Full Length Research Paper

# Prevalence and diagnostic aspects of sputum smear positive tuberculosis cases at a tertiary care institution in Rwanda

Claude Mambo Muvunyi<sup>1, 3\*</sup>, Florence Masaisa<sup>1, 3</sup>, Claude Bayingana<sup>1</sup>, Andre Musemakweri<sup>2</sup>, Leon Mutesa<sup>1</sup> and Teresa Carbonell Hernandez<sup>1, 4</sup>

<sup>1</sup>Department of Clinical Biology, Centre Hospitalier Universitaire-Butare, National University of Rwanda, Butare, Rwanda.

<sup>2</sup>Department of Internal Medicine, Centre Hospitalier Universitaire-Butare, National University of Rwanda, Butare, Rwanda.

<sup>3</sup>Department of Clinical Chemistry, Microbiology and Immunology, Ghent University Hospital, Belgium. <sup>4</sup>Department of Microbiology, Teaching Hospital "J. M. Aristegui", Matanzas, Cuba.

Accepted 12 October, 2015

Approximately one third of the world's population is infected with Mycobacterium tuberculosis and 9.27 million new cases of TB occurred in 2007. Developing countries disproportionately shoulder the global burden of disease with the highest estimated rates in the world, with an estimated 55% of global cases in Asia and 31% in the African region. The incidence of new sputum smear positive in Rwanda through recent national survey was an estimated 162 per 100 000 population. The aim of our study was to evaluate the prevalence of smear positive pulmonary TB among patients at the University of Butare Teaching Hospital, a tertiary health facility in South province, Rwanda. In addition, some aspects of the performance of the pulmonary TB diagnosis are discussed. The overall prevalence of sputum smear positive cases were 17.3% (63 0f 364) and most of the positive patients were within the age range 15 - 44 years. The highest percentage of TB was seen in the age group of 15 - 24 years compared with the lowest percentages in the age group below 14 years and above 45 years. A total of 63 (17.3%) suspects were found to have at least one positive. Of these, 56 (88.9% of those with one or more positive smears and 92% of those who fulfilled the case definition) were detected from the first specimen and 7 (11.1%) were positive on the second specimen but not the first. The third specimen did not have any additional diagnostic value for the detection of AFB. The prevalence of sputum smear positive cases of 17.3% increases with age up to the age 44 years. Our result show that examining two sputa smears was sufficient for the detection of AFB in our laboratory. Further research involving different laboratories from all of the regions of Rwanda is needed to reassess these findings.

Key words: Smear positive tuberculosis, prevalence, diagnostic aspects, Rwanda

## INTRODUCTION

Although a cure of Tuberculosis (TB) became available more than many years ago, TB still remains a major public health concern in both developed and developing countries. Approximately one third of the world's population is infected with *Mycobacterium tuberculosis*. Developing countries disproportionately shoulder the global burden of disease with the highest estimated rates in the world, with an estimated 55% of global cases in Asia and 31% in the African region. For example, in Rwanda, through recent national survey, incidence of new sputum smear positive was an estimated 162 per 100 000 population (Dye et al., 1999).

Tuberculosis bacteriology is one of the fundamental aspects of a national tuberculosis control programme and a key component of the DOTS strategy, yet the tuberculosis laboratory service is often the most neglected component of these programmes (WHO, 1998). Despite

<sup>\*</sup>Corresponding author. E-mail: cmuvunyi@nur.ac.rw. Tel: +250 0788493814. Fax: +250 530 328.

Table 1. Age and sex distribution of study patients.

Age group (years)	Male (%)	Female (%)	Total (%)
0 - 14	28 (7.7)	20 (5.5)	48 (13.2)
15 - 24	46 (12.6)	51 (14.0)	97 (26.6)
25 - 34	52 (14.3)	27 (7.4)	79 (21.7)
35 - 44	30 (8.2)	23 (6.3)	53 (14.6)
45 - 54	22 (6.0)	19 (5.2)	41 (11.3)
55	29 (8.0)	17 (4.7)	46 (12.6)
Total	207 (56.9)	157 (43.1)	364 (100)

recent advances in mycobacteriology, most developing countries still rely on the sputum examination in making a diagnosis of pulmonary TB and in monitoring of patients' progress under antiTB treatment (Rieder et al., 2007). The use of smear microscopy in patients suspected of tuberculosis presenting to health services is of great value in case detection and in reducing the spread of the infection throughout the population by treatment of such cases. Expansion and enhancement of DOTS have been implemented in Rwanda by the health ministry's national integrated programme to combat leprosy and TB since 1990. In Rwanda, the percentage of incident smearpositive cases being notified was only 24% (that is, 24%) case detection rate) in 2005 (WHO, 2005). Concerted efforts are being made to ensure that effective smear microscopy and DOTS are available nationwide. To our knowledge, few reports exist on the prevalence of smear positive pulmonary TB in Rwanda.

