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Demonstration of acid-fast organisms is a fool proof evidence of tuberculosis as various cytomorphological pictures may differentiate from other diseases.

*Objective:* To analyse the efficacy of Fluorescent stain Auramine-O over Ziehl-Neelsen stain in identifying tubercle bacilli in Lymph node aspirates.

*Material & Methods:* A total of 120 patients referred to the Department of Pathology, SNMC & HSK Hospital, Bagalkot for FNAC of palpable lymph node lesions suspicious for tuberculosis were taken for study. FNAC was done using the standard method.

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*Objective:*

- *To analyse the efficacy of Fluorescent stain Auramine-O over Ziehl-Neelsen stain in identifying tubercle bacilli in Lymph node aspirates.*

*Material & Methods: A total of 120 patients referred to the Department of Pathology, SNMC & HSK Hospital, Bagalkot for FNAC of palpable lymph node lesions suspicious for tuberculosis were taken for study. FNAC was done using the standard method. Smears from aspirates were subjected to routine cytological stains. Results were categorized into six (6) cytomorphological patterns:*

*(i) Epithelioid Granuloma with Langhans' giant cells and caseous necrosis.*

*(ii) Numerous epithelioid cells and granulomas in a reactive background.*

*(iii) Caseous necrosis with few epithelioid cells.*

*(iv) Caseous necrosis with few lymphocytes and histiocytes. No epithelioid cells.*

*(v) Only caseous necrosis without any cell type.*

*(vi) Tubercular abscess showing predominantly neutrophils along with epithelioid cells.*

*ZN stain, Pap stain & Auramine-O fluorescent stains were used to detect acid fast bacilli. A protocol for the examination of the positivity for AFB with a grading system was used.*

*Results: Among 120 cases of tubercular lymphadenitis, the most common cytomorphological pattern seen is pattern II & VI. Ziehl-Neelsen stain demonstrated 20.83% (25/120) positive cases whereas Auramine-O stain demonstrated 53.33% (64/120).*

*Conclusion: Fluorescent method in combination with cytomorphological pattern of Tubercular lymphadenitis is useful for evaluating lymph node lesions.*


*Keywords:* acid-fast bacilli; auramine-o; fnac; fluorescent microscopy.

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## I. INTRODUCTION

Tuberculosis (TB) is the major health problem in the world since 1993 when it was declared as a

global emergency by the World Health Organization (WHO). [9] Extra-Pulmonary tuberculosis (EPTB), particularly tuberculous lymphadenitis (TBL), continues to be a major health problem in developing countries.[11] India has the highest burden of tuberculosis. The World Health Organisation (WHO) statistics for 2015 give an estimated incidence of 2.2 million cases of TB for India out of a global incidence of 9.6 million. [13] Tuberculosis continues to be a major health problem in our country and is the single largest cause of loss in a healthy life year in the productive age groups. [7] FNAC can be an important tool in suspecting TB lymphadenitis based on identifying epithelioid granulomas and caseous necrosis. Smear examination is believed to be simple, cheap, quick, practicable and effective case finding methods for developing countries. As tubercular bacilli are very slow growing organisms, culture results are available after a period of three to six weeks. [7] Ziehl-Neelsen is the most extensively used procedure for the determination of Mycobacterium tuberculosis in smears. [7] Fluorescent staining by Auramine is another method of staining. In this a smear is made from the specimen and stained with fluorescent stain called Auramine-O. Auramine stain enters the wall of Mycobacterium Tuberculosis bacterial cell and makes them glow against dark background under UV light. [52] Lymphadenopathy is one of the most common extrapulmonary manifestations accounting to around 30-40% of TB. [6] FNAC can be an important tool in suspecting TB lymphadenitis based on identifying epithelioid granulomas and caseous necrosis. Conventional Ziehl-Neelsen (ZN) method of detection of acid - fast bacilli (AFB) is simple and rapid but lacks sensitivity ranging from 20% to 43%. Hence, the Fluorescent method for detection of AFB has proven more effective than the ZN method. [6] The most important advantage of fluorescence microscope technique is that the slides can be examined at a lower magnification, thus allowing the examination of a much larger area per unit of time. The tubercular bacilli stand out as bright objects against dark background in fluorescence microscopy which makes them easily identifiable hence causing less eye strain.

