Improved microscopy diagnosis of pulmonary tuberculosis using sodium hypochlorite concentration technique in Tanga, Tanzania

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Abstract: Pulmonary tuberculosis diagnosis commonly relies on the bacteriological examination of sputum. A cross-sectional hospital-based study was carried out to compare on "on the spot" sputum staining using sodium hypochlorite (bleach method) and routine Ziel-Neelsen (ZN) staining technique. Study candidates included individual patients presenting with cough <3 weeks (Group I) and ≥3 weeks (Group II). Sensitivity and specificity of the bleach method was calculated and compared at 100% using the ZN staining technique as the standard. A total of 171 patients (94 males, 77 females) with mean age 34.9 years (SD ±12.9) were recruited. Fifty-eight patients had coughed for <3 weeks while 113 had coughed for ≥3 weeks. Smear-positive TB in Group I was 13.8% (95% CI = 5-23) while in Group II was 25.7% (95% CI = 21-29). Using the bleach method, the prevalence of smear-positive TB in Group II was 28.3% (CI 95% = 20-36). This was an increase in smear-positivity rate of 15.6% as compared to the ZN technique. These results suggest that the use of bleach technique "on the spot" improve the sensitivity of tuberculosis diagnosis among patients with a history of coughing of over three weeks. However, further studies in different settings are recommended to validate the technique.

Key words: pulmonary tuberculosis, diagnosis, bleach, hypochlorite, Ziel-Neelsen, Tanzania

Introduction

Tuberculosis (TB) is a growing threat and a global health burden especially in developing world. The disease was declared a global emergency by World Health Organization since 1993. In 2003, 8.8 million new cases of TB were reported worldwide with 1.7 million deaths. Eight per cent of the new cases and 13.5% of the deaths were among individuals infected with HIV (WHO, 2006). In majority of developing countries, TB case-finding depends on self-referred of symptomatic individuals who attend health facilities and are then subsequently diagnosed as suffering from tuberculosis (L'Herminez, 1993). Sputum microscopy is the mainstay technique for the diagnosis of pulmonary tuberculosis as it is the most reliable, specific and objective method available especially in developing world. The current strategy for diagnosing pulmonary TB is to screen sputum for *Mycobacterium tuberculosis* of patients presenting with history of a cough for more than two weeks. However, many settings including Tanzania have not been able to put this new strategy fully function in their routine services. The use of the old strategy of screening sputum of patients presenting with history of cough for more than 3 weeks in still common among a number of practitioners in Tanzania. The cut off point of more than three weeks is too long and likely to contribute to patients to seek treatment outside the conventional healthcare facility. Moreover, this cut-off point leaves a proportion of patients unattended at earlier stage of the disease. For instance, a hospital-based study in Malawi has shown that 10% of patients with a history of a cough for less than three weeks had smear positive and culture positive TB compared to 22% who had a cough for more than three weeks (Banda *et al*, 1998). This suggests that, the current cut off point of a cough for more than three weeks to start screening for TB seem to be unrealistic.

The number of patients being diagnosed as having smear negative pulmonary tuberculosis has been increasing (Parry *et al*., 1995). The increase is due to several factors including the difficulty of patients to provide adequate early morning sputum specimens, quality of smear, time spent in examining the slide and co-infection with HIV / AIDS (Mendelson, 2007).

Tuberculosis diagnosis based on microscopy of smears made directly from sputum has a low sensitivity especially when performed in overburdened control programmes (Aber *et al*., 1980). Studies have already shown that liquefaction of sputum with sodium hypochlorite (NaOCl) and concentration of bacilli through

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centrifugation increases the sensitivity of direct microscopy (Corper & Nelson, 1949; Gebre *et al*., 1995; Miörner *et al*., 1996). Using this method, the case detection rate may be increased by more than 100% (Gebre *et al*., 1995). It has been observed that, the sensitivity of acid-fast smears is directly related to the relative centrifugal force achieved while concentrating the specimen (Rickman *et al*., 1980).

The use of sodium hypochlorite is ideal since it is cheap and available as household bleach, and also inactivates HIV and *M. tuberculosis* which could reduce the rate of nosocomial infections in laboratory workers (Rattan *et al*., 1994). However the bleach method is not widely used because sample preparation is laborious and requires access to centrifuge (Gebre *et al*., 1995). On the other hand, when sputum induction was carried out, there was an increased smear positive TB rates by 20% (Parry *et al*., 1995). In this study, we evaluated an improved method for sputum microscopy, which is based on liquefaction of the sample with household bleach, followed by its concentration by centrifugation prior to staining and microscopy. The bleach microscopy method was compared to standard direct microscopy.

