The quality of sputum smear microscopy diagnosis of pulmonary tuberculosis in Dar es Salaam, Tanzania

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This study was carried out to determine the rate of agreement or disagreement of microscopy reading and culture positivity rate among smear positive and negative specimens between peripheral tuberculosis diagnostic centres (PDCs) and Central Reference Tuberculosis laboratory (CTRL). In this study 13 PDCs in Dar es Salaam, Tanzania were involved. Lot Quality Assurance Sampling (LQAS) method was used to collect 222 sputum smear slides. A total of 190 morning sputum specimens with corresponding slides were selected for culture. First readings were done by technicians at PDCs and thereafter selected slides and specimens were sent to CTRL for re-examination and culture. Culture results were used as a gold standard. Of 222 slides selected, 214 were suitable for re-examination. Percentage of agreement of smear reading between PDCs and CTRL was 42.9% and 100% for positive and negative slides, respectively. Measure of agreement (Kappa statistic) was 0.5, indicating moderate agreement. Of 190 samples cultured, percentage of agreement between smear reading from PDCs and CTRL was 37% and 88.9% for smear positive and negative slides, respectively. Kappa statistic was 0.3 indicating poor-fair agreements. Comparison of smear reading from PDCs with culture showed sensitivity of 36.9% and specificity of 88.9%. Comparison of smear readings from CTRL with culture results showed sensitivity of 95.6% and specificity of 98.6%. In conclusion there was inadequate performance in diagnosis of TB using smear microscopy among peripheral diagnostic centres in Dar es Salaam. This calls for immediate and rigorous measures to improve the quality of smear microscopy. It is therefore important to strengthen the capacity of laboratory personnel in smear microscopy techniques through supportive supervision and training.

Key words: tuberculosis, sputum, quality assurance, microscopy, culture, Tanzania

Introduction

Tuberculosis (TB) is a major public health problem worldwide with a third of the world's population infected with *Mycobacterium tuberculosis* and about 8.2 million new TB cases occurred in the year 2000. More than 1.8 million deaths occurred in the same year, and more than 95% of those were in developing countries (Corbett *et al.*, 2003; WHO, 2002). Following the HIV/AIDS epidemic in Tanzania, TB case detection has increased from 11,812 in 1983 to about 65,000 cases in 2004 (Range *et al.*, 2001; Ministry of Health unpubl).The situation is worse in urban areas especially in Dar-es-Salaam which for almost two decades has contributed to about 25% of cases notified in 2006 (National Tuberculosis and Leprosy Control Programme, unpubl). TB control in countries like Tanzania will be effective and efficient when cases are diagnosed and treated early and correctly.

Despite the high prevalence of tuberculosis and limited resources for diagnosis, sputum microscopy is the mainstay for the diagnosis of infectious cases. Microscopic errors are likely to misclassify or misdiagnose cases as non cases and the vice versa and therefore compromise the national efforts to control tuberculosis For instance, human error due to fatigue and demotivation induced by the lengthy and monotonous process of examining the smears as well as low remuneration are likely to affect the performance of laboratory personnel. This could lead to failure in detecting TB patients or unnecessary treating non cases. It could be presumed that many patients are misclassified due to insufficient time for laboratory technicians to examine properly the large number of sputum smear for presence of acid fast bacilli (AFB). Furthermore, many technicians in peripheral medical services may be lacking the knowledge, skills and experience for proper examination of sputum for AFB.

Data from TB/HIV study conducted between 1991 and 1993 showed discrepancy in smear microscopy results between peripheral and reference laboratories (Range *et al.*, 2001). Most of the new or relapse pulmonary smear positive TB cases had negative sputum smears when the reference laboratories re-examined the sputa. Conversely, a smaller but not inconsequential number of new pulmonary smear negative and extra pulmonary cases were found by the reference laboratory to have positive sputum smears

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(Range *et al*., 2001). "False negative" results could be serious and common type of misclassification. Proper diagnosis will ensure that no over diagnosis and that only those require treatment are the ones that receive it. So far, only a few studies have attempted to assess the quality of microscopy diagnosis of tuberculosis in Tanzania (Basra *et al*., 2006). It was, therefore, important to assess the quality of smear microscopy diagnosis, especially, in areas of high incidence of Tuberculosis looking at both the technical and laboratory operation quality in selected health facilities in the City of Dar es Salaam. Objectives of the study were to determine the rate of agreement and disagreement of smear microscopy reading as well as to determine culture positivity rate among smear positive and negative specimens from peripheral diagnostic centres and CTRL.

