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Role of Fluorescent Staining and Ziehl-Neelsen Techniques in The Detection of Mycobacterium Tubercle Bacilli in Lymph Node Aspirate

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ABSTRACT

Background

Tuberculosis is the important disease of infectious etiology especially in the developing nations. It is a major health issue in India. FNAC is an economic and rapid tool having high diagnostic accuracy for LN tuberculosis. Present study was conducted to describe the role of fluorescent staining and Ziehl- Neelsen techniques in detecting Mycobacterium tubercle bacilli on FNAC samples of tubercular lymphadenitis.

Methods

This prospective study was carried out at Department of Pathology, Muzaffarnagar Medical College, U.P. It included 100 patients having enlarged lymph nodes which were suspected of TB. After FNAC, cytological examination was done and smears prepared were stained with ZN stain and Auramine Rhodamine (AR) stain.

Results

Twenty eight (28.0%) cases were found in 21-30 years age group followed by 20 cases (20%) in 31- 40 year age group and 11-20 year age group. Most were females forming female to male ratio 1.7:1. Cytomorphologically, four patterns were observed– Necrotizing granulomatous lymphadenitis in 41% cases, Granulomatous lymphadenitis in 29% cases, only Necrotizing lymphadenitis in 19% cases and Necrotizing lymphadenitis with suppuration in 11% cases. ZN staining showed AFB in 35% cases while AFB was not seen in 65% cases. Out of these 65% cases, fluorescent staining was positive in 29%, indicating significant association of fluorescent staining with ZN method.

Conclusions

Only with the cytological findings of lymph node, it is difficult to arrive at confirmatory diagnosis of TB. Fluorescence Microscopy (FM) increases the sensitivity for diagnosing TB in underdeveloped nations with high tuberculosis prevalence, such as India.

Keywords: cytology, fluorescent, mycobacterium tuberculosis, Z-N staining.

INTRODUCTION

Tuberculosis is primarily responsible for pulmonary tuberculosis.[1] In areas with high Mycobacterial infection, tuberculous lymphadenitis is the most prevalent TB.[2,3] Aspiration cytology can only provide the provisional diagnosis but there is a vast list of conditions which cause granulomatous lymphadenitis. That's why, confirmation requires demonstration of acid fast bacilli (AFB)which is done by Ziehl-Neelsen (ZN) technique but it can detect AFB in only 40-56% cases. Now-a-days, Fluorescent method is more sensitive and combination of AR and ZN staining yield better results.[4-6] As incidence of TB is very high in India, which imposes a great burden over the national economy. That's why it is necessary to make accurate diagnosis by more sensitive diagnostic method. Present study was aimed at describing the role of fluorescent method and Ziehl- Neelsen technique in detecting Mycobacterium tubercle bacilli in LN aspirate and to find out which method is more sensitive.

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METHODS

This hospital based study was a prospective study, done for 1 year from January 2021 to December 2021. This was carried out at cytology laboratory of Department of Pathology, MMCH. Clearance was taken from the Institutional Ethical Committee (MMC/IEC/2021/214). The study included 100 patients which were having lymphadenopathy suspected of TB. All patients with lymphadenopathy and characteristic history of TB like evening temperature, history of weight loss and chronic cough and Patient's willing to participate were included in the study. Study excluded cases not suspected of TB, where sufficient material was not available for special staining (ZN and fluorescence stain) and neoplastic lesions. A brief clinical history was obtained and thorough physical examination of the lymph node was done. After taking informed consent, FNAC was done. Smears were prepared and stained with Giemsa. On Giemsa stained smears, microscopic examination was done to assess cytomorphological features. At low magnification, primary cell population and pattern was evaluated. Detailed morphology for epithelioid cells and Langhans multinucleated giant cells was assessed under high power and final interpretation was given. Smears prepared were also stained with Ziehl-Neelsen stain and Auramine-Rhodamine stain for the direct demonstration of AFB. Grading was done as mentioned (Table 1) and (Table 2).

Table 1.Grading of Ziehl-Neelsen staining.[7]

Number of AFB	Result	Grading	Number of fields to be examined
More than 10 AFB per oil immersion field	Positive	3+	20
1-10 AFB per oil immersion field	Positive	2+	50
10-99 AFB per 100 oil immersion field	Positive	1+	100
1-9 AFB per 100 oil immersion field	Doubtful positive	Doubtful positive	100
No AFB per 100 oil immersion field	Negative	-	100

Doubtful positive were taken as scantily positive due to presence of occasional bacilli. Doubtful positive were taken as scantily positive due to presence of occasional bacilli.

