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A COMPARATIVE STUDY BETWEEN ZIEHL NEELSEN STAINING AND FLUORESCENCE MICROSCOPY IN THE DIAGNOSIS OF TUBERCULOUS LYMPHADENITIS ON FINE NEEDLE ASPIRATION CYTOLOGY

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 tuberculous lymphadenitis due to paucicellular nature of the disease. Fluorescent Microscopy 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 tuberculous lymphadenitis. Fine needle aspiration was done and the material was sent for Fluorescent Microscopy. The rest of the material was used for preparing H&E stained and Z-N stained smears. **Result:** Out of the total 80 cases, 47 cases were positive for AFB on ZN staining and 56 cases showed Fluorescent Microscopy positivity, thus proving the important role of Fluorescent Microscopy in diagnosing tuberculous lymphadenitis. This difference was also statistically significant (p value <0.05). **Conclusion:** Combined use of FNAC, ZN staining and Fluorescent Microscopy 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 tuberculous lymphadenitis.

KEYWORDS

Tuberculous lymphadenitis, Fluorescent Microscopy, 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. Incidence of tuberculosis is 2.2 million and prevalence is 2.5 million.[1]

Fine 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.[2] FNAC is a safe, simple, sensitive, rapid, convenient, cost effective, outpatient and minimally invasive procedure which is now acceptable and widely practiced.[3]

Conventional Ziehl - Neelsen (ZN) method for Acid-Fast Bacilli (AFB) plays a key role in the diagnosis and also for the monitoring of treatment in TB. Its major disadvantage is low sensitivity ranging from 20% to 43%. Mycobacterial culture is the reference method for the detection of tubercle bacilli but it is time consuming and requires specialized safety procedures in laboratories.[4]

Hence, a method for the identification of AFB which is more sensitive than the ZN method is required for early detection of cases. Thus arose the need for fluorescent staining. Fluorescent staining using Auramine is already in use for detection of AFB on sputum smears under the RNTCP. Therefore, an attempt to apply similar knowledge to lymph node aspirates was made for faster detection of extrapulmonary tuberculosis.[4]

The fluorescent staining is regarded as a more sensitive staining method for the detection of tubercle bacilli as it can detect bacilli when they are present in the concentration of 10^4/ml of sputum, while ZN stain requires 10^5 bacilli/ml to be detected and there is stronger binding of mycolic acid of bacilli with Auramine.[4] However no such data has been documented for lymph node aspirates.

AFB on ZN stained smears are seen as pinkish, thin, curved, coloured rods against a blue coloured background on oil immersion (1000X) and in Auramine stained smears as slender, bright yellow fluorescent rod shaped bacteria against a dark background on 400X.[4]

Fluorescent Microscopy greatly improves the diagnostic value especially in paucibacillary patients, that are likely to be missed on ZN stained smears. However, the fluorescent staining alone cannot be alternative method to ZN staining. It should be used as an adjuvant along with clinical history, haematological investigations and cytological features in lymph node aspirates. Still, for the cases which are negative, but strongly suspicious for tuberculosis, additional investigations must be undertaken like CB-NAAT, Xpert MTB tests etc. especially in a developing countries like ours where tuberculosis is highly prevalent and on a rise due to the HIV epidemic.[4]

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 tuberculous lymphadenitis. Sample size of the study was 80 cases and duration of study was 15 months. 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 Auramine-O staining.

RESULTS

The study included 80 suspected patients with clinical features of tuberculous lymphadenitis. The aspirate was blood mixed in 41.25% cases, purulent in 40% cases and cheesy white in 18.75% cases. Among 80 cases, the age of patients ranged from 3 years to 65 years with maximum number of cases in 21-40 age group, of which 36% were males and 64% were females. On fine needle aspiration cytology, various cytological patterns suggesting caseating granuloma were demonstrated in 55% cases, followed by features of granuloma without necrosis in 22% cases, acute suppuration in 13% cases and caseation without granuloma in 10% cases. The positivity for

Mycobacterium tuberculosis was 70% by Fluorescent Microscopy and 59% by conventional ZN staining method. 9 cases which were negative on ZN Staining were detected by Fluorescent Microscopy. This difference was statistically significant with p value <0.05. Sensitivity was 78.6% by ZN staining and it was increased to 93.6% by Fluorescent Microscopy. Specificity by ZN method was 87.5% and decreased to 63.6% by Fluorescent Microscopy. Positive Predictive Value by Fluorescent Microscopy was 78.6% and it was increased to 93.6% by ZN method. Negative Predictive Value by ZN staining was 63.6% and it was increased to 87.5% by Fluorescent Microscopy. This comparison illustrated that Fluorescent Microscopy is a better diagnostic tool to diagnose tubercular lymphadenopathy than the conventional ZN staining and microscopy.