Materials and Methods

Study population and design

The study was conducted at Bombo Regional Hospital in Tanga, north-eastern Tanzania between April and May 2004. The outpatient department (OPD) of the hospital serves 40-80 patients a day of which 8-14 are TB suspects. The annual number of TB notification for the entire region is about 3852 (MoH, 2005).

Study subjects were patients with cough attending the OPD. Patients were recruited in two groups: Group I consisted of patients referred to the investigator with a cough for less than 3 weeks from the OPD. This group submitted an "on the spot" sputum to be stained using the bleach method. Group II, consisted of screening consecutive TB suspects with a cough for ≥3 weeks using the bleach method and standard Ziel-Neelsen (ZN) method. Patients who qualified for inclusion criteria were examined and had their blood pressures, heart rates, respiratory rates, heart sounds, heights and body weights recorded.

Inclusion criteria were self referred adult patients aged 15 years and above attending Bombo Regional Hospital from 8:00-13:00hr between Monday and Friday. Patients recruited were those with a history of cough for less than three weeks and more than three weeks. Exclusion was made for patients aged less than 15 years or those who had received anti-tuberculosis chemotherapy within period preceding three months to the commencement of the study.

The sample size was calculated by Epi Info 6 on the assumption that the prevalence of smear positive TB in cough <3 weeks is 10% a sample size of 94 patients to give a 95% CI of ±7% was required. The prevalence of smear-positive TB in cough ≥3 weeks was assumed to be 22%; thus a sample size of 136 patients to give a 95% CI of ± 5% was required.

Sputum submission and processing

Patients were asked to produce an "on spot" sputum specimen in a 20ml screw plastic container (Cheesbrough, 1985) under the supervision of a trained nurse in the open air. An explanation on how to produce a good quality sputum sample with sufficient volume of sputum was given before collecting the sample. If a sputum sample was not produced within 15 minutes the patient was excluded from the study and was asked to consult the clinical officer for further management. Those produced a sputum sample were asked to return the following day for their results. Those who did not return were recorded as defaulters.

One smear from an "on the spot" specimen was prepared directly from the sputum; air dried and was stained with the routine ZN method. The rest of the sputum was treated with household bleach (Gebre *et al*., 1995; Rickman & Moyer, 1980). 2ml of sputum was mixed with equal volume of 3-5% sodium hypochlorite in a plastic falcon tube of 12ml. The sample was left to incubate at room temperature and shaken intermittently for 15min. before the addition of 6ml of distilled water to top it up to 10ml. The tube was then covered with parafilm paper before it was centrifuged at 3000 x g for 15 min (Cheesbrough, 1985; Gebre *et al*., 1995). The supernatant was removed and the pellet re-suspended in a few drops of the remaining fluid. A smear was prepared from the suspension and air-dried. A similar procedure was used to prepare sample from patients with cough for less than three weeks**.** All ZN stained

smears were examined semi-quantitatively using bright field microscopy at 1000x magnification.

Data analysis

Data was entered twice and cleaned using Epi-info version 6 (CDC, Atlanta, GA, USA). Statistical analysis was carried out using Chi-squired test with Yates correction and student *t*-test for normally distributed data and non-parametric tests for data not normally distributed.

Ethical consideration

This study was approved by the Ethical Committee of the National Institute for Medical Research, Tanzania. Patients were asked whether they wish to participate in this study before enrolment and an oral informed consent was obtained from each participant. Patients who had cough for > 3 weeks with no acid fast bacilli (AFB) in their smear were treated with Cotrimoxazole for ten days and asked to return back. If after ten days they were still coughing, they were sent to the clinical officer for routine screening procedures. Those patients identified to have positive smear were directly sent to the TB coordinator for appropriate anti-TB chemotherapy. However, those patients with cough for three or more weeks found to have no AFB in their smear were sent to a clinic for further investigations according to the national TB control programme policy.

Results

Smear positive was defined as more than 10 acid-fast bacilli (AFB) in 100 fields in more than two "on spot" smears. Smear negative was defined as those with no AFB in 100 fields in all three smears. On the other hand, indeterminate was defined as 1-9 AFBs in 100 fields in 1 smear.

