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ORIGINAL ARTICLE

Detection Rates of Ziehl-nelseen Staining Technique and Fluoresecnt Microscopy in The Examination of Sputum for Acid Fast Bacilli.

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ABSTRACT:

Background: Tuberculosis is endemic in developing countries but has re-emerged as a public health issue with the coming of Human immunodeficiency virus infection even in the developed world. **Objective:** To compare the detection rates of Mycobacterium tuberculosis in sputum specimens between Ziehl-Neelsen staining technique and Fluorescent microscopy. **Methods:** It was a retrospective study carried out from between June 2010 and May 2011 in a tertiary health centre in North-eastern Nigeria. In the first six months (June 2010-November 2010) detection of *Mycobacterium tuberculosis* was done using the Ziehl-Neelsen (ZN) staining technique and in the last six months (December 2010-May 2011) detection was by Fluorescent-Auramine technique of *Mycobacterium tuberculosis*.**Results:** One thousand four hundred and eighty six (1486) patients were used, with 764(51.4%) females and 722 (48.6%) males. Sixty nine (10.6%) were positive for *Mycobacterium tuberculosis* using the Ziehl Neelsen method while 122 patients (14.4%) were positive for *Mycobacterium tuberculosis* using the Fluorescent Microscopy method p=0.029. **Conclusion:** The six months comparison of the two methods (ZN and FM) showed that the detection rates of acid- fast bacilli in sputum specimens had increased from 10.6% to 14.4 % respectively which is statistically significant.

Key words: Ziehl-Neelsen staining , Fluorescent microscopy, sputum examination.

INTRODUCTION

The negative effects of tuberculosis on society are enormous. It is estimated that nearly one billion people will be infected with tuberculosis (TB), 200 Million will develop the disease and 35 million will die from TB during 2000-2020[1].

Direct microscopic examination for acid fast bacilli (AFB) by Carbol Fuchsin is currently the most widely used microbiological method for the diagnosis and confirmation of pulmonary tuberculosis (PTB), and when Positive defines the more infectious cases[2,3].This method is highly specific, fast and cheap for detecting AFB in sputum. The disadvantage of this method is its low sensitivity that vary from 45%-80% relative to culture[3-7].

The utilization of Auramine 'O' a fluorescent dye instead of Carbol Fuchsin was first proposed in the 1930s[8],but found widespread use in developed countries only about 30 years later after a thorough re-evaluation of the technique using a combination of Auramine 'O' and Rhodamine[9]. A study from Kenya showed superior sensitivity of fluorescent microscopy in comparison with bright field microscopy for low density smears[10]and fluorescent microscopy has proved to be as reliable as bright-field microscopy[11]. The advantage of fluorescence microscopy is the possibility to scan a sputum smear at 250x magnification rather than at 1000x magnification, allowing theoretical reduction of examination time of the same area to one sixteenth as the surface increases by the square of the diameter. Practically the

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examination time is reduced to about 10 fold with Fluorescent compared to bright-field microscopy using a 4-fold different magnification (250xVs 1000x)[12]. The disadvantages of the former include the higher cost of investment and maintenance and the lesser robustness of the fluorescence compared to bright-field microscope[13].

The current study was carried out to compare the detection rates of *Mycobacterium tuberculosis* in sputum specimens between the Ziehl-Neelsen staining technique and fluorescent microscopy at a tertiary health centre in the North-eastern Nigeria.

METHODS

The study was carried out in the department of medical microbiology, Abubakar Tafawa Balewa University Teaching Hospital (ATBUTH), Bauchi, Bauchi State, North-eastern part of Nigeria. The hospital receives referrals from Bauchi State in addition to other neighbouring states including Gombe, Jigawa, Yobe and Adamawa States.

It was a retrospective study carried out from June 2010 - May 2011. In the first six months (June 2010-November 2010) detection of *Mycobacterium tuberculosis* was done using the Ziehl-Neelsen (ZN) staining technique and the last six months (December 2010-May 2011) witnessed the introduction of the Fluorescent-Auramine technique of Mycobacterium tuberculosis detection at ATBUTH and hence patients were tested based on that. Information was retrieved from the laboratory register at the department of medical microbiology of the hospital. Standard method for the Ziehl-Neelsen(ZN) staining technique was used during the procedure,[14] also; Standard method was used for the newly adopted method of Fluorescent Microscopy (FM) using Auramine and Rhodamine dyes.[15]

Descriptive statistics were summarized for patients' demography. The data obtained were analysed using statistical package for social sciences (SPSSTM) computer software version 16.0. Chi-square and Fishers exact test were used to compare the two methods, at 95% confidence interval. P value < 0.05 was considered to be statistically significant.

