected cases of EPTB.

MATERIALS AND METHODS

This retrospective study was conducted at VBCH

Hospital/NAMO Medical College, Silvassa. Out of 1236 total FNAC, 138 cases were selected for study over a period of 1 year and 6 months. Patient's age, gender, site of lesions, gross examination of aspirate, cytomorphological patterns, stain for AFB (acid-fast bacillus), and the result of CBNAAT assay results were studied

FNAC was done using a 22 gauge needle, under strict aseptic precautions after taking consent from patients or guardians. One for H&E stain (Fixative) and two dry slides for Giemsa and Z-N stain respectively. Second pass sample collected in sterile vacutainer CBNAAT(EXPERT MTB/RIF Cepheid, USA).

Cytomorphological findings were categorized into different morphological patterns i.e. Granuloma with Caseaous necrosis(Type 1), Granuloma without Caseous necrosis(Type 2), Only necrosis without granuloma (Type 3), Neutrophils with necrosis (Type 4).

For each Z-N staining microscopy with known positive and negative slides were included. A minimum of 100 oil immersion fields were seen before reporting a smear as negative.

CBNAAT is a semiquantitative nested real-time PCR that was performed according to the manufacturer's instructions. A semi-quantitative estimate the concentration of bacilli can be defined on the cycle threshold (ct) value as ct value high <16; Medium:16-22; Low 22-28; very low >28.

Data were analyzed with the use of appropriate statistical software MS-EXCEL.

Approval from the Institutional Ethics Committee was taken for conducting of the present study.

Inclusion Criteria:

1. Patients having clinical features of extra-pulmonary tuberculosis.
2. Any purulent aspirate in FNA irrespective of the clinical suspicion.
3. Patients giving consent for the study.

Exclusion Criteria:

1. Scant aspirate in which material could not be sent for CBNAAT
2. Patients who did not give consent or were uncooperative for the procedure.

RESULTS

This study was carried out for 1 year and 6 months in the Department of Pathology in the cytopathology section at VBCH, tertiary care hospital. Out of 1236 FNAC samples aspirated on OPD bases, 138 cases were diagnosed as EPTB and analyzed.

Table: 1 Age and gender distributions in EPTB cases(n=138)

Age(years)	Male	Female	Total (%)
1-10	2	4	6(4.34%)
11-20	6	12	18(13.05%)
21-30	32	37	69(50.0%)
31-40	14	18	32(23.19%)
41-50	4	4	8(5.79%)
>50	2	3	5(3.63%)
Total	60(43.48%)	78(56.52%)	138(100%)

Table: 2 Distribution of cases as per involved Anatomical sites

Site of aspiration	No. of cases (%)
Cervical	88(63.76%)
Submandibular	13(9.43%)
Supraclavicular	11(7.98%)
Axillary	10(7.24%)
Pre auricular	06(4.34%)
Submental	05(3.63%)
Temporo Occipital	03(2.18%)
Inguinal	02(1.44%)
Total	138(100%)

Table3: Fine Needle Aspirate material nature

Sr.No	Nature of aspirated materials	No. of cases (%)
1	Blood mixed pus	54(39.13%)
2	Pus(purulent)	53(38.40%)
3	Cheesy(Thick grey white)	18(13.04%)
4	Creamy	13(9.43%)
Total	-	138(100%)

Table 4: Results of cytological patterns, AFB & CBNAAT

Sr. no	Type of patterns	No. of Cases (%)	AFB positive	CBNAAT positive
1	Epithelioid granulomas with necrosis (Type 1)	68 (49.27%)	56 (79.41%)	65 (95.58%)
2	Epithelioid granulomas without necrosis (Type 2)	52 (37.68%)	41 (78.84%)	50 (96.15%)
3	Only necrosis without granuloma (Type 3)	5 (3.62%)	3 (60.0%)	5(100%)
4	Neutrophils with necrosis (Type 4)	13 (9.42%)	9 (69.23%)	12 (92.30%)
Total	-	138	109 (80.74%)	132 (95.65%)

DISCUSSION

The annual global incidence of EPTB has been increasing in the last decade due to the changing TB control practices, the spread of HIV, and population growth. Early diagnosis of EPTB is important because of high mortality. The diagnosis of EPTB poses a particular challenge for clinicians because of its atypical presentations, paucibacillary nature, and lacking health facilities in rural areas that are used by about 70% of the populations in developing countries like India [14]. Fine needle aspiration cytology (FNAC) with or without guidance is a simple outpatient diagnostic procedure used for the diagnosis of palpable and deeply seated lesions. But this procedure has several limitations and pitfalls as explained earlier. To address this issue there was a need for a simple and rapid diagnostic tool and a new diagnostic test, CBNAAT was developed.[15]

In our study, out of 138 patients, a maximum no. of patients were presented in 21-30years. Less number of cases were seen in extreme ages. Female patients (56.52%) were more commonly affected. The most commonly involved anatomical site observed was the cervical region (63.76%). The most commonly observed cytomorphology pattern was epithelioid granulomas with caseous necrosis(49.27%). Similar findings were reported by Purohit et al [6], Majed Momin et al[17], Shairoly singh et al.[18]

Table 5: Cytological pattern and AFB positivity comparison with other studies.