A total 175 patients were recruited, however, only 171 (97.7%) were covered in this analysis. Of these, 58 (33.1%) were included in Group I and 117 in Group II. Four patients in Group II did not complete the study and therefore were not included in the analysis. The mean age of the patients was 34.9 years and this included 34.0 years in Group I and 35.3 years in Group II. Sixty-seven (59.3%) of the patients were illiterate. Males and females accounted for 55% and 45% of the patients, respectively. Only 49.5% of the patients had formal occupation.

Using ZT technique, tuberculosis was identified in 13.8% and 23.8% of the patients with history of cough for <3 and ≥3, respectively (Table 1). When using a bleach technique, tuberculosis was diagnosed in 28.3% of the patients with history of ≥3week cough.

In Group II, 110 (97.3%) patients submitted the "on the spot" sputum specimens, 3 (2.6%) patients were "unable". A total of 109 (93.2%) patients had submitted the early morning sputum specimen while 106 (90.6) produced the "on the spot" sputum specimen. Twenty-seven (23.8%) patients were smear-positive by the three smear case-finding strategy, while 32 (28.3%) were smear-positive by the bleach method in Group II patients. Those with negative smear in Group II were 81 (71.7%). In Group I, 8 (13.8%) patients were considered to be smear positive after been independently assessed by the technician using a traditional Ziel-Neelsen staining technique. On the other hand 50 (86.2%) were negative.

The significant risk factors associated with smear-positive identified in this study were weight loss, haemoptysis, body mass index (BMI) and increased respiratory rate (Table 2). However, low BMI, gender, age and having received treatment for cough previously, and increased pulse rate were not statistically associated with smear-positivity. Although cough was the leading symptom in both groups, increased respiratory rate was almost similar in the two groups. However, majority of patients in Group II had more days (46.02 days) of cough than those in Group I (12.34 days).

Table 1: Tuberculosis cases detected in patients with cough for <3 and ≥3 weeks

Duration of cough	Positive	Negative	Total	95% CI
Cough <3 weeks	8 (13.8%)	50(86.2%)	58	5-23
Cough ≥3 weeks	27 (23.9%)	86 (76.1%)	113	13-28
Cough ≥3 weeks by bleach single smear	32(28.3%)	81(71.7%)	113	20-36

Table 2: Risk factors associated with smear positive when considering bleach method alone

Variable		Smear positive		Smear negative		*P*-value	Odds ratio (level of significance)
		N	%		%		
Sex	Female	14	40.0	63	49.6		
	Male	24	60.0	64	50.4		
Mean age		31	12.04	35	13.2	0.7	
Body mass index		17.12	2.4	19.19	2.7	0.0003	
Weight loss	Yes	35	87.5	67	53.0	0.0004	
	No	5	12.5	60	47.2		
Haemoptysis	Yes	15	37.5	21	16.5	0.003	
	No	25	62.5	106	83.5		
Cough	Yes	16	40.0	43	33.9		1.3 (0.58-2.9)
	No	24	60.0	84	66.1		
Chest pain	Yes	14	35.0	58	45.7		1.04 (0.28-1.43)
	No	26	65.0	69	54.3		
Cough treatment	Yes	29	72.5	98	77.2		1.47 (0.67-3.26)
	No	11	27.5	29	22.8		
Mean respiratory rate		24		21		0.0001	

The characteristics of the patients are summarised in Table 3. The number of patients who had attended elsewhere was 78 of which 3 were in Group I and 75 were in Group II. The TB smear positive cases detected in group II patient by a single bleach smear was 32 (28.3%) with (95% CI =20-36) as compared to those in Group II 27 (23.8%) with (95% CI= 13-28).

The mean body weight and height of the patients was 47.6kg (SD=9.2) and 1.595m, respectively and was similar in both groups. Mean respiratory rate and heart rate were 22.129 and 79.848 per minute, respectively. More patients in Group II were found with enlargement of lymph node, skin lesions and oral thrush (Table 4).

Table 3: Subjective characteristics of the study population shown as number (%) of patients

Symptoms	Cough <3 weeks	Cough >3 weeks	Total
Cough	21 (36.2%)	39 (33.6%)	60 (34.5%)
Attend elsewhere	3 (5.2%)	75 (66.4%)	78 (69%)
Weight loss	22 (38%)	84 (74.3%)	106 (62%)
Haemoptysis	12 (20.7%)	24 (41.4%)	36 (21.1%)
Fever	49 (84.5%)	80 (71.8%)	129 (75.4%)
Chest pain	17 (29.3%)	57 (50.4%)	74 (43.3%)
Night sweats	16 (27.6%)	56 (49.6%)	72 (42.1%)
Short of breath	28 (48.3%)	70 (62%)	98 (57.3%)
TB contact	10 (17.2%)	18 (16%)	28 (16.4%)

