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RESEARCH ARTICLE

COMPARISION BETWEEN CONVENTIONAL ACID –FAST STAINING AND LEDs MICROSCOPY IN THE DIAGNOSIS OF PULMONARY AND EXTRA-PULMONARY TUBERCULOSIS

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ABSTRACT

Background: Tuberculosis (TB) continues to be one of the world's most important infectious causes of morbidity and mortility. The backbone of TB diagnosis worldwide continues to be smear microscopy. For microscopic detection of acid fast bacilli (AFB), fluorescence microscopy (FM) using Auramine staining has been shown to have 10% higher sensitivity compared to routine light microscopy used with Ziehl-Neelsen (ZN) staining, without compromising specificity.
Objectives: This study is carried out to diagnosis of pulmonary and extra-pulmonary Tuberculosis by Fluorescence microscopy and comparing the smear sensitivity and specificity of conventional Light microscopy with ZN (Gabbet's method) and FM with LED microscopes.
Materials and Methods: About 100 samples were included in the study. These samples belonged to suspected cases of pulmonary and extra-pulmonary Tuberculosis. Samples were stained both by Gabbet's and Fluorescent staining as per standard protocol.
Results: Out of total 100 samples included in the study, 71 were sputum samples and 29 were of other body fluids. Of all, 18 were found to be positive by both FM and ZN staining.25% (4/18)of the samples belong to female patients and 75%(14/18)were from male patients with an incidence rate of who tested positive.
Conclusion: Use of FM helped in rapid detection of tubercle bacilli from various body fluids when the organisms were scanty.

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INTRODUCTION

Tuberculosis (TB) continues to be one of the world's most important infectious causes of morbidity and mortility. An estimated 9.4 million people develop TB disease each year and approximately 1.7 million die from the disease. (WHO 2010) In 2011, out of the total 9 million cases of TB recorded worldwide 2.3 million were found in India with a 0.32 million mortality rate. (Revised National Tuberculosis Control Program, 2006) One of the key step in TB control is case detection. Although advances in diagnostics are leading to the introduction of new tests (Small and Pai, 2010), the backbone of TB diagnosis worldwide continues to be smear microscopy. Thus, increasing the sensitivity of smear microscopy could have a large impact on global TB case detection rates. (Steingart *et al*., 2006) For microscopic detection of acid fast bacilli (AFB), fluorescence microscopy (FM) using Auramine staining has been shown to have 10% higher sensitivity compared to routine light microscopy used with Ziehl-Neelsen (ZN) staining, without compromising specificity. (Steingart *et al*., 2006) FM is also more time efficient, with one large study reporting FM to take 25% of the time required for ZN examination. (Bennedsen and Larsen, 1996) Light emitting diode (LED) microscopy is a novel diagnostic tool developed primarily to allow resources – poor parts of the world access to the benefits of FM. (Hanscheid, 2008) compared to conventional mercury vapor fluorescence microscopes are less expensive and have lower maintenance requirements. The diodes are very durable, do not require warm-up time, and do not contain toxic products. Importantly, they are reported to perform equally well without a darkroom. (Shenai *et al*., 2011) Many of the benefits of LED technology would also be appealing to laboratories in high-income countries if LED microscopy is equivalent in performance to conventional FM. Indeed, the World Health Organization (WHO) recently recommended that conventional FM be replaced by LED-FM in all settings where fluorescence microscopy is currently used, and that LED-FM in all settings where fluorescence microscopy is currently use, and that LED FM be phased in as an alternative to conventional ZN microscopy in all settings. (WHO, 2010) This study is carried out for diagnosis of pulmonary and extra-pulmonary

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Tuberculosis by Fluorescence microscopy and comparing the smear sensitivity and specificity of conventional Light microscopy with ZN (Gabbet's method) and FM with LED microscopes.

MATERIALS AND METHODS

About 100 samples were included in the study for a period of 2 months. These samples belonged to suspected cases of pulmonary and extra-pulmonary Tuberculosis. Samples included sputum, broncho-alveolar lavage(BAL) fluid, Bronchial wash (BW) and various body fluids like CSF, Ascitic fluid, Pleural fluid, Synovial fluid, which were processed as per the standard protocol. Samples were stained both by Gabbet's and Fluorescent stain.