Type of patterns	Present study		Shilpa G et al [19]		Majed Momin et al[17]		Shairoly singh et al[18]	
	Patterns (%)	AFB positive (%)	Patterns (%)	AFB positive (%)	Patterns (%)	AFB positive (%)	Patterns (%)	AFB positive (%)
Epithelioid granulomas with necrosis (Type 1)	49.27%	79.41%	51.2%	19%	40%	37.5%	52.4%	72%

Epithelioid granulomas without necrosis (Type 2)	37.68%	78.84%	34.2%	7%	22.5%	10%	40.5%	62%
Only necrosis without granuloma (Type 3)	3.62%	60.0%	4.3%	52%	10%	7.5%	2.4%	52%
Neutrophils with necrosis (Type 4)	9.42%	69.23%	10.3%	83%	27.5%	15%	4.8%	73%

In the present study overall stain for AFB was positive in 80.74% of cases. The percentage of positivity of AFB staining (Z-N stain) is most commonly seen with epithelioid granulomas with caseous necrosis (79.41%) followed by epithelioid Granulomas without caseous necrosis (78.84%), neutrophils with necrosis (69.23%) & only necrosis without granuloma (60.0%).Shairoly singh et al[18]showed 72% with granulomas with caseous necrosis followed by Granulomas without caseous necrosis(62%), neutrophils with necrosis(73%) & only necrosis without granuloma(52.0%)[18] which was similar to the present study. whereas Majed Momin et al[17] observed 37.5% AFB positivity with Epithelioid granulomas with necrosis and only 15% with Neutrophils with necrosis. Shilpa G et al observed only 26% AFB positivity with granulomatous pattern whereas 83% with only necrosis.[19]

The sensitivity of AFB was 80.74% in our study, comparable to that of Brijesh Thakor et al (80%) [20] but less than of Delyuzar et al (93.65%)[21]. AFB lacks sensitivity due to the paucibacillary nature of fine needle aspirates and the presence of nonviable bacilli due to either the harsh decontamination process or caseous lesion in the lymph node tissue containing dead bacilli. The specificity of AFB in our study was 100% similar to Ligthelm et al [22](100%); studies by Tadesse et al[23] and Delyuzar et al[21] showed AFB specificity to be 87.5%and 77.99% respectively.

Table 6: CBNAAT results compared with other studies

Sr .no	Study done by	Country (year)	CBNAAT sensitivity %(95%CI)	CBNAAT specificity%(95%CI)
1	Lightelm et al[22]	South Africa(2011)	96.6%	88.9%
2.	Vadwai et al[27]	India.(2011)	80.6%	99.6%
3	Singh KG et al[24]	India(2017)	91.0%	90.0%
4.	Tortoli et al[28]	Italy(2012)	81.5%	99.8%
5.	Shilpa G et al [19]	India(2018)	37.0%	80.0%
6	L Aruna et al [21]	India(2018)	65.0%	92.45%
7.	Present study	India(2019)	95.6%	100%

In our study CBNAAT test showed a Sensitivity of 95.6% and a specificity 100% which was comparable with study by Ligthelm et al (sensitivity-96.6%, specificity of 88.9%) [22] and Singh KG et al, (sensitivity 91%, specificity 90%) [24].whereas a study done by Vadwai et al observed 80.6% sensitivity and 99.6% specificity in his study [27]. The reason for false negative CBNAAT test results may be due to the limited number of bacilli in the FNA sample, prolonged storage of the sample before XPERT testing, and the amount of material sent. The possible cause for CBNAAT negativity in six (6/138) cases may be due to cheesy material might be the solid nature of the cheesy material which usually has a very low bacillary load in nature compared to liquid caseous material which has a high bacillary load or might be insufficient material. Because low bacillary load and its detection limit of 131cfu/ml might be the reason for CBNAAT negativity in these patients. Hence increased false negatives on CBNAAT. So, CBNAAT's negative result can still have Tb or MOTT. The main limitation of our study was not able to compare the Culture report which is considered the gold standard. Although only 1.44% (2/138) were Rifampicin resistant in the present study detection helped in further management. whereas the study conducted by Majed Momin et al [17], Tadesse M et al[23], and Komanapalli SK et al [25] 2.5%, 4.7%,2.1% Rifampicin resistant cases respectively. Whereas study conducted by Singh KG et al [24]and Shilpa G et al [19] observed no rifampicin-resistant cases in their populated study groups.

CONCLUSION

To Conclude, Diagnosis of EPTB is difficult due to its atypical presentation and requires high clinical suspicion and special diagnostic procedures. FNAC with imaging proves as a very useful first-line investigation. Gene X-pert testing with rifampicin resistance test in aspirated samples has shown significantly higher levels of sensitivity and specificity for the diagnosis of EPTB as compared to cytomorphology alone. WHO recommended CBNAAT over conventional tests in patients suspected of having extrapulmonary TB but CBNAAT is costly, requiring technical expertise, power backup, maintenance, and calibration [26]. In areas of resource availability, it may be used to diagnose Rifampicin resistance in suspected cases, immunocompromised individuals (PLWHA), and children and it should be always supplemented with FNAC. Also conclude that combined techniques proved themselves as an effective tool in OPD basis diagnosis and early initiation of treatment to prevent morbidity, mortality, and economic loss.

Findings: Nil;
Conflict of Interest: Non-initiated
Permission from IRB: Yes

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