*Strain:*

Depending upon the cytomorphological appearances, TB lymph node aspirates were subdivided further into Six (06) patterns: [6].

1. Epithelioid granulomas with Langhans' giant cells and caseous necrosis.
2. Numerous epithelioid cells and granulomas in a reactive background.
3. Caseous necrosis with few epithelioid cells.
4. Caseous necrosis with few lymphocytes and histiocytes. No epithelioid cells.
5. Only caseous necrosis without any other cell type.
6. Tubercular abscess showing predominantly neutrophils along with epithelioid cells.

## II. MATERIAL & METHODS

*Sources of data:*

- The present study is a prospective study based on FNAC sampling of 120 cases of suspected tubercular lymphadenitis over a period of Dec 2016 to May 2018 (18 months) in the Department of Pathology, S.N Medical College & H.S. Kumareshwar Hospital, Bagalkot.
- Ethical clearance has been obtained from the "Institutional Ethical Committee" of S. N.

*Medical College, Bagalkot:*

After clinical examination, with prior consent, fine needle aspiration will be done on all referred cases of clinically diagnosed tuberculous lymphadenitis. Air dried smear will be subjected to Ziehl-Neelsen, Auramine- O and Giemsa Stain. Prior fixation in 95% ethyl alcohol will be done for H&E and Papanicolaou stain.

- Auramine-O Stain & Papanicolaou stains are also seen under Fluorescent Microscope.
- Light microscopy & Fluorescent Microscopy findings will be analysed.

*Inclusion criteria for the study group:*

- Clinically Diagnosed cases of Tuberculous Lymphadenitis.

*Exclusion criteria for the study group:*

- Non-cooperative Patient.
- Cytological Diagnosis other than Tuberculous Lymphadenitis.
- Diagnosed cases of T.B Lymphadenitis who are under anti tubercular treatment.

As per study done by Kulkarni. M.H et al, Sensitivity of ZN and Auramine stain of 58% and 81% respectively .

Statistical analysis: Data will be analysed using SPSS software. Proportion and percentage is used for qualitative data. Sensitivity, Specificity, Positive predictive value, Negative Predictive value and Diagnostic accuracy for Auramine-O Stain will be calculated.

Patients were clinically evaluated and an informed consent was obtained for the FNAC procedure. The limitations and complications of FNAC were explained to the patients.

Air dried & heat fixed smears used for ZN stain & Auramine-O fluorescent stain. The slides were examined under 10x & 40x & the ZN stain with oil immersion field.

*Interpretation of aspiration was done as follows:*

- Assessment of the adequacy of material in the smear.
- Cytomorphological features: overall cell population & predominant pattern were
- assessed by examination under low power. The individual cell morphology for
- epithelioid cell morphology, Langhans' type of giant cell, lymphocytes etc. was
- studied under high power.

Grading of Ziehl-Neelsen staining [7]

- More than 10 AFB per oil immersion field Positive 3+
- 1-10 AFB per oil immersion field Positive 2+
- 10-99 AFB per 100 oil immersion field Positive 1+
- 1-3 AFB per 100 oil immersion field Doubtful Positive.
- No AFB per 100 oil immersion field Negative.

Doubtful positive cases were taken as scantily positive due to the presence of occasional Bacilli.

*Interpretation:*

-Smears were examined carefully in a linear pattern or three horizontal sweeps by scanning at least 50-100 fields before reporting as negative or positive. Observation done under lower power magnification using fluorescent microscope.

Bacilli appear as bright yellow to orange against a dark background.

Positive smears are graded into four categories based on the grading system used by Central TB Division, Directorate General of Health Services, Ministry of Health and Family Welfare, RNTCP Manual for sputum smear fluorescence microscopy. New Delhi 110011.

Grading of Auramine-Rhodamine staining [60] 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.

Internal quality control measures taken for reagents and smears. The containers of all reagents with the date first opened were noted. Any material found to be unsatisfactory, for instance poor quality of reagents, expired reagents, change of colour of the reagents on exposure to atmospheric air, scratched slides etc. were discarded.