Therefore, the aim of our study was to evaluate the prevalence of smear positive pulmonary TB among patients at the University of Butare Teaching Hospital, a tertiary health facility in South province, Rwanda. In addition, some aspects of the performance of the pulmonary TB diagnosis are discussed

#### MATERIALS AND METHODS

This retrospective study was conducted at the department of microbiology, University of Butare Teaching Hospital after obtaining clearance from the ethical committee. This is a largest 418- bed tertiary-care, teaching hospital serving as a reference center for respiratory diseases located in the south province, Rwanda. The hospital averages 7,595 Patient admissions and almost 33,304 outpatient clinic and emergency room visits annually. In this study, using TB register and laboratory sputum register, we review laboratory records of four hundred and forty six patients who were TB suspect during the period from January 2007 to December 2008. Information regarding demographic data e.g. age, sex and sputum smear results were recorded using a standardized data collection form.

The data were independently entered by two persons and then compared. In cases of discordances, the physical register was checked and corrections made where necessary to produce a finalized data set. All patients having symptoms suggestive of pulmonary tuberculosis (cough for >3 weeks, chest pain, low-grade fever, night sweats) are routinely instructed to submit three early

morning sputum samples for the detection of acid-fast bacilli (AFB) and Ziehl -Neelson or Kinyoun technique was performed on each specimen in compliance with standard bacteriological procedure according to WHO guidelines. Laboratory technician's process three sputum samples for all suspects irrespective of the number of positive smears, as only the physician is allowed to make the decision to start treatment.

In Rwanda, two types of staining are used in the laboratory for AFB: Kinyoun stain had been used until May 2008 before the implementation of Ziehl-Neelson stain in all Rwandan laboratories. Patients are considered smear positive if they have at least two smear -positive specimens. Technicians keep all smear positive slides and an equal number of smear negatives for quality control. Patients who contributed fewer than three sputum AFB specimens were excluded from the analysis. Eighty two files did not satisfy entry criteria due to incomplete information. Data processing and statistical analysis were performed using SPSS software (Windows version 16.0). The results were expressed as percentage, with significance at 5%.

#### RESULTS

A retrospective study of 364 suspects fulfilling the inclusion criteria were studied over the 2-years period for AFB; made up of 207 (56.9%) male and 157 (43.1%) females.the age ranges was 5 - 80 years with a mean of 32.3 years (Table 1) . The overall prevalence of sputum smear positive cases were 17.3% (63 0f 364) and most of the positive patients were within the age range 15 - 44 years. The highest percentage of TB was seen in the age group of 15 - 24 years compared with the lowest percentages in the age group below 14 years and above 45 years (Table 2).

The prevalence rates of smear-positive pulmonary TB for 2007 and 2008 were 17.9% and 16.7% respectively (Table 3). A total of 63 (17.3%) suspects were found to have at least one positive smear and 61 (17%) fulfilled the case definition (at least two positive smears). Of these, 56 (88.9% of those with one or more positive smears and 92% of those who fulfilled the case definition) were detected from the first specimen and 7 (11.1%) were positive on the second specimen but not the first. The third specimen did not have any additional diagnostic value for the detection of AFB as shown in Table 3.

## DISCUSSION

TB is a global issue and is a great concern in Rwanda. The main requirement for TB control is the rapid and accurate identification of infected individuals (Garg et al., 2003). For the detection of TB, microscopy examination of sputum still remains the first step and is an insensitive tool in providing quick information to the clinician in this setting (Leonard et al., 2005; Van Deun et al., 2002). There have been few reports of the prevalence of sputum smear -positive pulmonary TB in Rwanda. The present study, which employed a retrospective laboratory-based study, provided unique information on the prevalence of TB in suspect patients in the developing community.

Table 2. Age distributio	n of sputum smear results.
--------------------------	----------------------------

Age group (Years)	Number tested	Sputum positive (%)	Sputum negative (%)
0 - 14	48	2(0.5)	46 (12.6)
15 - 24	97	29 (8.0)	68 (18.7)
25 - 34	79	20 (5.5)	59 (16.2)
35 - 44	53	8(2.2)	45 (12.4)
45 - 54	41	2(0.5)	39 (10.7)
55	46	2(0.5)	44 (12.1)
Total	364	63(17.3)	301 (82.7)

Table 3. Sputum smear results by timing of sample.

