Original Article

Comparative study of Ziehl-Neelsen (Z-N) Staining Versus Fluorochrome Stain for Pulmonary Tuberculosis

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Abstract

Background: *Tuberculosis is a highly infectious disease and has the highest burden with it. Diagnosis of tuberculosis in many countries is still dependent on microscopy. For developing countries with a large number of cases and financial constraints, evaluation of rapid and inexpensive diagnostic methods has great importance. The bacilli in the sputum can be detected microscopically by ZN stain and fluorochrome stain.*

Objective: *The purpose of the present study was to compare the efficacy of flurochrome(FI) stain with Z-N stain in the diagnosis of pulmonary tuberculosis.*

Methodology: *This cross sectional study was done in the Department of Microbiology at Sir Salimullah Medical College, Dhaka and National Institute of Chest Disease & Hospital (NIDCH), Dhaka during the period of January 2014 to December 2015 for a period of 1(one) year. Sputum samples from suspected MDR-TB patients were collected by purposive sampling technique from OPD of Sir Salimullah Medical College (SSMC) and NIDCH. Microscopy, liquid culture in liquid MGIT 960 media were done for MTB diagnosis.*

Result: this study *shows the comparison of results of microscopic examination of Fluorochrome and Z-N stained sputum smear. Both Fluorochrome and Ziehl-Neelsen stains showed predominant positive results, that is, 3+ in 50% and 50.94% samples respectively, followed by 2+ in 30.65% and 32.07% samples, 1+ in 14.5% and 13.2% samples and scanty in 4.84% and 3.77% samples respectively. On the other hand, Fluorochrome stain gave negative report in 38 (38%) samples, whereas Z-N staining showed 47 (47%) negative results.*

Conclusion: *Fluorochrome stain yielded more positive result than Z-N stain under microscope in smear of sputum.*

Key Words:
MDR-TB, Fluorochrome, Ziehl-Neelsen (Z-N)

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Introduction

Tuberculosis (TB) remains a major global health problem. It ranks as the second leading cause of death from an infectious disease worldwide. The latest estimates included that there were 8.6 million new TB cases in 2012 and 1.3 million TB deaths. These large numbers of cases and deaths notwithstanding, 20 years on from the 1993 World Health Organization (WHO) declaration of TB as a global public health emergency, major progress has been made. Globally, the TB mortality rate (deaths per 100 000 population per year) has fallen by 45% since 1990 and TB incidence rates (new cases per 100 000 population per year) are falling in most parts of the world. In the 18 years since the launch of a new international strategy for TB care and control by WHO in the mid-1990s (the DOTS strategy) and the subsequent global rollout of DOTS and its successor (the

Stop TB Strategy), a cumulative total of 56 million people were successfully treated for TB between 1995 and 2012, saving approximately 22 million lives.[1]

Microscopical technique which is available now is fluroscence staining, it is simpler and more rapid as it screens the smear in 40×. This advantage would be more beneficial in overburden laboratories in low resource settings where culture facilities are not available. Even the cost of this microscopy method can be reduced by use of LED microscope instead of fluroscnece microscope which contains the bulb with life up to 50,000 hours.[2]

Also processing of samples for decontamination and concentration method can also increase the sensitivity of Z N stain and fluorescence stain as compared to direct smear preparation from slide. The aim of this study was to compare both Z N stain and fluorescence stain and also to compare the results of smears both before and after decontamination procedure.

Methodology

This cross sectional study was done in the Department of Microbiology of Sir Salimullah Medical College, Dhaka and National Tuberculosis Reference Laboratory (NTRL), Dhaka during the period of January 2014 to December 2014. Suspected cases of MDR-TB patients who were attended at the OPD and IPD of NIDCH and SSMC were selected as study population. Patients were excluded who were undergoing treatment or having extra-pulmonary tuberculosis or were new pulmonary tuberculosis cases. The sediment of processed sputum was used for microscopic examination by Ziehl-Neelsen (Z-N) staining and flurescent Auramine staining. Early morning sputum samples were collected in clean, sterile, leak proof, wide mouth containers. Before collecting specimens, each patient was interviewed and informed written consent was taken from patients or legal guardian of patients and relevant information were recorded systematically in a pre-designed data sheet.

Results:

This study shows the comparison of results of microscopic examination of Fluorochrome and Z-N stained sputum smear. Both Fluorochrome and Ziehl-Neelsen stains showed predominant positive results, that is, 3+ in 50% and 50.94% samples respectively, followed by 2+ in 30.65% and 32.07% samples, 1+ in 14.5% and 13.2% samples and scanty in 4.84% and 3.77% samples respectively. On the other hand, Fluorochrome stain gave negative report in 38 (38%) samples, whereas Z-N staining showed 47 (47%) negative results. So, Fluorochrome stain yielded more positive result than Z-N stain under microscope in smear of sputum.