RESULTS

Majority of the patients tested were females with 764 (51.4%) out of the total number of 1486 patients in the study. The age ranged from 2 years to 90 years with a mean of 35.8 $\pm$1.51 years. Age group of 20-29 years has the highest frequency of occurrence while on the other hand age group 0-9 years has the lowest frequency of occurrence (Table 1).

Sixty nine patients (10.6%) were positive for *Mycobacterium tuberculosis* using the Ziehl Neelsen method while 122 patients (14.4%) were positive for *Mycobacterium tuberculosis* using the Fluorescent Microscopy method (X^2= 4.98,df=1,P=0.029. (Table 2).

However, no significant relationship was found between the age or sex of the patients in detecting *Mycobacterium tuberculosis* by ZN method or the FM method (X^2 =1.000, df =1, P= 0.373; X^2 =0.011, df =1, P=0.922).

Table 1: Some Demographic characteristics of the patients studied

Age Group (years)	**Frequency**	**(%)**
0-9	14	0.9
10-19	110	7.4
20-29	385	25.9
30-39	357	24.0
40-49	212	14.3
50-59	132	8.9
60-69	78	5.2
70 and Above	60	4.0
* Adult	138	9.3
Total	**1486**	**100.0**
Sex	**Frequency**	**Percentage**
Male	722	48.6
Female	764	51.4
Total	**1486**	**100.0**

*Was entered as adult with no age specification

Table 2: Positivity of AFB Detection Using The Ziehl-Nelseen Method Versus Fluorescent Microscopy.

		Results			
		Positive	Negative	Total	PRE.
Methods	Ziehl Neelsen Technique	**69**	579	648	**10.6%**
	Fluorescent Microscopy	**122**	716	838	**14.4%**
Total		191	1295	1486	12.8%

X^2 = 4.98, df=1, p=0.029.

DISCUSSION

There was no statistically significant difference between the two methods based on gender. This is in contrast to studies by Desai et al[16] and Bhanvalikar et al [17] who found the prevalence rates of TB to be higher in males than females in India. The lack of significant difference between the sexes found in this study might be due to the fact that both sexes had similar risks of contracting the disease, or it might be due to chance occurrence alone.

Sputum positivity was higher in the age groups of 20- 39years than other age groups. These are the productive individuals in the society and they are more vulnerable to the tuberculosis infection. This finding was similar to the findings of several other workers[10,18-20.]

Our study showed a detection rate with ZN of 10.6% while that of FM was 14.4%. This reached statistical significance. Many comparative studies.[19-22] have shown the reliability of FM over ZN method in the diagnosis of pulmonary tuberculosis. FM was found to be more sensitive in terms of detection of mycobacterium because it is done under lower power magnification (400x) compared to ZN method in which 1000x magnification is used. So, FM is less time consuming and allows a large number of sputum specimens to be examined in a given time. The importance of increase in detection rate of *Mycobacterium tuberculosis* from 10.6% to 14.4% using ZN and FM methods respectively cannot be over emphasized. It shows that there is improvement in the laboratory diagnosis of pulmonary tuberculosis at ATBUTH Bauchi after acquiring fluorescent microscope. This will help in commencing the treatment of tuberculosis in time. The extrapolation of the above statements is that there would be reduction in terms of infectivity, morbidity and mortality due to this deadly scourge.

Our study has some limitations. The study was done over a short duration of one year with six months allotted to each method. In some cases age was entered as adult with no specific value thereby hampering categorization. We recommend that further studies should be carried out simultaneously on patients' samples to find out the actual sensitivity and specificity of the tests.

Based on our findings we recommend that, whenever possible fluorescent microscopy should be adopted in tertiary, secondary or even comprehensive health centres in processing specimens for acid- fast detection in suspected cases of tuberculosis for better management of the disease. This would go a long way in reducing the burden and consequences of this deadly scourge.