Table 2. Grading of Auramine-Rhodamine staining.[8]

Fluorescence staining using 20X objective	Reporting
>100 AFB/20fields	Positive, 3+
11-100 AFB/50fields	Positive, 2+
1-10 AFB/100fields	Positive, 1+
1-3 AFB/100fields	Doubtful positive/repeat
No AFB per 100fields	Negative

*Comparative grading was done by dividing the number of AFB in FM objective with this magnification correction factor. Therefore, the comparative grading is used in the present study (Table 3).

Table 3. Magnification correction factor.[8]

FM objective magnification (power)	Magnification correction factor*
20X	10
25X	10
40X	5
45X	4
63X	2

The Excel data was loaded and analyzed using the SPSS version 18.0. Mean and standard deviation was given to show quantitative data (numerical variables), whereas frequency and percentage are part of qualitative (categorical variables) data. Student t-test was applied to compare the mean values of two groups, while their frequency differences were calculated by the chi-square test. p value of less than 0.05 was considered statistically significant.

RESULTS

Twenty eight cases (28.0%) were found in 21-30 years age group followed by 20 (20.0%) cases each belonged to 11-20 year age group as well as 31- 40 years age group. Fourteen (14.0%) cases belonged to 41-50 years age group, 10 (10.0%) cases were above 50 years and 08 (8.0%) cases belonged to 0-10 years age group. The majority were females (63 cases, 63.0%) while males were 37 (37.0%) (Table 4). On cytological examination, Necrotizing granulomatous lymphadenitis was present among 41.0% and Granulomatous lymphadenitis was present among 29.0% cases. (Image A) Necrotizing granulomatous lymphadenitis with suppuration was seen among 11.0% cases and only Necrotizing lymphadenitis among 19.0% cases. In ZN staining, smear positivity was observed in 35 cases (35%) out of which grade 1 AFB

Table 4: Distribution of study population according to age and sex.	
Variables	**Frequency (%)**
Age groups (Years)	
0-10	08(8.0)
11-20	20(20.0)
21-30	28(28.0)
31-40	20(20.0)
41-50	14(14.0)
> 50	10(10.0)
Gender	
Female	63(63.0)
Male	37(37.0)

positivity was seen among 30 (30.0%) cases, grade 2 among 04 (4.0%) cases and grade 3 AFB positivity was seen in 01 (1.0%) case. (Image B &C) In 65 (65.0%) cases AFB was not seen. With Auramine-Rhodamine Fluorescent staining, tubercle bacilli was seen in 63 cases (63%). Grade 1 AFB was seen among 36 cases (36.0%), Grade 2 among 19 cases (19.0%) and Grade 3 was seen in 08 cases (8.0%). (Image D) In 37 cases, AFB was not seen.

In this study, 65 cases were negative on ZN staining. Out of these 65 cases, 29 cases came positive on fluorescent staining, indicating that fluorescent method is more sensitive in detecting the tubercle bacilli. On statistical analysis, p-value was 0.00001, indicating significant association between ZN and fluorescent method (Table 5).

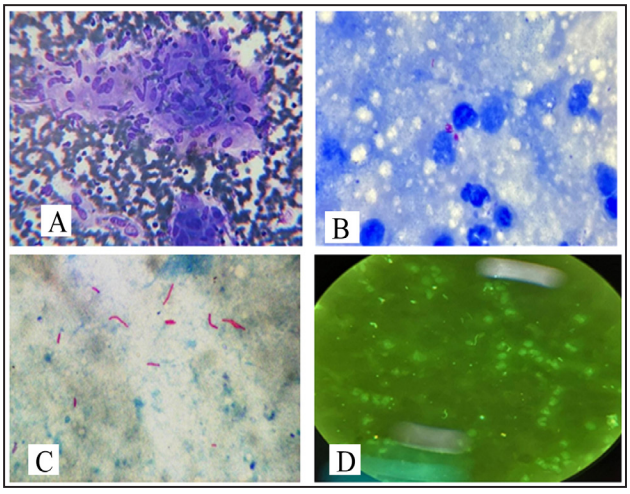

Figure 1. Image (A) - Photomicrograph showing epithelioid cell granuloma over hemorrhagic background (Giemsa stain,400X). Image (B) -Photomicrograph showing AFB Grade 2+ (ZN stain, 1000X). Image (C) - Photomicrograph showing AFB Grade 2+ (ZN stain, 1000X). Image (D) -Photomicrograph showing AFB Grade 3+ (A-R stain, 200X).