Table I

Comparison of Result of Fluorochrome Stain and Ziehl-Neelsen Stain Grading (n=100).

Grading	Fluorochrome Stain n (%)		Ziehl-Neelsen Stain n (%)	
	Positive	Negative	Positive	Negative
	62 (62)	38 (38)	53 (53)	47 (47)
S	03 (4.84)		02 (3.77)	
1+	09 (14.5)		7 (13.2)	
2+	19 (30.65)		17 (32.07)	
3+	31 (50)		27 (50.94)	
Total	100 (100)		100 (100)	

Table-II

Comparison of Results of Smear Microscopy Ziehl-Neelsen staining and fluorochrome (n=100).

Result	Ziehl-Neelsen Staining	Fluorochrome Staining
Positive	53	62
Negative	47	38
Total	100	100

Discussion

Bangladesh has a long history of research and demonstration projects on TB. The detection of Acid fast bacilli is often considered as the evidence of the infected stage. Thus, the laboratory plays a critical role in the diagnosis of TB.[3] In developing countries, microscopy of the specimen is by far the fastest, cheapest, and most reliable method for the detection of AFB.[4,5] However fluorescent staining has been added in Revised National Tuberculosis Control Program (RNCP) because of more sensitive and rapid results and can be used in field areas.

In our study, Fluorochrome yielded slightly higher positivity than Z-N stain (62% vs.53%) on smear microscopy of sputum. Murray *et al*. [6] reported 93% positive by auramine staining and 73% positive by Z-N method; these observations were much higher than present study. However, several other studies showed opposite results. Laifangbam *et al*[7] showed that positivity rates for Z-N and Auramine-O (AO) were 36.1% and 72.2% respectively. Desai et al.[8] reported *M. tuberculosis* positive samples detected by Fluorescent staining were higher (36.27%) in comparison to Z-N staining (19.11%). Goyal and Kumar[9] showed fluorochrome staining was found more efficient (14.69%) than Z-N stain (7.47%) in AFB detection from cases of pulmonary tuberculosis. Smear negative cases were also predominant in the studies conducted by Scott *et al*.[10] (72%), Helb *et al*.[11] 2010 (72%) and Zeka *et al*.[12] 56%). This discrepancy might be related

to case selection, geographical and technical variations. As open pulmonary cases are more prevalent in Bangladesh there is more chance of spread of infection.

The results of present study indicate that Auramine staining of sputum smears in is a more sensitive method of sputum microscopy for demonstration of AFB in sputum specimen, compared to Ziehl-Neelson staining. The use of 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 Zeihl Neelson stained smears. The method is economical in both time and expense and recommended for laboratories handling large number of sputum specimens.[13] Fluorescent staining is superior to that of ZN staining in the presence of a low bacterial load as seen in smears with diagnostic cytomorphologial featured tuberculosis, in problem areas like AIE (acute inflammatory exudates alone or with occasional granulomma, AFB positivity by ZN staining is nearly as good as the fluorescent method because bacterial load is high).[14,15] Using fluorescent microscopy, the tubercle bacilli when examined under ultra violet illumination, the bacilli appeared as a bright rod against a dark back ground. Since there was a contrast, the bacilli were readily seen and therefore in very less time large area could be examined. Images were then captured with the digital camera and enhance through imaging processing techniques.[16] While in ZN staining acid fast bacilli appeared bright red rods in blue background. In this also image were captured. The potential benefits of automated screening for tubercle bacilli are: rapid, acute, inexpensive diagnosis; the ability to screen large number of people; increased resources to monitor patients; and reduction in health risk to staff. Thus the study reveals that sputum stained by the florescent method is useful and reliable for pulmonary tuberculosis. Since the fluorescent microscopy is costly some laboratories cannot afford to buy florescent microscopy, so in these laboratories Ziehl-Neelson staining is most employed.[16] Auramine stain offers much contrast and bacilli appears as brilliant yellow against dark background. Moreover, in Fluorescence microscopy with 40 magnification objective is used for examination, thereby much larger area of the smear to be scanned than Z-N smear resulting in more rapid and large number of specimens could be examined in a given time.

Conclusion

Fluorescent stain is a more efficient over ZN stain in detecting Tubercle bacilli in sputum. Since screening is done under low power of magnification, fluorescence has been found to be less time consuming compared to ZN method in the diagnosis of tuberculosis. Hence, it has been advocated to be methods of choice where the large numbers of sputum smears are to be examined.

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