Materials and Methods

Settings and design

This cross sectional study involved thirteen peripheral diagnostic centres in Dar es Salaam and was conducted during March 2005. A list of all government health facilities with TB diagnostic facilities in Dar es Salaam was made. From that list three health facilities were selected randomly in each Municipality to make a total of 9 health facilities. Another two facilities were selected randomly from a list of all private heath facilities which provide TB diagnosis services. Muhimbili National hospitals and IDC were selected purposely.

The involved peripheral diagnostic centres (PDCs) included the Muhimbili National Hospital, Amana, Mwananyamala and Temeke Hospitals; Magomeni, Bandari, Tandale, Ebrahim Haji and Mnazi Mmoja Health Centres; and Tambuka Reli, Vituka and Vingunguti dispensaries.

In each diagnostic centre all smear slides were stored. Slides were selected according to the WHO guidelines for external quality assessment for AFB smear microscopy (WHO, 2003). Number of slides required for the study was calculated using annual volume of work for each diagnostic centre, positivity rate and expected performance (sensitivity) compared to the controller of 80% set by the National Tuberculosis Leprosy Programme of Tanzania. A minimum sample size for culture specimens was calculated by using Epi-info version 6 programme. A CTRL culture positivity rate of 52% was taken into consideration.

CTRL is the type one TB laboratory, and apart from other functions, CTRL supervises, evaluates and provides a programme of quality assurance for the diagnostic services of the National Tuberculosis Control Programme. CTRL also performs direct smear microscopy and culture for its catchments area as well as collect and help evaluate the laboratory data obtained throughout the country and participate in epidemiological research on tuberculosis. In this study CTRL was used for quality assurance purposes.

Collection of slides and specimen for culture

Laboratory technicians from each diagnostic centre were requested to keep all smear slides from all TB suspects from the routine samples for a period not exceeding four days. An instructional manual for preserving slides was developed and distributed to the study laboratories. Collection of slides and specimens were done after every two days. The technician responsible for collection of slides was neither responsible for the reading of slides nor collection of peripheral results. Slides were randomly selected by an independent technician from CTRL from a list of all slides obtained. A sample of slides was re-examined directly and results were compared to that of peripheral diagnostic centres.

Specimens for culture were obtained from a list of all morning specimens from each diagnostic centre. This list was made on a second visit and random sampling was done at CTRL to get specimens for culture. Collection of sputum samples for culture with its smeared slides were then done on the following visit. Before being transferred to CTRL, each specimen was labelled with an identification (ID) number corresponding to the ID on the study form. The lists of all selected slides were made by CTRL technicians in a separate special study forms. The results of slides reading from PDCs were not shown to CTRL technicians. At CTRL, all slides were read blindly by two technicians, and slides with discrepant results were re-read for the third time by another technician. Results of PDCs were compared with that of CTRL by the study scientists. Culture results were compared with smear results from CTRL and results from PDCs.

Data analysis

Epi Info version 6 was used for data entry and analysis. Culture positivity rate among smear positive and negative results from PDCs and CTRL was calculated and factors associated with discrepant results were

noted. The CTRL culture results were used as a gold standard. The percentage of false positive and negative were calculated. Kappa (κ) statistic was used to calculate the rates of agreement, disagreement and reproducibility of microscopy reading results of PDCs and CTRL. The inter-observer variability was assessed on the basis of κ-values of <0.40, 0.40-0.60, 0.61-0.80 and >0.80 indicating respectively, poor-fair, moderate, substantial and almost perfect agreement between assessors.

Results

Results were available for 187 out of the 190 slides from specimens brought for culture. Three slides brought to CTRL were not suitable for re-examination and therefore were discarded. Percentage of agreement for smear positive and negative results between PDCs and CTRL was 35.6% and 88.7%, respectively. Measure of agreement, κ-statistic was 0.3 indicating poor-fair agreements (Table 1). The comparison of smear results between PDCs and CTRL show sensitivity and specificity of 35.6% and 88.7%, respectively.

Percentage of agreement of culture results and smear results from PDCs was 51.5% and 81.5% for positive and negative slides, respectively. Kappa value was 0.3 which indicate poor-fair agreement (Table 2). The comparison of smear and culture results between PDCs'and CTRL showed sensitivity of 36.9% and specificity of 88.9%. Percentage of agreement of smear results from CTRL and culture was 95.6% for positive slides and 98.6% for negative slides. The κ-statistic was 0.9 indicating perfect agreement (Table 2). Culture positivity rate among smear positive slides were 95.6% for CTRL and51.5% for PDCs. Culture positivity rate among smear negative results were 1.4% and 18.5% for CTRL and PDCs, respectively.