Gabbet's staining method: Flood the smear with concentrated carbolfuschin for 10mins. Wash with water. Flood with Gabbet's methylene blue for 3mins. Wash with water. Air dry the smear and examine under oil immersion objective by light microscopy.

Fluorescence staining method: Heat fixed smears were flooded with Auramine O (AO) for 15minutes, then rinsed with sterile water; decolorize with acid-alcohol for 2 minutes, then rinsed with sterile water; counter stained with potassium permanganate for 2-4 minutes, then rinsed with sterile water and allowed to air dry and examine with LED microscope.

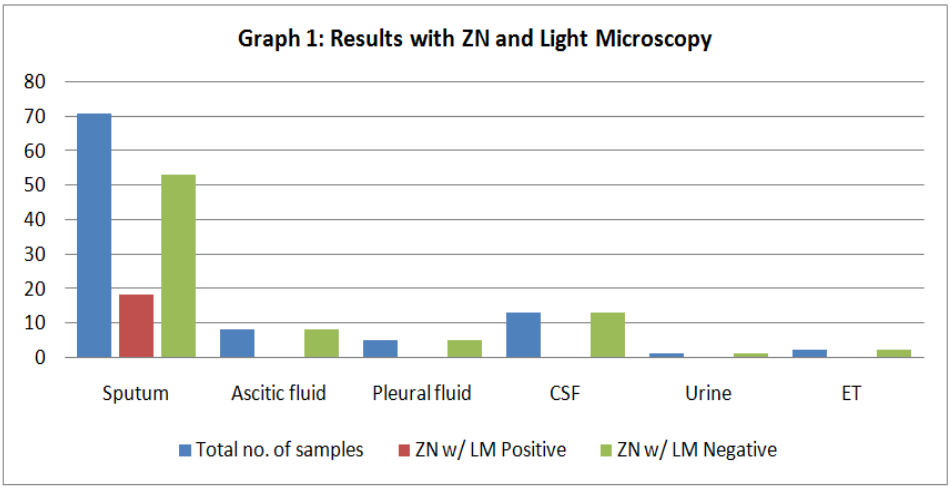
OBSERVATION AND RESULTS

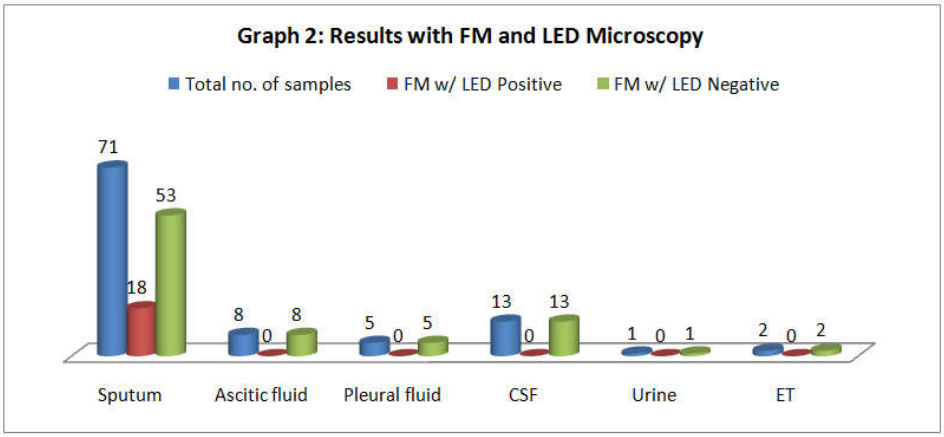
Out of total 100 samples included in the study, 71 were sputum samples and 29 were of other body fluids such as Ascitic fluid-8, Pleural fluid-5, CSF-13, Urine-1, and ET-2.

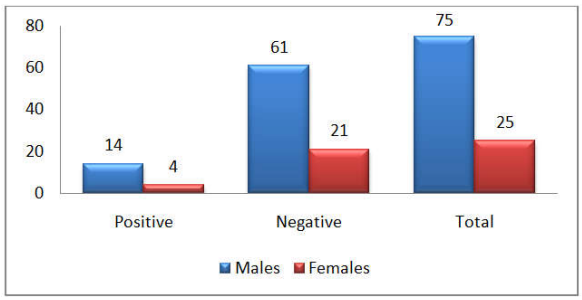
Of all the 100 samples 18 were found to be positive by both FM and ZN staining, that is, 18%of the samples were positive for tubercle bacilli. All the 18 samples were positive for tubercle bacilli were of sputum and none of the other body fluids were positive. 25% of the samples belong to female patients with an incidence rate of 4 out of 18 who tested positive. 75% of the suspected cases were male patients with an incidence rate of 14 out of 18 who tested positive.