Table I Age Wise Distribution Of Cases

Age	Number	Valid Percent
<10 years	7	8.75
11-20 years	22	27.50
21-30 years	23	28.75
31-40 years	14	17.50
41-50 years	10	12.50
>50 years	4	5.00
Total	80	100

Table II Location Wise Distribution Of Cases

	Location	Valid Percent
Supraclavicular	4	5.00
Submandibular	9	11.25
Axillary	5	6.25
Cervical	58	72.5
Preauricular	1	1.25
Submental	1	1.25
Inguinal	2	2.50
Total	80	100

Table III Cross Tabulation Of Cytomorphological Patterns With Zn Staining And A-o Staining Positivity

Cytomorphological Patterns	ZN staining Positivity	A-O Staining Positivity	Total
Acute Suppuration	2	2	10
Caseating Granuloma	36	38	44
Granuloma without Necrosis	2	8	18
Caseation without Granuloma	7	8	8
Total	47	56	80

Table IV Comparison Of Sensitivity, Specificity, PPV And NPV Between ZN Staining And FM

Parameter	ZN vs IF	IF vs ZN
Sensitivity	78.6	93.6
Specificity	87.5	63.6
PPV	93.6	78.6
NPV	63.6	87.5
P Value	<0.001	<0.001

DISCUSSION

Out of 80 cases in our study, 44 cases were diagnosed as Caseating Granuloma on FNAC. ZN staining for AFB was able to detect only 36 (81.8%) cases, while 2 more cases i.e 38 (86.4% cases) were detected by Fluorescent Microscopy. So Fluorescent Microscopy was proved to be more effective. Similar findings were present in a study done by Kailash Chand Sharma et al[8] where they found 52 cases of Caseating Granuloma. Among them 20 cases showed ZN staining positivity and 34 cases showed Fluorescent Microscopy positivity.

18 cases in our study that were diagnosed as Granuloma without Necrosis, 2 cases were found to be positive for AFB by ZN staining while Fluorescent Microscopy was able to detect 8 cases of tuberculosis. Here again Fluorescent Microscopy come out to be more effective than ZN staining, by picking 6 more cases to be positive for tuberculosis.

Findings in favour of our study were present in a study done by Kailash Chand Sharma et al[8]. Out of 24 cases of Granuloma without Necrosis, 5 cases were found to be positive by ZN staining and 10 cases showed Fluorescent Microscopy positivity.

In our study out of 80 cases, 8 cases were diagnosed as Caseation without Granuloma. Out of which 7 cases were found positive by ZN staining while all 8 cases was found to be positive for TB by Fluorescent Microscopy. Here Fluorescent Microscopy picked 1 more case of Caseation without Granuloma as compare to ZN staining. Similar findings were present in a study done by Kailash Chand Sharma et al[8] where they detected more number of TB cases by Fluorescent Microscopy among cases of Caseation without Granuloma.

Out of 80 cases in our study, 10 cases were diagnosed as Acute Suppuration on FNAC. Out of which 2 cases were found to be positive by both ZN staining and Fluorescent Microscopy.

In our study sensitivity was 78.6% by ZN staining and it was increased to 93.6% by Fluorescent Microscopy, which showed Fluorescent Microscopy is more sensitive than ZN staining. Similar observation was made by Dr Alpesh M. Maru et al[6] where they found sensitivity for ZN staining was 43.1% which was increased to 87.7% in Fluorescent Microscopy. Supreet Kaur Kalra et al[7] in their study, also found Fluorescent Microscopy to be more sensitive than ZN staining where they found 73.5% sensitivity by Fluorescent Microscopy and 36.7% by ZN staining. Study done by Akash Bhat et al[5] also found similar results in which sensitivity by ZN staining was 33.3% and increased to 45.5% by Fluorescent Microscopy.

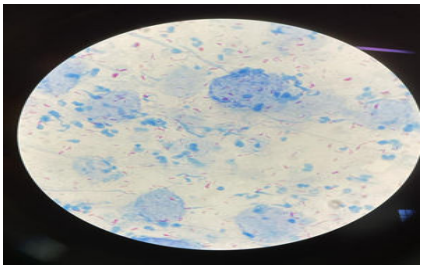
We observed specificity by ZN method was 87.5% and decreased to 63.6% by Fluorescent Microscopy. This showed ZN staining to be more specific than Fluorescent Microscopy. Similar result was observed in study done by Akash Bhat et al[5] in which specificity was 88.8% by ZN method and decreased to 81.8% by Fluorescent Microscopy.