Table 5. Comparison of the conventional ZN method with the Fluorescent method.		
Conventional ZN method	**Fluorescent Method**	
	Positive	Negative
Positive	34	1
Negative	29	36
p-value	< 0.00001*	

DISCUSSION

In present study, majority (28.0%) of cases were in 21-30 years with mean age of 29.20±14.28 (0-66) years. Maru et al.[9] reported that in their study, age ranged from 1-70 years, 28.5 years being the mean age. Most commonly affected age group in current study is similar with the other studies like Dagar V et al.[10] (mean age of 29.4 years), Ergete and Bekele.[6] (mean age of 22.8 years) and Aalmeen G et al. [11] (mean age of 23.7 years). An individual is energetically most active during 20-40 year age group and therefore the chances of exposure are also more, explains maximum cases of TB in this age group. In present study, there were 63.0% females and 37.0% males with 1.7:1 female to male ratio. This is similar with other study like Vimal S et al.[12] in which males patients were 46 and 71 were females thus forming M: F ratio of 1:1.54. Similarly, in the study done by Chand P et al.[13] females were predominating with female male ratio being 1.38:1. Other studies like, study by Fazal et al.[14] (female male ratio being 1.6:1), Bhatta S et al.[15] (F:M ratio of 1.1:1) and Wadhwa R et al.[16] (F:M ratio of 1.3:1) also found similar results. Mani Krishna et al.[17] also observed female predominance accounting for 60.2% but differed from the research done by Maru et al.,[9] in which more males were seen i.e. 61.53% cases. The increased occurrence among females can be related to poor nutritional condition and poorer level of living in emerging nations, especially in socio-economically weaker portions.In present study, out of total 100 cases, 35% cases were AFB positive and 65% cases were negative on ZN staining while on fluorescent staining, positivity was seen in 63% cases thus indicating that more cases were found positive with fluorescent staining. Out of 63 cases which were positive on fluorescent staining, 29 cases had ZN negativity but fluorescent positivity. Thirty seven cases were negative

for both ZN and fluorescent stain. It is undoubtedly sure that more TB cases were diagnosed by the fluorescent stain in comparision to ZN stain with a p-value of 0.00001 showing statistically significant association. Dagar V et al.[10] in their study found that AFB positivity was 36.5% with ZN stain and it was increased to 51.3% with fluorescent stain indicating significant difference between these two methods. Jadhav et al.[18] found that in 52 cases of tubercular lymphadenitis, 41 cases (78.84%) had positive smear results for acid fast bacilli using the ZN stain and 47 cases (90.38%) had positive results using the fluorescent stain method, clearly stating that in comparison to ZN stain, Auramine fluorescence staining is more sensitive in detecting AFB. Aalmeen G et al.[11] observed that out of 151 cases, 61 cases (40.39%) were positive for AFB with ZN staining and 85 cases (56.29%) were positive with Auramine – O fluorescent stain indicating that the smear positivity was increased from 40.39% with conventional ZN stain to 56.29% on Auramine-O fluorescent stain. Ulukanligil et al.[19] in their study observed that 67.6% cases were ZN positive and 85.2% cases were fluorescent positive. Similar result was observed by Singh N Petal.[20] Study carried by S J Murry et al.[21] showed that 93% cases were found positive on fluorescent stain and 73% cases were positive with ZN stain. Jain et al.[22] also noted 32% cases positive with ZN stain and 41% cases positive with fluorescent stain. All the mentioned studies showed findings which are comparable with the present study. This was also in agreement with research conducted abroad by Zailani SB et al.[23] as well as by Mistry Y et al.,[2] Dagar V et al.,[10] they found statistically significant difference in the detection of tuberculosis by these two techniques. However, the results of the current investigation were at odds with those of Subramani P et al.[3] who found no appreciable differences in the outcomes of the two staining techniques. Studies conducted in the past to determine the sensitivity of ZN staining and AR staining techniques for the demonstration of AFB from a variety of clinical specimens, including pus, sputum, fine needle aspirate and other body fluids revealed that AR technique was 86.6% more sensitive than ZN, with a more pronounced difference in extrapulmonary samples.[22]

CONCLUSIONS

Fluorescence microscopy has advantage over light microscopy of being quick, more sensitive and inexpensive technique for diagnosing TB in underdeveloped nations with high tuberculosis prevalence, such as India. Patients presenting with superficial lymphadenopathy may have a more accurate diagnosis of tuberculous lymphadenitis when using FNAC in conjunction with other ancillary test like fluorescent stain and ZN stain.

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