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Pathology

COMPARATIVE STUDY OF FLUORESCENCE AND AFB STAIN IN FNA SAMPLES OF EXTRAPULMONARY TUBERCULOSIS

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ABSTRACT The extrapulmonary tuberculosis (EPTB) is difficult to diagnose due to its pauci-bacillary nature. Aim of the present study is to do the comparative analysis of Fluorescence and Ziehl-Neelson stain upon FNA samples in clinically suspected cases of EPTB. **Methods:** Presentstudy was retrospective study of 100 cases of clinically suspected EPTB referred from Respiratory Medicine OPD. After procedure of FNA, smears were stained with routine H&E, PAP stain, ZN stain and fluorescence stain. Results were obtained after detailed study. **Results:** Out of 100 cases, presumptive tuberculosis was diagnosed in 54 cases showing either epithelioid cell granulomas or caseous necrosis or both upon morphology, while overall 26 cases were positive on ZN stain and 45 cases were positive on fluorescence. **Conclusions:** FNAC is the cheapest and simplest method to diagnose extrapulmonary tuberculosis, however those smears where TB cannot be diagnosed on FNAC like suppurative lesions, reactive lymphadenitis and low cellularity,fluorescence stain plays a key role for the correct diagnosis thereby significantly reducing the morbidity and mortality.

KEYWORDS : EPTB, FNAC, ZN, Fluorescence.

Introduction

Tuberculosis is one of the world's deadliest communicable diseases. Yearly, worldwide around 10 million people fall ill with TB according to Global Tuberculosis Report 2019 of World Health Organisation.1Annually, one fourth of the global incident TB cases occur in India and India has continued to top the list of TB burden.1,2Tuberculosis is caused by Mycobacterium tuberculosis and it commonly involves lungs but TB can affect any organ or system of the body. Extrapulmonary tuberculosis according to WHO classification criteria is an infection by M. tuberculosis affecting tissues and organs outside the pulmonary parenchyma.3,4In India, 10-15% of total TB cases are of extrapulmonary tuberculosis commonly affecting the pleura, lymph nodes, gastrointestinal tract and other organs with a significant mortality rate (25-50%).5Cytology and conventional smear microscopy have been used as the initial diagnostic tools for tuberculous lymphadenitis in resource poor settings.6Fine needle aspiration cytology is a simple and rapid diagnostic technique, but with low specificity due to common cytomorphological features between tuberculous and non-tuberculous cases.7,8Conventional smear microscopy lacks sensitivity due to the pauci-bacillary nature of fine needle aspirates (FNA).6Mycobacterial culture is a gold standard for diagnosis of TB and because drug susceptibility testings are not always available in resource poor settings, their results may take 4-8 weeks or even longer and it is expensive too.4Considering these limitations, more rapid and reliable methods like Fluorescence Microscopy are needed so that bacilli can be picked up within no time.

Koch first described the tubercle bacilli in 1882 which is now called as Mycobacterium tuberculosis. Mycobacteria are known to comprise a large group of acid-fast, alcohol-fast, aerobic or microaerophilic, non-spore forming, non-motile bacilli. 9However, the Ziel-Neelsen method for acid-fast bacilli plays a key role in the diagnosis and also for the monitoring of treatment in tuberculosis. Its major disadvantage is that it requires more than 5000-10000 bacilli/ ml turning to low sensitivity ranging from 20% to43%. 10, 11, 12Newer molecular techniques such as polymerase chain reaction (CBNAAT), although rapid, are costly to be routinely used in developing countries where most TB cases occur. 13Newer investigative methods like Fluorescent Microscopy plays an important role for detection of Mycobacteria because lower magnifications are used along with its less time consumption to examine the smears. Fluorescence microscopy using Auramine-Rhodamine (AR) or Papanicolaou (PAP) staining has been considered to be superior to ZN staining.14, 15The method is quick and inexpensive. The efficacy of autofluorescence and fluorescence in the diagnosis of extrapulmonary tuberculosis was evaluated for this purpose.

With this aim, the present study was carried out andthe results of FNA smear cytology were compared with ZN stain and Fluorecscence microscopy findings.

