ORIGINAL ARTICLE

Comparative Study of Direct Sputum Microscopy with Different Sample Pre-Treatment Procedures for Examination of Acid Fast Bacilli

Abdusemed Abdurahman, BSc[1], Getnet Beyene, BSc, MSc[1] and Negussie Wodajo, BSc, MSc[1].

ABSTRACT: A comparative study of three methods of identifying **Mycobacterium tuberculosis** *by microscopy was performed on the sputa of suspected pulmonary tuberculosis patients in Jimma Health Center from November 14 to December 6, 1998. Direct sputum microscopy was compared with microscopy after sodium hypochlorite pre-treatment overnight sedimentation and sodium hypochlorite pre-treatment overnight centrifugation techniques. One hundred sputum samples were examined for acid-fast bacilli by direct microscopy, sodium hypochlorite overnight sedimentation and hypochlorite centrifugation methods and gave 22 %, 37 % and 41 % smear positivity respectively. The increment of positive results obtained by the two concentration methods over the direct method was statistically significant ($P < 0.05$, $\chi^2 = 8.36$ and 5.41 for centrifugation and sedimentation methods respectively). The difference in the proportion of positive results obtained by the two concentration techniques was not statistically significant ($P > 0.05$, $\chi^2 = 0.336$). Therefore, employing a concentration method improves the efficiency of TB laboratories, and an overnight sedimentation method can be an alternative method for rural laboratories where there is no electric supply to use electrical centrifugation.*

INTRODUCTION

Tuberculosis (TB) is a serious health problem in most developing countries. According to the reports of the World Health Organization, there were about 9 million new cases of TB with 3 million deaths in 1995 (1,2). About 500 children die every day due to TB (3). The spread of HIV has further aggravated the situation. There is a ten times increased risk of developing TB in HIV-infected individuals (4). In 1995 about one-third of the 17 million HIV-infected people world wide were also co-infected with *M. tuberculosis*, of which 70% lived in sub-Saharan Africa.

In the developed nations, laboratory diagnosis of TB is based on rapid and sensitive detection methods such as nucleic acid amplification techniques, serological tests (5) and rapid drug susceptibility testing techniques (6,7). Since these techniques are expensive and require skilled manpower, most developing countries can not afford to use them for routine laboratory services. Culture is the reference method for the detection of tubercle bacilli. Culture of mycobacterium

[1]*School of Medical Laboratory Technology, Jimma Institute of Health Sciences, P.O. Box 378, Jimma, Ethiopia.*

from clinical specimens is more sensitive than microscopy allowing the biochemical identification of the species with excellent specificity and sensitivity. However, its growth rate on all culture media makes detection very slow with results taking 4-6 weeks (8). Thus, the only possible method in most developing countries is classical direct sputum microscopy.

At present, the number of diagnosed cases by direct microscopy is about 50% or less of the estimated cases (9). This is due to the poor quality of the general laboratory service where microscopic examination of sputum for tubercle bacilli is routinely carried out with insensitive methods that requires the presence of at least 10,000 organisms/ml of sputum (7). As a result, only about 30-40% of all newly developed cases in many developing countries are diagnosed microscopically (9). This failure to identify many new cases urged the national TB control program (NTCP) to establish a new strategy for direct sputum microscopic examination (10,11). The suggested strategy is sputum microscopy of spot morning instead of the old trend of morning sputum for 3 consecutive days. This new strategy is intended to increase patient compliance and reduce patient inconvenience especially for those patients whose residence is far from health centers. The disadvantage of this strategy is the lower quality of spot specimens as compared to the morning sputum as the latter may contain overnight accumulation of bacilli.

Another approach to improve the microscopic technique is the use of sodium hypochlorite (NaOCl) digestion as a sample pre-treatment procedure. In a study designed to compare direct sputum smear microscopy with that prepared after liquefaction of sputum with NaOCl, house bleach, and centrifugation of sputum, statistically significant increment (108-125%) in positivity rate was observed by the sample pre-treatment procedure (12).

The aim of this study was to assess diagnosis of TB by concentration techniques using NaOCl in comparison with direct sputum microscopy.

MATERIALS AND METHODS

Sample collection and processing. The study was conducted on the sputum of suspected pulmonary TB patients who came to Jimma health center between Nov. 14 and Dec. 4, 1998. All patients suspected to have pulmonary TB with laboratory request for AFB were included. Labeled sputum cup was provided for each patient after instruction on how to produce the right specimen. In the investigation, a total of 100 sputum samples were collected.

Direct and Concentration methods: Smears were prepared on slides according to the WHO recommendation (13,14).

One ml of sputum from the same sample was added into two labeled 10 ml screw capped test tubes and mixed with equal volumes of commercially available 4-5% NaOCl. One of the test tubes was processed and centrifuged according to the procedure described by Gebre N. *et. al.* (12), while the other was left overnight at room temperature. The supernatant was discarded and a drop of pellet was added onto a slide for each method, dried and stained with Ziehl-Neelsen stain (14).

The slides prepared by the three methods were coded without the knowledge of the technician in charge in order to avoid bias during examination of the slides. The technician examined a single slide for each sample by scanning for AFB using the standard method.

Statistical analysis: Descriptive statistics was used for data analysis and the Chi-square method was used to determine statistical significance.

RESULTS

Of the 100 specimens, 22%, 37% and 41% were found to be smear positive for the direct microscopy, sodium-hypochlorite liquefaction-overnight sedimentation, and hypochlorite liquefaction-centrifugation methods respectively (Fig. 1).