Table 4: Objective characteristics of the study population as shown by number of patients (%)

Character	n=58	N=113	N=171
	Cough <3 weeks	Cough > 3 weeks	Total
Oral thrush	4 (6.9%)	22 (19.5%)	26 (15.2%)
Skin lesions	5 (8.6%)	19 (16.8%)	24 (14.0%)
Lymph node enlargement	9 (15.5%)	48 (42.5%)	57 (50.4%)
X-ray done	7 (12.1%)	26 (23.0%)	33 (19.3%)

When comparing a single bleach smear microscopy with three standards ZN smear in Group II patients, bleach smear positive were 26 (81.3%) and indeterminate 6 (18.8%). The bleach smear negative was 100% (77), indeterminate smear positive 1 (25.0%) and smear negative 2 (50.0%). The sensitivity and specificity were 100% [95% CI=100%], Positive Predictive Value (PPV) was 88.9% (95% CI 79%-99%) and Negative Predictive Value (NPV) was 95.1% (95% CI 91-100%). The bleach result positive smears were 29 (100.0%), while negative smears were 3 (3.6%). Both the sensitivity and specificity were 100%. PPV was 90.6% (95% CI 81%-100%) while NPV was 100% (95% CI 94%-98%).

Discussion

The prevalence of smear-positive TB in patients with a cough of less than three weeks was similar to that reported in Malawi (Harries *et al*., 1997). The proportion of smear-positive disease detected in this study was found in the cough of less than three weeks that is not normally considered as a TB suspect group. These patients are infectious TB patients who would not have routinely been detected. It is not known whether these smear-positive cases would have returned to health care facilities later on. All the smear-positive TB patients detected in the short duration group by a single bleached smear were also found to be independently positive by a single ZN smear. Facilities for Mycobacterium culture as in many developing world are not available, and therefore cultures are not routinely performed. However, a previous study (Banda *et al*, 1998) has shown that, 35% smear-positive patients with a cough for less than three weeks had pulmonary tuberculosis. Although cultures are not routinely carried out in majority of the developing countries, when performed over 99% of the smear positive cases turn to be culture positive.

Direct smear microscopy does not discriminate between tubercle bacilli and other mycobacteria, however this is not a major problem in developing world because majority of patients with AFB in sputum smears have TB as atypical mycobacteria does not present in sufficient concentration in sputum to be detected by direct microscopy (Lipsky *et al.*, 1984). It is also possible that the direct smear microscopy may be a false positive result because of contamination by environmental bacteria or cross-contamination.

In this study, the short duration group did not statistically differ from the long duration group with respect to age, sex and previous TB contact. On the other hand having less period of illness, less visits elsewhere; less being treated for cough previously correlated with cough for <3 weeks than the long duration cough. This would appear to support a shorter illness period and subjectively verify the history of a cough for less than three weeks.

Many patients with long duration cough failed to submit all three sputum samples. This led to 12.4% patients being classified as indeterminate. However with the bleach method only a few (3.5%) were classified as indeterminate using the International Union Against Tuberculosis and Lung Diseases definition for smear-positivity and negativity. The bleach method increased smear-positivity by 13% with >100% sensitivity and specificity. Previous studies found increased smear-positivity by 14% with 100% sensitivity and specificity (Allwood *et al.*, 1997). Possibly it is in this group where a high number of patients presented with oral thrush were found. In most cases oral thrush is associated with immune suppression demonstrating some of these patients could have contacted HIV infection.

The traditional approach of waiting for three sputum smear samples before any of them is examined leads to delay in diagnosis and treatment. Previous studies have shown

an increased risk of nosocomial transmission of tuberculosis in hospital workers (Harries *et al*, 1997, 1998) due to the current procedure of waiting for three sputum smears. The findings of this study suggest that, bleach method could widely be applied as routine procedure in all laboratories that perform direct microscopy only and its use would definitely lower the risk of laboratory infection since sodium hypochlorite is a potent disinfectant and accelerate patient prompt treatment. Moreover the rapid identification of smear-positive TB has the potential to increase patient access to diagnosis and treatment and significantly improve the efficiency of passive case finding both in economic and human terms. It is obvious that patients with a short history of cough would benefit from pulmonary tuberculosis screening strategies with sputum examination at an early stage of less than the common three week period. Moreover, with the high sensitivity and specificity of the bleached sputum microscopy method, it is time to revisit the policy regarding tuberculosis screening in Tanzania.

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