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REFERENCE

1. Floyd K, Blanc L, Raviglione M, Lee JW. Resources required for global Tuberculosis control. Sci 2002;95:2040-2041.
2. Sumoskori A, Hotaling JE, Fitzgerald M, O'Donne lD, Persons LM, Salfinger M. Lessons from a proficiency testing event for acid-fast microscopy. Chest 2001;10:250-257.
3. Bruchfeld J, Aderaye G, PalmeI B, Bjorvatn B, Kallenius G, Linquist L. Sputum concentration improves diagnosis of tuberculosis in a setting with a high prevalence of HIV.Trans R Soc Trop Med Hyg 2000;94:677-684.
4. Murray SJ, Barret A, Magee JG, Freeman R. Optimization of acid-smears for the direct detection of Mycobacteria in clinical samples. J Clin Pathol 2003;56:613-615.
5. Peterson EM, Nakasone A, Platon-Deleon M, et-al.Comparison of direct and concentrated acid fast smears to identify specimens culture positive for Mycobacterium spp.J P Clin Microbio 1999;37:3564-3568.
6. Woods GL, Pentony E,Boxley MJ, Gatson AM. Concentration of sputum by centrifugation for preparation of smears for detection of acid-fast bacilli does not increase sensitivity of the fluorochrome stain.J Clin Microbiol 1995;33:1915-1916.
7. Seacenu CA, Pfeiffer HC,McleanT.Evaluation of sputum smears concentrated by centrifugation for detection of acid fast bacilli.J ClinMicrobiol 1993;31:2371-2374.
8. Hagemann PKH. Fluorezez Farbang von Tuberktein MitAuramin.Munch Med Wscchc 1935;55;1066-1068.
9. Truant JR, Thomas W. Flourescence microscopy of tubercle bacilli stained with Auramine. Henry Ford Hospital Med Bull 1962; 10: 287-296.
10. Githul W, Kitul F, Juma ES, Obwana DO, Mwai J, Kwamanga D. A comparative study on the reliability of the fluorescent microscopy and ZN method in the diagnosis of pulmonary tuberculosis. East Afr

Med J. May 1993; 70 (5): 263-6.
11. Kubica GP. Correlation of acid-fast staining methods with culture results for Mycobacteria. Bull Int Union Tuberc 1980;55:117-124.
12. Smithwick RW.Laboratory manual for acid-fast microscopy.2nded Atlanta GA,USA:US Public Health Service,1976.
13. TomanK.Tuberculosis case finding and chemotheraphy.Questions and Answers 1sted Geneva:WHO.1979.
14. Allen BW. Tuberculosis bacteriology in developing countries. Med Lab Scien 1984;41:400-409.
15. Winn W, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P, et al. Koneman'scolor atlas and text book of diagnostic microbiology. Baltimore and Philadelphia: Lippincott Williams & Wilkins; 2006.
16. Desai K, Malek S, Mehtaliya C. Comparative study of Z-N staining v/s fluorochrome stain from pulmonary and extrapulmonary tuberculosis. Gurajat Medical Journal 2009; 64 (2) 32-34
17. Banvaliker JN, Gupta R, Sharma DC, Goel MK, Kumari S. HIV seropositivity in hospitalised Pulmonary Tuberculosis patients in Delhi. *Indian J Tub*erc 1997;44:17-9.
18. Rajasekaran S, Uma A, Kamakshi S, Jayaganesh D, Senlhanizhehelvan A, Savitri S, *et al*. Trend of HIV infection in patients with Tuberculosis in rural South India. *Indian J Tuberc* 2000;47:223-6.
19. Prasanthi K, Kumari AR. Efficacy of fluorochrome stain in the diagnosis of pulmonary tuberculosis co-infected with HIV. *Indian Journal of Medical Microbiology, (2005) 23 (3):179-185.*
20 Singh NP, Parija SC. The value of fluorescence microscopy of Auramine stained sputum smears for the diagnosis of pulmonary tuberculosis. Southeast Asian J Trop Med Public Health .1998 Dec; 29(4):860-3.
21. Kivihya-Ngugga LE, Van Cleeff MR, Githul WA, Nganga LW, Kibuga DK, et-al. A comprehensive comparison of Ziehl-Neelsen and fluorescence microscopy for the diagnosis of tuberculosis in resource-poor-urban setting. Int J Tuberc Lung Dis. 2003; 7(12):1163-71.
22. Laifangbam S, Singh HL, Singh NB, Devi KM, Singh NT. A comparative study of fluorescent microscopy with Ziehl-Neelsen staining and culture for the diagnosis of pulmunary tuberculosis. Katahmandu Univ Med J (KUMJ). 2009; 7(27):226-30.

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