The increase in the number of positives samples obtained after employing NaOCl concentration methods compared to the direct method was statistically significant ($p<0.05$, $\chi^2=8.365$ and 5.409 for centrifugation and sedimentation methods respectively). The difference in the

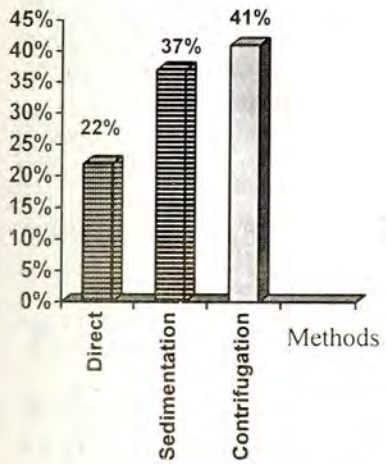

Fig. 1. Percentage of positive slides for direct sputum microscopy, after NaOCl treatment-centrifugation and NaOCl treatment-overnight sedimentation.

proportion of positive results obtained by the two NaOCl concentration techniques was not significant ($p > 0.05$, $\chi^2 = 0.336$).

DISCUSSION

Comparison of the results obtained by direct smear and NaOCl pre-treatment-centrifugation techniques revealed that there were 22 positives by both methods and an additional 19 positives by the concentration method that were negative by the direct smear microscopy. Similarly, there were 22 samples that were positive by both the direct and NaOCl pre-treatment overnight sedimentation techniques and additional 15 positive by the latter method.

This result shows pronounced relative efficiency of the concentration technique over the direct smear preparation. The number of positive result was increased by 86.4% after NaOCl pre-treatment-centrifugation method and by 68.2% after NaOCl pre-treatment sedimentation method. This rise was shown to be statistically significant ($p< 0.05$) which is comparable with the previous study conducted in 3 diagnostic centers of developing countries (12).

Previous studies revealed that better yield of bacilli can be obtained using low relative centrifugal force (RCF) for sputum treated with NaOCl than that treated with other digestion methods (NaOH, dithiothreiotol) [12,15]. In this study, the overall result showed the equal effectiveness of either centrifugation or overnight sedimentation method since the difference in the proportion of positive results obtained by the two concentration techniques showed no statistical significance ($p>0.05$). This could make it highly applicable and effective in the diagnosis of TB in rural laboratories where there is shortage of centrifuge and electricity.

In the present study, NaOCl concentration techniques were not evaluated against the standard, culture method due to material and time constraints. However, the results of the previous study showed that the sensitivity of NaOCl concentration method was 69% where as that of direct method was 31% (12). This improved sensitivity of NaOCl method is believed to be due to the change in surface properties of mycobacteria and/or denaturation of sputum constituents leading to flocculation and subsequently

increasing the sedimentation rate of mycobacteria. Moreover, use of NaOCl concentration method minimizes the area of smear to be examined so that the time needed to examine one slide is lower than that of smear prepared by direct method. The importance of concentration method is emphasized, especially in overburdened TB laboratories where the technician can not endure to examine large number of smears prepared by direct method. Despite its importance the sample preparation is more laborious as compared to the direct method.

In conclusion, this study shows that the concentration techniques employing house hold bleach pre-treatment for examination of AFB can significantly improve the quality of TB diagnosis since it increases the number of smear positive cases. Moreover, the reagent in the pre-treatment procedure is relatively cheap and readily available in local markets as house bleach. Thus, use of NaOCl pre-treatment and centrifugation in laboratories with electric supply and centrifuges as well overnight sedimentation techniques in those laboratories without these facilities is recommended. Moreover, the NTCP should implement this newly modified laboratory technique for the fight against TB.

ACKNOWLEDGEMENT

This study was financially supported by the Research and Publications Office of Jimma Institute of Health Sciences.

REFERENCES

1. Ministry of Health. Manual of TB and Leprosy Control program, 1st edn, Addis Ababa, 1997.
2. Maher D, *et. al.* Treatment of tuberculosis: Guidelines for National Programs, 2nd edn, Global TB program, WHO, Geneva, 1997.
3. AHRTAG. TB and Children: The Missing Diagnosis. Child Health Dialogue, London, April-June 1996.
4. Maher D, Harries AD. TB/HIV: A Clinical Manual: Global TB Program, WHO, Geneva, 1996.
5. Wilson S. Molecular Methods in the detection of Mycobacteria, Biomedical Scientist, 1998, 42: 290-191.
6. Rauiglone MC, O'Brien RJ. Tuberculosis. In: Fauci AS (ed.), Harrison's Principles of Internal Medicine, 14th edition, McGraw-Hill Inc., New York, 1997.
7. McNerney R. Diagnosis: Present difficulties & prospects for the Future. African Health. 1996; 9: 22-23.
8. Dhir V, Stupple M. New Approach to Laboratory Diagnosis. African Health. 1997; 20: 18.
9. Styblo K. Tuberculosis and its Control: Lessons to be learned from past experience and implication for Leprosy Control Programs. Ethiop. Med. J. 1983; 21: 101-120.
10. Kochi A. Tuberculosis Control: is DOTS the health breakthrough of the 1990s? World Health Forum. 1997; 18: 225-232.
11. World Health Organization. Tuberculosis Program: framework for effective TB control, Geneva, 1994.
12. GebreN *et al.* Improved microscopical diagnosis of pulmonary tuberculosis in developing countries. Trans. R. Soc. Trop. Med. Hyg. 1995; 98:191-193.
13. World Health Organization. Manual of Basic techniques for a health laboratory, Geneva, 1980.
14. Ebersole L. Acid-fast stain procedure. In: Clinical Microbiology Procedure Handbook, Isenberg HD (ed), American Society for Microbiology, Washington, D.C. 1992.
15. Miorner H. *et. al.* Diagnosis of pulmonary TB. Lancet. 1994; 344:127.