*Standardisation of AO fluorescent staining:*

- Known positive and negative smears of about 8-10 sputum samples were stained & studied

for standardization of Auramine-O staining.

- It included proved and confirmed Grade 1+ to 3+ positive smears and two negatives smears.

## III. RESULTS

In the present study, the age of the patients ranged from 3 months to 80 years. Sex Ratio: Male: Female=1.06:1. Evening rise of Fever, loss of weight (10% loss of weight in 3 months), lymphadenopathy are prominent features. Loss of weight is the most common clinical presentation seen.

Fever (Evening rise of temperature) shows 46.67% cases. Loss of weight (10% loss in last 3 months) 51.67% cases. Lymphadenopathy 31.67% cases. Fever Loss of weight Lymphadenopathy 8.33% cases.

Most common Lymph node involvement is the cervical group of lymph nodes in 62.5% cases.

Single group of lymph nodes were involved in 107cases.Generalized lymphadenopathy was seen in 13 cases involved. Matted group of lymph nodes was noticed in 55 cases. Nature of the aspirate was blood mixed. Most common cytomorphological pattern seen is Caseous necrosis with few lymphocytes and histiocytes. No epithelioid cells.
In clinically diagnosed cases,
Auramine-O shows

- Sensitivity=76% PPV=29.69%,
- Specificity=52.63% NPV=89.29% Diagnostic Accuracy =57.5%

whereas ZN shows

- Sensitivity = 70%
- Specificity=97.1%

at 95% confidence limit.

## IV. CONCLUSION

It shows that Auramine-O is more sensitive than ZN stain in the diagnosis of Tubercular Lymphadenitis especially in Paucibacillary cases & less specific in diagnosis of tuberculosis in lymph node aspirates.

The Auramine-O stain detected acid fast bacilli in a greater number of cases when compared to Ziehl-Neelsen stain. A statistically significant differentiation was seen in the detection of acid-fast bacilli by the AO stain with a significant p value. No acid-fast bacilli were observed in 56 cases (Auramine-O, ZN, Pap).

Cases POSITIVE p-value*

ZN Stain 25(20.8%) ,Auramine -O stain 64(53.3%) p=0.0001(Significant) *Based on Z test.

## V. DISCUSSION

Koch first described the tubercle bacilli in 1882, which is now known as mycobacterium tuberculosis. Mycobacteria are now known to comprise a large group of acid-fast, alcohol-fast, aerobic or microaerophilic, non-spore forming, non-motile bacilli.[17] Ghali et al. (1984) first demonstrated auto fluorescence of pneumocystic carinii in Pap stained smear. Their results indicated eosin to be responsible for the autofluorescence. Its value in the fluorescent microscopic diagnosis is well established. In Pap stained smear the slightly curved, beaded tubercle bacilli may be readily identified. [17] An advantage of fluorescent microscopy lies in the ease with which bacilli can be detected due to color contrast, allowing for a larger area of the smear to be scanned at lower magnification when compared with conventional ZN staining. Since the Pap stain is routinely used in cytology, it saves the time and material that would be involved in

any extra staining and also avoids the use of toxic or carcinogenic substances such as phenol and rhodamine, which are used in A-R staining method.[10].

Goyal. R. et al. (2013) reported that Fluorescent Microscopy greatly improves the diagnostic value of sputum smear especially in patients with low density of bacilli that are likely to be missed on Ziehl Neelsen stained smear. [7].

Thakur. B et al. (2013) also found that, Conventional ZN method for AFB plays a key role in the diagnosis & monitoring of treatment of Tuberculosis. Its major disadvantage is low sensitivity, time consuming, and oil immersion use. Fluorescent microscopy plays an important role for detection of MTB because lower magnifications are used as well as less time consuming.[5] Tuberculosis is a worldwide public health problem in spite of the fact that the causative organism Mycobacterium tuberculosis was discovered 100 years ago. [16] Globally, it is estimated that one-third of the population is asymptomatically infected with tuberculosis. [16].

