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Field Evaluation of Auramine O LED Fluorescent Microscopy Compared with Existing Method and Culture

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Research Article

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ABSTRACT

Microbiological diagnosis is that the major drawback in limited resources settings for effective treatment of wasting TB disease. Despite the recent advancement in diagnostic methods, the smear microscopy remains the gold standard for the diagnosis of tb in high burden countries at Designated microscopic centers.

According to the NTEP National Tuberculosis Elimination Program smear microscopy is the gold standard for primary diagnosis of mycobacterium tuberculosis. Fluorescence Auramine O staining technique provides a more efficient option for the detection of Mycobacterium tuberculosis positive smears. This study therefore aimed toward assessing the diagnostic performance of microscopy Auramine Staining (FM) and Ziehl-Neelsen (ZN) staining techniques within the diagnosis of white plague with MGIT Liquid culture to test the viability.

Materials and Methods: The Pulmonary sputum samples from field particular at vulnerable population like HIV, Smokers, Health care workers , Malnutrition which were registered at IRL was used in the comparative study which was carried out at IRL Intermediated reference Laboratory Karnataka 350 samples were selected and smeared in two for every sample ,stained with ZN, Auramine O and therefore the same sample were Digested and decontaminated ,inoculated in Liquid culture MGIT (Mycobacterium Growth Indicator Tube) to test the Efficiency of the stain and its Viability in different vulnerable population .

Results: In the 350 samples analyzed, 124 (35.4%) , 81 (23.1%), and 145 (41.4%) were positive for Mycobacteria with Auramine O, ZN, and MGIT Liquid culture , respectively. Within 124(35.5%) positives of Auramine O 35(28.2%) were 1+, 49 (39.51%) were 2+,40 (32.25%) were 3+. The mean reading time of Auramine O Fluorescent microscopy was thrice faster than

the ZN technique with a good Background and sharp fluorescent shining bacilli. The sensitivity and specificity of fluorescent staining to it of Liquid MGIT assay were 84.5% and 100%, respectively, while those of ZN staining were 54.8% and 100%, respectively.

For a routine laboratory test in a poor resource-limited setting, our study has demonstrated that fluorescence Auramine O staining technique will be a more sensitive test for the diagnosis of tuberculosis as compared to the traditional ZN technique.

INTRODUCTION

Tuberculosis caused by mycobacteria which remains a major public unhealthiness with approximately one-third of the world's population affected. In 2017, 10 million people were infected with tuberculosis and 1.6 million died from the disease. Over 95% of tuberculosis deaths occur in low- and middle-income countries [1]. A faster, simpler, more accurate, and fewer expensive means of diagnosis of tuberculosis is vital for the control of people infected with the disease as wells as preventing its spread within the community [2]. Various investigations are often used to help within the diagnosis of tuberculosis, and these include chest radiographs, clinical suspicion, staining for acid-fast bacilli, culture for mycobacteria, and macromolecule amplification assays smear microscopy is that the foremost preferred and rapid test that's widely used for the detection and diagnosis of white plague [2,3]. The bacilli within the sputum are often detected either by ZN or fluorescence staining techniques. Sputum microscopy is beneficial to assess the response to treatment and to see a cure or failure at the tip of treatment.

In many developing countries, the diagnosis of tuberculosis is typically supported the ZN staining technique[4]. The sensitivity of microscopy by ZN method, however, is reported to be low and variable, ranging from 20% to 80%, often depending on the diligence with which specimens are collected, smears are made, and stained smears are examined [4–7]. This procedure leaves an enormous number of cases undetected, especially if it becomes the only means of diagnosis. FM was introduced to spice up the outcomes of smear microscopy. The sensitivity of conventional FM provides far better yield and detection of positive smears than the ZN and takes less time to perform [8–10] there's however a lingering doubt about the specificity of FM as there's the likelihood of false positives which may result to the incorporation of fluorochrome dyes by inorganic objects [11, 12].

With of these cost constraint could be a limitation of FM especially in low and ,medium limited settings or within the peripheral DMC District microscopic centers .In Karnataka the diagnosis of TB by use of conventional microscope ZN is overcoming a change with FM smears and stain while the utilization of all molecular techniques require large resource and Laboratory technician Their is currently no documentation or evidence on the appliance for diagnosis of consumption. Hence this paper compares the diagnosis and it application with three methods of detection for Tuberculosis.

MATERIALS AND METHODS

The Pulmonary samples from vulnerable groups were registered at IRL for culture and DST were selected for the study (Table 1).

Table 1. Category of Samples.