Material and methods

Present studywasthe retrospective study conducted in the Department ofPathology, Government Medical College, Akola. All those clinically suspected cases of EPTB referred from Respiratory Medicine, ENT and Surgery OPDs were included in this study. So in total, we had included 100 cases. While those already diagnosed, recurrent and follow up cases of EPTB were excluded.

Procedure:

After obtaining detailed history and examining the patients, FNAC specimens were collected from 100 cases by performing 2-3 passes of 23-24 gauge needle attached to 5 ml syringe. Four smears were prepared from each aspirated material. Two were fixed with 95% isopropyl alcohol each for H & E and PAP staining. These smears were evaluated for adequacy and for the presence of epithelioid cell granulomas with or without caseous necrosis or only caseous necrosis. Third smear was for ZN staining. ZN stained smears were examined for bright pink beaded curved bacilli on bluish background and were reported as Positive/Negative for acid fast bacilli and the fourth one was for fluorescence microscopy. Smears for Fluorescence Microscopy were prepared with Auramine O stain which appears bright yellow fluorescent curved bacilli which were reported as positive and those cases showing similar fluorescence by crystals or fungal bodies were reported as negative.

Results

One hundred cases with clinical suspicion of tuberculosis subjected to FNAC, ZN stain and Fluorescence microscopy were studied. Most of the cases (27%) were found in the age group of 21-30 years with female preponderance(20%). The youngest patient was 4 year old male child and 88 years old male was the oldest patient. Overall, females (69%) showed predominance over males (31%) as shown in Table 1.

Table 1: Age and sex wise distribution of total cases

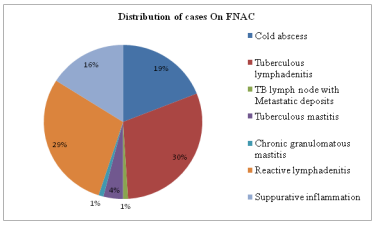
Age (Years)	Number Of Patients	Males	Females
0-10	07	3	4
11-20	20	6	14
21-30	27	7	20
31-40	22	5	17
41-50	9	2	7
51-60	7	4	3
61-70	5	2	3
71-80	2	2	0
81-90	1	0	1
Total	100	31	69

Table 2: Site wise distribution of total cases

Site	No of patients	%
Cervical	63	63
Post auricular	8	8
Breast	8	8
submandibular	6	6
Inguinal	3	3
Axilla	2	2
Other (Back, arm, nape of neck, abdominal wall)	10	10
Total	100	100

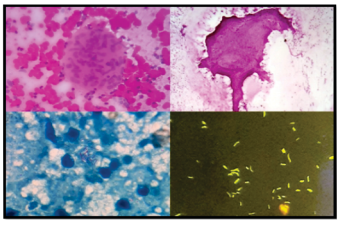
The most common site tobe involved was cervical neck swelling (63%) followed by post-auricular (8%) & breast (8%) and submandibular swelling (6%) as shown table 2.

Figure 1:FNAC Diagnosis



Out of 100 clinically suspected cases, majority cases were of tuberculous lymphadenitis comprising 30%, followed by reactive lymphadenitis (29%).Among FNAC diagnosed cases of tuberculosis, cases of tuberculous lymphadenitis showing ill to well defined granulomas and caseous necrosis (Figure 2- A)were 30%, cold abscess showing only caseous necrosis(Figure 2-B) were 19%. One case was with Tuberculous lymphadenitis with associated metastatic deposits of squamous cell carcinoma. Breast was involved in 8 cases as tuberculous mastitis (4) and granulomatous mastitis (1) and remaining were given as suppurative inflammation showing plenty of polymorphs and histiocytes as shown in Figure 1.