Table 1. Results of ZN w/ LM and FM w/LED in various samples collected

Sample Type	Total no. of samples	ZN w/ LM Positive	ZN w/ LM Negative	FM w/ LED Positive	FM w/ LED Negative
Sputum	71	18	53	18	53
Ascitic fluid	8	0	8	0	8
Pleural fluid	5	0	5	0	5
CSF	13	0	13	0	13
Urine	1	0	1	0	1
ET	2	0	2	0	2
Total	100	18	82	18	82







Graph 3. Sex wise distribution

DISCUSSION

The operational benefits of LED microscopes over conventional light microscopes would certainly be of interest for laboratories of high-income as well as low and middle income settings. Working without the need for a dark room using LED microscopes could significantly improve workflow and maximize space utilization in the lab. However, these benefits would not be sufficient to adopt LED FM in high-income settings unless the sensitivity and reading efficiency associated with conventional LM ZN were maintained with LED FM. In this LED evaluation, we found no difference in diagnostic accuracy. When smears were stratified by their specimen type, their diagnostic accuracy remained equivalent. The analysis was also performed by specimen and not by patient. While this is consistent with most other studies in this field, we recognize that the lack of independence between specimens arising from the same patient may overestimate the precision of accuracy estimates. ZN stain can detect bacilli when they are in the order of 10^5/ml of the sputum, where as a more sensitive AO stain can detect in the order of 10^4/ml of sputum. In the present study, out of the 100 samples examined, 18% cases were detected by ZN and FM staining methods respectively. Almost similar results have also been reported by studies done by SarojGolia *et al*. (10.57% and 16.56% were positive by ZN and AO staining) 2013 (SarojGolia *et al*., 2013), and Suriakumar *et al*, 2012 (Prasanthi *et al*., 2005). Whereas higher smear positivity rates were shown by Prasanthi *et al*, 2005(50% by ZN, 69% by AO) (Suria Kumar *et al*., 2012) and Ulukanligil *et al* (67.6% by ZN, 85.2% by AO) (Desai *et al*., 2009). In the present study, out of the 100 samples examined, none of the cases detected AFB in body fluids by both ZN and FM staining methods respectively. But results have been reported by studies done by K. Desai *et al* (out of 28 extra-pulmonary samples 6(21.42%) were positive by FL stain and 4(14.28%) were positive by ZN staining (Desai *et al*., 2009). These results show FL staining technique is more sensitive in detection of AFB in sputum as well as extra-pulmonary samples as compared to ZN stain. Similar results were obtained by Githuri *et al* (80%by fluorescent microscopy and 65% by ZN staining) (Githui *et al*., 1993), Ulukanligil *et al* also obtained 85.2%positivity by fluorescent microscopy and 67.6%by ZN method. (Ulukanligil *et al*., 2000) Similar results obtained by S J Murray *et al* (93%by FL microscopy and 73%by ZN method) (Murray *et al*., 2003), K. Prashanthi *et al.* (69%by FL microscopy and 50% by ZN method) (Suria Kumar *et al*., 2012) and Jain *et al.* (41%by FL microscopy and 32%by ZN method) (Jain *et al*., 2002).

Conclusion

After conducting this study we can conclude that fluorescence microscopy with LED microscopes has various diagnostic advantages over conventional ZN staining. Increased sensitivity of fluorescence microscopy allows more time efficient screening of sputum and other body fluids. This is highly advantageous, particularly when high numbers of samples are to be screened per day, because the majority of laboratory time is spent confirming negative smear results. Use of FM helped in rapid detection of tubercle bacilli from various body fluids when the organisms were scanty. This was possible as the tubercle bacilli stood out as bright objects against a dark background in fluorescence microscopy which made them easily identifiable hence causing less eye-strain. In simple words the bacilli "looked at us" instead of we trying to look for them. Although these advantages are more apparent in practice, a larger sample load needs to be assessed before we can come to more narrowed down conclusion.

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