ZN stain is commonly used throughout the world and still remains the standard method against which new tests must measure. [8] . The Utilization of auramine-O, fluorescent dye instead of carbol fuchsin, was first proposed in 1930 but found widespread application in industrialized countries only. [9].

Study conducted by Osman. A.N et al. (2014) revealed that Fluorescent staining is regarded as a more reliable method due to more intensive binding of mycolic acid of the bacilli to phenol auramine-o, so that the bacilli can stand out sharply against black background to allow rapid & accurate screening under low power microscope.[9].

A Patient with positive smears carries the greatest no. of tubercle bacilli and is the most infectious and hence the most important patient to be detected early. Fluorescence microscopy using fluorochromes (Auramine-Rhodamine) is more sensitive and rapid as compared to conventional microscopy using Ziehl-Neelsen (ZN) staining. [18].

Holani et al have successfully demonstrated presence of tubercle bacilli in saliva using fluorochromes staining. [18] Pap stain allows prospective as well as retrospective analysis of cases in which material for staining by other methods is not available and obviates need to restain existing slides. [18].

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FNAC of lymph nodes is a safe, simple and inexpensive definite diagnostic procedure to render a prompt diagnosis, especially in lymph node aspirates, where biopsies are not done commonly. The limitations are with necrotic lymphadenopathy, heterogenous swelling with limited representative aspirates, where if the clinician is unsatisfied with the cytological diagnosis further workup like biopsy is required in order to make a reliable diagnosis. [14].

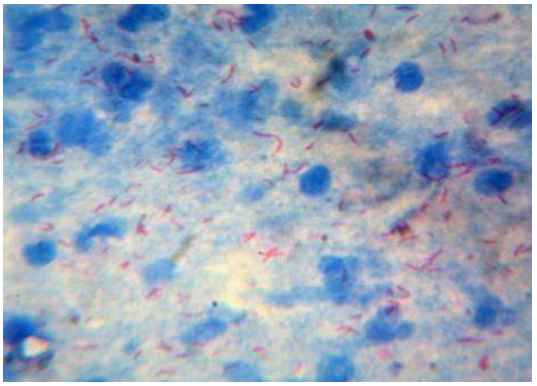

*Fig. 1:* AFB Positive 3+ (ZN Stain)

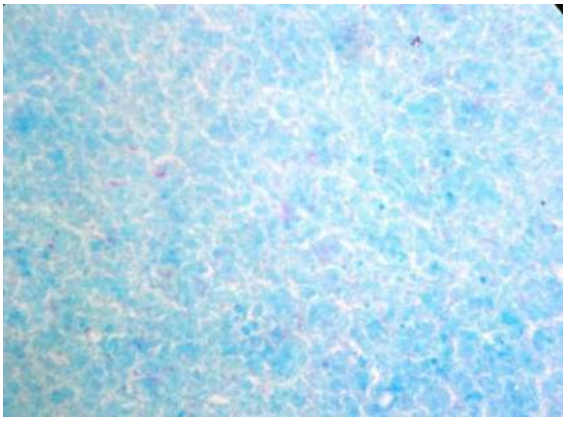

*Fig.2:* AFB Positive 2+ (ZN Stain)

*Fig. 3:* Auramine- O Stain 2+ (20X)

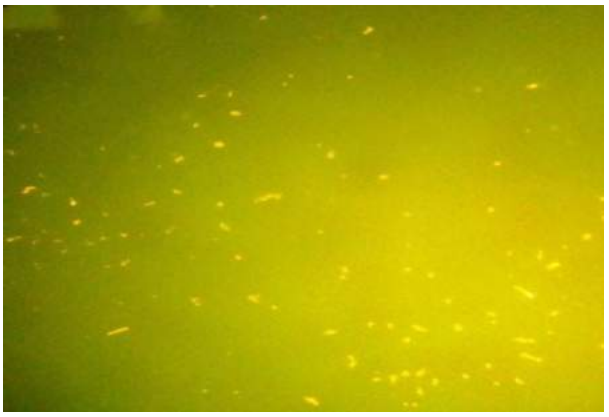

*Fig.4:* Auramine- O Stain 3+ (20X)

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