Figure 2: Different cytomorphological features of Tuberculosis



A- Granuloma consisting of epithelioid cells (H & E, 400X), B-Caseous necrosis (H & E, 200X), C- ZN Stain show positive acid fast bacilli (1000X-arrow and arrow head).D- Bright yellow fluorescent mycobacterial bacilli (100X, Fluorescence microscopy)

Table 3: Comparison of FNAC diagnosis with ZN stain and Fluorescence microscopy:

Diagnosis	Number of cases	ZN stain		Fluorescence Microscopy	
		positive	Negative	Positive	negative
Tuberculosis (54)					
Cold abscess	19	9	10	14	5
Tuberculous lymphadenitis	30	16	14	24	6
TB lymph node with Metastatic deposits	01	0	1	1	0
Tuberculous mastitis	04	1	3	2	2
Chronic granulomatous mastitis	01	0	1	0	1
Reactive lymphadenitis	29	0	29	2	27
Suppurative inflammation	16	0	16	2	14
Total	100	26	74	45	55

Out of 100 cases, 26% cases were positive for acid fast bacilli while 45% cases were positive for fluorescence. Among 54 cases of tuberculosis diagnosed on FNA, 26% cases were positive for acid fast bacilli and 41% cases were positive for Fluorescence microscopy. Among 29 cases of reactive lymphadenitis, all were ZN negativehowevertwo cases were fluorescence positive while out of 16 cases of suppurative inflammation, all were ZN stain negative and two cases were fluorescence positive as shown in table 3.

DISCUSSION

The present study was retrospective study on the diagnosis of extrapulmonary tuberculosis by fluorescence microscopy in comparison to FNAC and ZN stain in a tertiary care hospital.

In this study, we compared the age and sex wise distribution of total cases with different studies. Out of 100 cases, majority was in the age group of 21-30 years & female predominancewas reported which was similar to the studies conducted by Konamapalli S K et al3, Majed M et al12, Lavanya G et al.16 as shown in table 4, 5.

Table 4: Comparison of age wise distribution with other studies:

Study	Age group	% of cases
Present study	21-30	27.0
Konamapalli SK et al 3	21-30	26.4
Lavanya G et al.16	21-30	27.8
Majed M et al12	21-30	37.5

Table 5: Comparison of sex wise distribution with other studies

Study	Male (%)	Female (%)
Present study	31.0	69.0
Majed M et al12	32.5	67.5
Lavanya G et al.16	42.0	58.0
Konamapalli SK et al3	48.0	51.0

We also compared anatomical site distribution of cases. The most common site involved in this study was cervical lymph node which was also noted by the study done by Majed M et al12 and Lavanya G et al.[16]

It is generally believed in literature that the Auramine-Rhodamine fluorescent technique yields more positive results than the conventional Ziehl-Neelsen method. This has been proved by the experiences of Traunt et al 18, Needham et al 19, Kuper et al 20 and Braunstein et al 21. In the present study, out of a total of 100 cases, 54% cases were Tuberculous lesions on FNAC, while 26% cases were positive on ZN stain and 45% cases were positive on fluorescence microscopy which was similar to the findings of Thakur B et al17 (ZN stain-26.7% & Fluorescence-42.2%) while Konamapalli SK et al3 stated 69% fluorescence positive cases. Annam et al 22proved on lymph node aspirates that the positivity rates were 44.11% and 81.37% by Ziehl-Neelsen and fluorescent techniques respectively and the total AFB positivity rate was 36.5% cases on ZN stain and was increased to 51.3% on fluorescent stain studied by Dagar V et al23.

CONCLUSION

As accepted worldwide, FNA is a safe, cost effective, non invasive and rapid method to diagnose tuberculosis based upon cytomorphological features and ZN stain. However, it becomes very difficult to diagnose TB in suppurative lesions, reactive lymphadenitis and low cellularity.

In such context if in a single prick; cytology smears, ZN stain and fluorescence microscopy are carried out, rapid diagnosis of TB is possible reducing false negative diagnosis thereby reducing morbidity and mortality related to TB. So, coupling FNAC with ZN stain and with fluorescence microscopy increases diagnostic accuracy for tuberculosis. The use of fluorescence microscopy greatly improves diagnostic value especially in patients with low bacillary load those are likely tobe missed on ZN stain as they get easily caught on fluorescence. Its superiority is ascribed to the ease with which bacilli are seen because of the greater contrast between bacilli and background. With fluorescence microscopy larger number of smears can be screened in a short period of time. Hence to achieve WHO goal of eradication of TB by 2025- use of Fluorescence microscopy should be promoted in all rural hospitals and tertiary care centers across the whole country. This will reduce diagnostic cost spent upon costly investigations like PCR and time spent upon gold standard TB culture so that we can help reducing the global burden of tuberculosis.

Declarations:
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Ethical approval- Done

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