Vulnerable Group	Samples
HIV	134
Smokers	75
Health care workers	25
Malnutrition	116
Total	350

The 350 smears were flooded with filtered 0.1% auramine for a minimum of 20 minutes. They were then rinsed with water and drained. Acid alcohol decolorizing solution (0.5%) was applied on the smear for 30 to 60 seconds, rinsed with water, and drained. They were then flooded with 0.5% permanganate counterstain for a maximum of 1 minute and rinsed with water. The smears were allowed to air dry and examined microscopically using the dry (40x) objective lens of an LED illumination-based fluorescence microscope (Labomed) [13-15]. With the 350 smears were used for ZN staining with 1% filtered carbol fuchsin is poured on to the full slide. The slide is gently heated with carbol fuchsin thereon, until vapours rise don't boil. Carbol fuchsin is left on the slide for five minutes. The slide is gently rinsed with H_2O until all free carbol fuchsin stain is washed away. Currently, the smear on the slide looks red in colour. 25% of vitriol solution is poured onto the slide and allowed in and of itself for 2–4 minutes then The slide is gently rinsed with H_2O and tilted to empty off the water. A properly decolourised slide appears light pink in color .If the slide continues to be red, oil of vitriol is reapplied for 1–3 minutes rinsed gently with water .The back of the slide is cleaned with a swab dipped in sulfuric acid solution, 0.1% counter stain is poured onto the slide and left for 30 seconds.

Then the slide is rinsed gently with H_2O and allowed to dry. The slide is examined under the sunshine microscope using x40 lens to select out the appropriate area then examined under x100 lens by employing a drop of immersion oil.

METHODS

In the 350 samples analyzed, 143 (40.85 %) in Auramine FM, 81 (23.1%) in ZN Microscopy, and 145 (41.4%) were positive in MGIT Liquid culture respectively. With these 143 positive sample in the Auramine FM on different vulnerable population was found to be in HIV 43 (30%) , smokers 39 (27%), Health care workers 13 (9.09%),Malnutrition 48 (33.5%) and mean reading time of Auramine LED Microscopy was 3 times faster than the ZN technique with superb acceptance (1.5min: 4.6min). The sensitivity and specificity of fluorescent staining to it of Liquid MGIT assay were 98.6% and 100%, respectively, while those of ZN staining were 55.8% and 100%, respectively (Figure 1).

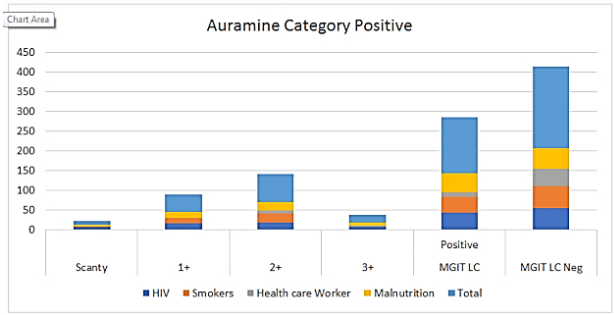

Figure 1. Auramine positive in different Vulnerable group with LC.

DISCUSSION

Tuberculosis may well be a significant public unhealthiness everywhere the country to keep with ages, with such plenty of advances in treatment and management, still tuberculosis can be a public problem in India with adverse social and economic conditions. Current recommendations for the control of tuberculosis emphasize early case detection, treatment of patients and there by limit the transmission of the bacilli.

The main stay for its control is that the rapid and accurate identification of the infected individuals. The detection of AFB is taken under consideration because the evidence of infective stage. The laboratory plays a critical role in diagnosis of consumption of high sophisticated equipment of other diagnostic tests that use molecular and immunological methods are developed. While molecular methods overcome the insensitivity of smear method, the time required for culture and retrieval of a specimen from the positioning of infection require well-prepared laboratory and well-trained personnel the most effective rapid method is that the detection of acid-fast bacilli by microscopy.

In developing countries, microscopy of sputum is much and away the fastest, cheapest and more reliable method for diagnosis of consumption. The estimated detection limit of microscopy is 107 bacilli/ml of sputum. In immunocompromised patients like HIV infected cases there's major impact on the pathogenesis of tuberculosis it directly attacks the critical immune mechanisms involved in protection against tuberculosis.

ZN stain can detect bacilli which when put on to culture were seen minimal positivity, where as a more sensitive Auramine stain can detect only bacilli which can grow on to culture so by this study we describe the efficiency out of 350 samples examined 143 (40.85 %), 81 (23.1%) TB cases were FM staining and ZN staining methods respectively. Where the sensitivity and specificity of fluorescent staining there to of Liquid MGIT assay were 98.6% and 100%, respectively, while those of ZN staining were 55.8% and 100%, respectively.

CONCLUSION

Sputum examination for the tubercle bacilli is typically conducted for patients clinically and radio logically suspected of Tuberculosis disease. However, the routine method of sputum examination, that is, ZN staining isn't sensitive enough and same suspected cases aren't confirmed.

More over they continue to be undiagnosed and fail to urge treatment. Hence our study concludes that Fluorochrome staining with LED is more efficient over ZN stain in detecting Tuberculosis bacilli in sputum, especially the paucibacillary cases and also FM LED has been found to be less time consuming as compared to ZN method (1000x) within the diagnosis of TB.

FM with LED is simpler to use, quicker and cheaper especially in centers where large numbers of sputum specimens are processed. the sole disadvantage with Fluorescent staining is that the value of LED microscope is comparatively high and will not be affordable for developing countries. Whenever it's possible, cytosmear examination should be done by Fluorescent method by gradually replacing the normal microscopes with LED Fluorescent ones.

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