Of 222 smeared slides collected, results were available for only 214 slides. Percentage of agreement of smears results between PDCs and CTRL was 42.9% for positive slides and 100% for negative slides. Kappa statistic was 0.5 indicating moderate agreement (Table 3).

Table 1: Comparison of smear results between peripheral diagnostic centres and CTRL

Smear results PDCs	Smear results CTRL		
	Positive	Negative	Total
Positive	16 (35.6%)	16 (11.3%)	32 (17.1%)
Negative	29 (64.4%)	126 (88.7%)	155 (82.9%)
Total	45 (100%)	142 (100%0	187 (100%)

Table 2: Comparison of smear and culture results between peripheral diagnostic centres (PDCs) and Central Tuberculosis Reference Laboratory (CTRL)

Smear Result PDCs	Culture Positive	Culture negative	Total
Positive	17 (51.5%)	16 (48.5%)	33 (100%)
Negative	29 (18.5%)	128 (81.5%)	157 (100%)
Total	46(24.2%)	144(75.8%)	190
CTRL			
Positive	43 (95.6%)	2 (4.4%)	45 (100%)
Negative	2 (1.4%)	140 (98.6%)	142 (100%)
Total	45 (24.1%)	142 (75.9%)	187

Table 3: Comparison of smear results between peripheral diagnostic centres (PDCs) and Central Tuberculosis Reference Laboratory (CTRL)

	CTRL			Total
		Smear Positive	Smear Negative	
PDCs	Smear Positive	18 (42.9%)	0	18 (8.4%)
	Smear Negative	24 (57.1%)	172 (100%)	196(91.6%)
	Total	42 (100%)	172 (100%)	214 (100%)

Discussion

Sputum smear microscopy for acid fast bacilli is the widely available, easily accessible, rapid and affordable method for diagnosis of pulmonary tuberculosis. The quality of tuberculosis laboratory services therefore, has major influence for both patients and the monitoring and evaluation of the National Tuberculosis Control programme.In this study, the percentages of agreement in both smear results and culture results between CTRL and PDCs showed a low kappa statistic indicating poor-fair agreement. On other hand, comparison of CTRL smear results to culture results showed high and acceptable percentage of agreement. Moreover, culture positivity rate was lower for smear positive slides and higher for smear negative slides from PDCs than for smear results from CTRL.

The most important element in the diagnostic test is the accuracy of the test in terms of specificity and sensitivity. The results of the sensitivity and specificity of smear and culture results between PDCs and CTRL differ from results of a previous study in Dar es Salaam which showed an overall sensitivity and specificity of 88.5% and 100%, respectively (Basra *et al*., 2006). This indicates that there are variations in quality of smear microscopy between tuberculosis laboratories in Dar es Salaam and most likely throughout the country. This is probably due to overwhelming burden of tuberculosis coupled with insufficient staff.

The false smear-negative and smear-positive rates observed in this study are higher than that reported from a similar study in Malawi (Mundy, 2002) where false positive and negative rates for AFB microscopy were less than 2%. Our results indicate that the PDCs are increasingly making incorrect diagnoses. The overall results showed that about one fifth (29/157) of patients were misdiagnosed as non cases and therefore not treated. This under reading has also been reported in other several studies. While a study in Tanzania showed a false negative of 24% that of Kenya showed false negative of about 25% (Basra *et al*., 2006; Hawken *et al*., 2001).

Misdiagnosis contributes to low coverage for early treatment, suffering and ultimate death. This could be one of the reasons for increased transmission of the disease in Dar es Salaam (Eldholm *et al*., 2006). Moreover, false negative exaggerate a true magnitude of smear negative tuberculosis which is said to be fuelled by HIV/AIDS.

In conclusion, laboratories at peripheral diagnostic centres in Dar es Salaam show inadequate performance in diagnosis of TB using smear microscopy. It is obvious that rigorous measures to improve the quality of smear microscopy diagnosis are urgently required.

Acknowledgements

We are grateful to Mrs Levina Lema and Dr. Mbazi Senkoro for their critical review of the early version of the manuscript. We thank the Regional Tuberculosis and Leprosy Coordinators for Temeke, Mwananyamala and Ilala for their support. We are grateful to all district tuberculosis and leprosy coordinators and laboratory personnel from the 13 PDCs for their enthusiasm and technical support. This study received financial assistance from the Ministry of Health and Social Welfare, Tanzania.

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