One study done by Dr Alpesh M. Maru et al[6] was in contrast to our study, it stated that Fluorescent Microscopy was more specific than ZN staining where specificity by ZN method was 21.3% and increased to 55.6% by Fluorescent Microscopy.

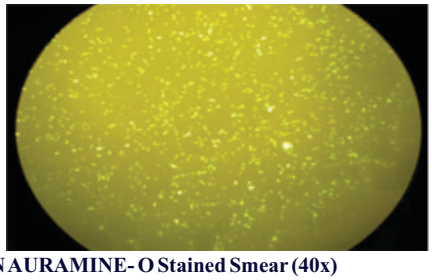
One more study by Supreet Kaur Kalra et al[7] stated that both ZN staining and Fluorescent Microscopy were equally specific.

In our study Positive Predictive Value by Fluorescent Microscopy was 78.6% and it was increased to 93.6% by ZN method which showed Positive Predictive Value for ZN staining was higher. Similar result was observed in study done by Akash Bhat et al[5] in which Positive Predictive Value was 33.3% by Fluorescent Microscopy and increased to 45.5% by ZN staining.

In the present study Negative Predictive Value by ZN staining was 63.6% and it was increased to 87.5% by Fluorescent Microscopy which showed Negative Predictive Value for Fluorescent Microscopy was higher. Similar result was observed in study done by Akash Bhat et al[5] in which Negative Predictive Value was 81.8% by ZN staining and increased to 88.2% by Fluorescent Microscopy.



AFB ON ZN Stained Smear (oil Immersion, 100x)Pictograph 2



AFB ON AURAMINE- O Stained Smear (40x)

CONCLUSION

The diagnosis of tuberculous lymphadenitis 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 in resource limited settings to diagnose tuberculosis based upon cytomorphological features and ZN staining. Our study found FNAC, ZN staining and Fluorescent Microscopy to be mutually complementary diagnostic tests. Fluorescent Microscopy was more sensitive and had higher Negative Predictive Value whereas ZN staining was more specific and had higher Positive Predictive Value. In a developing country like India, incorporation of Fluorescent Microscopy 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.

REFERENCES

1. Komanapalli SK, Prasad U, Atla B, Vasundhara N, Yendhluri D. Role of CBNAAT in diagnosing extra-pulmonary tuberculosis in correlation with FNA in a tertiary care centre. Int J Res Med Sci 2018;6:4039-45.
2. Aljafri AS, Khalil EA, Elsiddig KE, El Hag IA, Ibrahim ME, Elsafi ME et al. Diagnosis of tuberculous lymphadenitis by FNAC, microbiological methods and PCR: a comparative study. Cytopathol 2004;15:44-8.
3. Lalitharani N, Bagiyalakshmi V. Incidence of Tuberculosis among cases of peripheral lymphadenopathies and techniques useful in the diagnosis of Tuberculous lymphadenitis by FNAC- Our experience from a tertiary heath care centre. Ind J Appl Res 2017;7:168-9.
4. Kalra SK, Paul S, Singh SP. A Comparative study of Fluorescence Microscopy with Ziehl Neelsen Staining for Detection of Acid Fast Bacilli in Lymph Node Aspirates. Int J Sci Res 2018;7:789-95.
5. Bhat A, Jayaprakash M. Comparison of fluorescent and Ziehl Neelsen Staining in Fine Needle Aspirates of Tuberculous Lymphadenitis. J Cont Med A Dent 2020;8:70-4.
6. Maru AM, Kapadia DN, Makavana H, Lakum NR. Comparison of Z.N. staining and fluorescent microscopy in detection of M.Tuberculosis bacilli in Fine needle aspiration smears. Trop J Pathol Microbiol 2018;4:242-7.
7. Kalra SK, Paul S, Singh SP. A Comparative study of Fluorescence Microscopy with Ziehl Neelsen Staining for Detection of Acid Fast Bacilli in Lymph Node Aspirates. Int J Sci Res 2018;7:789-95.
8. Sharma KC, Yadav A. Ziehl Neelsen and Auramine Rhodamine staining: A comparative evaluation from North India. J Med Sci Clin Res 2020;8:610-3.