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**Research Article**

# Determination of fluorescent stain and Ziehl - Nelson stain for the demonstration of acid fast bacilli in cytological specimens

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## ABSTRACT

**Background:** Tuberculosis is infectious disease caused by mycobacterium tuberculosis. There are various methods for the diagnosis of tuberculosis such as examination of tubercular bacilli by Ziehl – Nelson (ZN), Auramine – Rhodamine (AR), Kinyoun staining. The present study was conducted to know the prevalence, clinical characters, nature of specimens and tuberculosis confirmation by ZN & fluorescence staining technique using AR in 2101 clinically suspected cases of tuberculosis.
**Methods:** This study was conducted as prospective study in Tertiary care hospital. Present study was done clinically suspected tubercular patients from January to July 2015. On all received samples ZN stain and fluorescent stain was applied.
**Results:** Among the clinically suspected patients 689 (32.79%) was diagnosed with tuberculosis. Fever (81.27%) was chief clinical complaint. Among the received specimens maximum cases was of sputum (pulmonary) tuberculosis (88.27%). Tuberculosis was diagnosed in (16.75%) cases with ZN staining and with fluorescent staining in (32.79%) cases.
**Conclusions:** In present study fever was chief clinical complaint. Maximum cases of tuberculosis are of pulmonary tuberculosis than extra pulmonary and AR fluorescent staining is more sensitive.

**Keywords:** Tuberculosis, *Mycobacterium tuberculosis*, Acid fast bacilli, Ziehl – Nelson staining, Fluorescent Auramine Rhodamine staining

## INTRODUCTION

Tuberculosis is one of the important communicable diseases which remain a world-wide public health problem despite the fact that the causative organism was discovered more than 100 years ago and highly effective drugs and vaccine are available. 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.[1]

In stained smears of pathological material, *M. tuberculosis* is seen as slightly bent rods, 2-4 µm long and 2-5 µm wide, which may be evenly stained or beaded and granular. On solid or liquid media the bacteria tend to be parallel and form long threads or cords.[2]

Commonly fever is low grade and appearing late each afternoon and then subsiding and night sweats, with progressive pulmonary involvement, increasing amount of sputum, at first mucoid and later purulent. Some degree of hemoptysis is present in about half of cases. Extra pulmonary manifestations of tuberculosis are legion and depend on the organ system involved.[3]

First proposed utilization of Auramine O, a fluorescent dye, instead of carbol fuchsin, in the 1930s, but this staining technique found widespread application in

industrialized countries only same 30 years later, after a thorough reevaluation of the technique, using a combination of Auramine & Rhodamine was done.[4]

Although isolation and identification of mycobacterium tuberculosis in clinical specimens is a definitive proof of tuberculosis infection, microscopy remains the most rapid technique for evaluation of a clinical specimen. Many laboratories prefer Rhodamine Auramine method of staining AFB over carbol fuchsin stain because the fluorescing bacilli are more readily detected. Auramine stained smears are scanned under low magnification than fuchsin stained smears, thus permitting a large area of the smear to be examined in shorter period of time.[5]

Currently there is scant data regarding the ZN and fluorescent staining on pulmonary and extra pulmonary samples from this region so this study was planned to know prevalence, clinical characters of patients diagnosed with tuberculosis and efficiency to ZN and fluorescent staining in diagnosis of tuberculosis.

## METHODS

This study was conducted in Department of Pathology and Department of Pulmonary Medicine of Rural Institute of medical sciences and research Saifai Etawah (U.P), India from January to July 2015.

For the present study was Sputum, pleural fluids, ascetic fluid, FNAC were collected from a total 2101 patients with clinically suspected tuberculosis. Patient with palpable swelling suspected to be tubercular in nature, FNAC was performed in cytology section of pathology department. Sputum was collected from a total 1949 patients with clinically suspected tuberculosis in pulmonary department. Decontamination of the samples was carried out 5% Hypochloride. After decontamination for 30 minutes, test tubes were centrifuge for 5 minutes at 3000 rpm. Supernatant was discarded & sediments left were used for smear preparation whenever required. Two smears were prepared from each sample. One smear was stained by routine Ziehl – Nelson method and other by fluorescence stain Auramine – Rhodamine. By Ziehl – Nelson stain AFB stain pink curve / straight beeded rods against blue background & by Auramine – Rhodamine stain AFB appears as bright reddish – yellow fluorescing rods against a dark back ground.

## RESULTS

In the present study a total of 2101 consecutive clinical sample from 2101 clinically suspected cases of tuberculosis, received in the department of pathology and Department of Pulmonary Medicine. Tuberculosis was confirmed in (73.43%) Males which were more than females (26.56%) and the ratio were 2.76:1 (Table 3). Fever (81.27 %) was chief clinical complaint. Among the received specimens pulmonary tuberculosis cases was more (88.67%). Tuberculosis was diagnosed with ZN in (16.75%) cases and with fluorescent staining in (32.79%) cases (Table 4).

**Table 1: Clinical presentation of patients diagnosed with Tuberculosis.**

| Symptoms | No. of Cases presented | Percentage |
|---|---|---|
| Fever | 560 | (81.27%) |
| Cough | 517 | (75.03%) |
| Sputum | 446 | (64.7 3%) |
| Weight loss | 391 | (56.74%) |
| Night sweats | 255 | (37.01%) |
| Haemoptysis | 158 | (22.93%) |
| Dyspnoea | 105 | (15.23%) |
| Anorexia | 79 | (11.46%) |
| Non specific | 58 | (8.41%) |

**Table 2: Nature of sample in suspected cases of tuberculosis.**

| Nature of sample | No of sample | Percentage |
|---|---|---|
| Sputum | 1949 | 92.76 % |
| FNAC | 86 | 4.09 % |
| Body fluids (Ascitic, peritoneal fluid) | 66 | 3.14 % |
| Total | 2101 | |

**Table 3: Sex wise distribution in AFB positive cases.**

| Nature of sample | Male | Female | Total |
|---|---|---|---|
| Sputum | 471 (77.08%) | 140 (22.08%) | 611 (88.67%) |
| FNAC | 33 (45.83%) | 39 (54.16%) | 72 (10.44%) |
| Fluid | 02 (33.33%) | 04 (66.66%) | 06 (.87%) |
| Total | 506 (73.43%) | 183 (26.56%) | 689 |

## DISCUSSION

Tuberculosis is one of the commonest chronic diseases affecting people throughout the world. Incidence of tuberculosis is low in developed countries but in developing country like India it is still increasing reason may be due to rise in incidence of HIV and multidrug resistant tuberculosis. Since T.B is highly infectious in early stage and treatment is long & cost effective, early diagnosis and accurate (correct) treatment is mandatory.

In India prevalence of tuberculosis is 400 per 100,000 population.[6] In present study prevalence of tuberculosis was (32.79 %).

**Table 4: Distribution of AFB positive results with ZN and AR methods according to kind of clinical specimen.**

| S. No. | Kind of Specimen | Total No.of cases | Total No. of AFB Positive smear | ZN Positive | AR Positive | ZN Positive AR Negative | ZN Negative AR Positive |
|---|---|---|---|---|---|---|---|
| 1. | Sputum | 1949 | 611 (31.34%) | 315 (16.16%) | 611 (31.34%) | 00 | 296 (15.18%) |
| 2. | Extra Pulmonary | | | | | | |
| a | FNAC | 86 | 72 (83.72%) | 33 (38.37%) | 72 (83.72%) | 00 | 39 (45.34%) |
| b | Misc. Body Fluid (AF, PF) | 66 | 6 (9.09%) | 04 (6.06%) | 06 (9.09%) | 00 | 02 (3.03%) |
| | Total | 2101 | 689 (32.79%) | 352 (16.75%) | 689 (32.79%) | 00 | 337 (16.03%) |

The grading for the number of observed bacilli was recorded according to the recommendations of the IUATLD (1978) & is given in table VIII below.

**Table 5: Quantification wise distribution of cases.**

| | | Sputum | FNAC | FLUID |
|---|---|---|---|---|
| Paucibacillary | Negative | 1338 | 14 | 60 |
| | 1-3 AFB/100 oil immersion field | 35 | 38 | 3 |
| | 4-9 AFB/100 oil Immersion field | 131 | 34 | 3 |
| Multibacillary | +(10-99 AFB/100 oil Immersion field) | 72 | 0 | 0 |
| | ++(1-10 AFB/ oil Immersion field) | 120 | 0 | 0 |
| | +++(> AFB/ oil Immersion field) | 253 | 0 | 0 |
| | Total | 1949 | 86 | 66 |

The ratio of males to female in this was 2.76:1 which shows that males were more effected by tuberculosis in comparison to female reason behind that may be because they move to outside for their earning so more exposure to the bacilli. The clinical presentation of tuberculosis is usually fever, cough, sputum, night sweat, haemoptysis, dyspnoea, wt loss, anorexia. But some time delay in diagnosis has often been attributed to atypical clinical presentation and radiological presentation. In present study fever (81.27%) (Table-1) was chief clinical symptom which is consistant with Rizvi[7] and Korzeniewska KM[8] in their studies.

In present study maximum no. of samples that we received was of sputum (92.76 %) (Table -2) which again signifies that no. of pulmonary tuberculosis is still increasing .The diagnosis of tuberculosis is confirmed by the demonstration of Tubecular bacilli. Mycobacteria are slender rod shaped, nonmotile, nonsporing, and aerobic bacterium measuring 2 to 10 um in length. It has lipid coat which makes it difficult to stain but once stained cannot be decolurised with alcohol. Thus, termed as acid fast bacilli (AFB) as they retain carbon fuschin staining (AFB stain or ZN stain) even after washing with acid alchoal. Flourscence staining utilize same approach as Zn staining but carbon fuschin is replaced by flourscent dye as Auramine which as primary stain followed by counter stain (potassium permagnate) employed to highlight stain organism for easier recognition for the diagnosis of tubercular bacilli in the samples examined. In present study we have diagnosed mycobacterium in (16.75%) with ZN stain whereas in same study we have diagnosed bacilli in (32.79 %) with flourscent AR stain (Table-4) which confirm flourscent stain is more sensitive in comparision to ZN stain for the diagnosis of tuberculosis. Our results were similar with. Prasanti K,[9] Cheng AG,[10] Sethi S,[11] Singh NP,[12] Ulukanligil M,[13] and Jain A[14] in their studies which also found AR stain was more sensitive in comparison with ZN stain for the diagnosis of tubercular bacilli. Thus it is concluded that, for detection of AFB in paucibacillary specimen, AR is much more sensitive than ZN staining technique. They are Paucibacillary in extrapulmoary samples (Table -5). This is confirming in present study Chakravoty S[15] also favoured that in his study.

## CONCLUSION

Tuberculosis is highly infectious disease which is common among rural population in India. Males are more infected with tuberculosis and pulmonary tuberculosis is common. It usually present with fever, cough, sputum

and wt. loss. Fluorescence microscopy is a useful, rapid, and reliable & is quite economical tool for the diagnosis of mycobacterium bacteria in specimens for AFB in comparision to ZN staining. Auramine - Rhodamine fluorescent staining is more sensitive than ZN staining demonstration of AFB in various clinical samples. It must be used in place of ZN stain for the diagnosis of tubercular bacilli. Considered for use in laboratories that handle large and few number of specimens.

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## REFERENCES

1. David HL: bacteriology of the myco bacterioses. US department of health, education and welfare, PHS, CDC, Washington DC. 1976.
2. Wayne LG: Microbiology of tubercle bacilli. Am Rev Respir Dis. 1982;125:31.
3. Mc Adam A, Sharpe A. Infectious diseases. In: Kumar V, Abbas Abul K, Fausto N, editor. Robbins & cotran Pathologic Basis of disease, 7th ed. New Delhi: Elsevier India privates ltd. 2004:381-6.
4. Hagemann P.K.H. Fluoreszenz farbung von Tuberkelbakterien mit Auramin - Munch med Wschr. 1938;85:1066 - 8.
5. Heubner RE, Good RC, Tokars JI: current practices in mycobacteriology: results of a survey of state public health laboratories. J clin Microbiology. 1993;31:771 - 5.
6. Mandal AK, Chaudhary S. Tuberculosis. In: Mandal AK, Chaudhary S, editor. Text book of pathology, Isted. NewDelhi: Avichal Publisher. 2010: 70-5.
7. Rizvi N, Shah R.H, Inayat N, Hussain H. Differences in clinical presentation of pulmonary tuberculosis in association with age. Journal of Pakistan medical Association. 2003;53(8).
8. Koreniewska KM, Muller KJN Black W. Tuberculosis in young and elderly. Cheast. 1994;106:28-32.
9. Prasanthi K, Kumari AR. Efficacy of fluorochrome stain in the diagnosis of pulmonary tuberculosis coinfected with HIV. Indian Journal of Medical Microbiology. 2005;23:179-85.
10. Cheng AG, Chang A, Farwell DG, Agoff SN. Auramine orange stain with fluorescence microscopy is a rapid and sensitive technique for the detection of cervical lymphadenitis due to mycobacterial infection using fine needle aspiration cytology: a case series. Otolaryngol Head Neck Surg. 2005;133:381-5.
11. Sethi S, Sharma M, Sengupta C, Mohandas K,Sharma SK. Enhanced detection of Mycobacteria stained with rhodamine auramine at 37 degrees C. Indian J Pathol Microbiol. 2003;46:521-23.
12. Singh NP, Parija Sc. The value of fluorescence microscopy of Auramine stained sputum smears for the diagnosis of pulmonary T.B, Southeast Asian J Trop Med Public Health. 1988;29:86 0-3.
13. Ulukanligil M, Aslan G, Tasci S. A comperative study on the different staining methods and number of specimens for the detection of acid fast bacilli. Mem Inst Oswaldo Cruz. 2000; 95:855-8.
14. Jain A, Bhargava A, Agarwal SK: a comparative study of two commonly used staining techniques for acid fast bacilli in clinical specimens. Indian. Journal of tuberculosis. 2003; 49:161-2.
15. Allen Chakravoty S, Sen M, Tyagi T. Diagnosis of extrapulmonary tuberculosis by smear, culture and PCR using universal sample processing technology. Journal of clinical Microbiology. 2005;43:4357-62.

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