	At least two	At least one positive,	PXX N	NPX N	NNP <sub>*</sub>	Total with three
Year	positive, N (%)	N (%)	(%)	(%)	N (%)	results
2007	31 (16.8)	33 (17.9)	31 (93.9)	2 (6.1)	-	184
2008	30 (16.7)	30 (16.7)	25 (83.3)	5 (16.7)	-	180
All	61 (17)	63 (17.3)	56 (88,9)	7 (11,1)	-	364

PXX, first sputum positive irrespective of the second and third sputum; NPX, first sputum negative, second positive irrespective of the third sputum; NNP, first and second sputum negative and third sputum positive <sup>\*</sup> Percentage positive from "at least one positive"

In the present study, our finding with the overall prevalence of sputum smear-positive of 17.3% is in agreement with some studies that showed different rates ranging from 12 to 20% (Harries et al., 2000; Katamba et al., 2007). However, higher rates of sputum smear-positive cases have been reported in some studies carried out in developing countries. In studies from Ibanda, Nigeria, Onadeko et al. (1975), Kolawole et al. (1975) found high percentage of positive smear cases in 30 and 57% respectively. High rates of positive smear cases have been also reported by other many authors in their studies carried out in African regions (Bruchfeld et al., 2002; Habeenzu et al., 2007). These variations in the rate could be related to systematic differences in the various population samples. One of the reasons could be the advent of HIV/AIDS which has been shown to be associated with the increase of the prevalence of pulmonary tuberculosis in some countries with high incidence of HIV infection. Although our findings could be explained by a low prevalence of HIV in our country, there is no evidence to support this assertion as our patients were not tested for HIV infection as part of their management.

In this study, there was a high rate of smear positive cases in the 15 to 44-year-old group. Previous studies have also reported the highest rate of smear positive tuberculosis cases in this age group (Holmes et al., 1998). This might be as a result of the fact that individuals of this age in their life tend to be more active and are more likely to interact with other people than the elderly and the very young.

In the present study, we tried to assess the contribution of each sputum specimen collected for the detection of

AFB by microscopy in our laboratory. *M. tuberculosis* was detected from the first smear specimen with relatively high rate as shown in Table 3, with a small proportion being detected from the second specimen. It is clear that for the overwhelming majorities (100%) of patients from whom three smear specimen were assessed, the first and the second specimen were proved to be diagnostic. The third specimen was of no value for microscopic examination in smear-negative patients. The analysis of our records shows that under routine conditions, evaluating TB suspects with two sputum smears was as effective as with three sputum smears and is accompanied with less laboratory work and thus, reductions in the cost related to the TB workup. Furthermore, this strategy could leave more time for the examination of each slide.

Several recently published reports have shown results similar to the observation in the present study (Gopi et al., 2004; Leonard et al., 2005). Ozkutuk et al. (2007), in a 4year retrospective study in Turkey, found that the collection of two sputum specimens was almost always adequate to make a diagnosis (100%), irrespective of the quality of the specimen obtained. Mathew et al. (2002), in a 5-year retrospective study in New Jersey, found that the majority of patients whose AFB smears were negative and for whom TB isolation was discontinued were identified with the first sputum AFB smear.

Craft et al. (2000) reviewed 4 years' worth of data at the University of North Carolina and concluded that "modifying the smear policy from three to two negative smears would have resulted in no increased risk of spreading TB and would decrease the number of days patients are unnecessarily placed under airborne precautions" in their institution. Our findings and those mentioned above raise the question of whether three sputum AFB smears are necessary before discontinuing TB isolation. In addition, given limited resource in our setting, it is mandatory to investigate strategies to optimize the detection of cases further pointing the impracticality of the current recommendations in routine clinical practice.

In conclusion, the overall prevalence of sputum smear positive cases of 17.3% with the most vulnerable amongst the age ranges 15 - 44. Although we did not evaluate the contribution of each sputum specimen for the detection of AFB in this study directly, our result show that examining two sputa smears was sufficient for the detection of AFB in our laboratory. Further research involving different laboratories from all of the regions of Rwanda is needed to reassess these findings.

## ACKNOWLEDGEMENTS

We thank all technicians from the bacteriology unit for their collaboration and technical assistance.

#### REFERENCES

- Bruchfeld J, Aderaye G, Palme IB, Bjorvatn B, Britton S, Feleke Y, Kallenius G, Lindquist L (2002). Evaluation of outpatients with suspected pulmonary tuberculosis in a high HIV prevalence setting in Ethiopia: clinical, diagnostic and epidemiological characteristics. Scand. J. Infect. Dis. 34: 331-7.
- Craft DW, Jones MC, Blanchet, CN, Hopfer RL (2000). Value of examining three acid-fast bacillus sputum smears for removal of patients suspected of having tuberculosis from the "airborne precautions" category. J. Clin. Microbiol. 38: 4285-7.
- Dye C, Scheele Š, Dolin P, Pathania V, Raviglione MC (1999). Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. WHO Global Surveillance and Monitoring Project. JAMA 282: 677-86.
- Garg SK, Tiwari RP, Tiwari D, Singh R, Malhotra D, Ramnani VK, Prasad GB, Chandra R, Fraziano M, Colizzi V, Bisen PS (2003). Diagnosis of tuberculosis: available technologies, limitations, and possibilities. J. Clin. Lab. Anal. 17: 155-63.
- Gopi PG, Subramani R, Selvakumar N, Santha T, Eusuff SI, Narayanan PR (2004). Smear examination of two specimens for diagnosis of pulmonary tuberculosis in Tiruvallur District, south India. Int. J. Tuberc. Lung Dis. 8: 824-8.

- Habeenzu C, Mitarai S, Lubasi D, Mudenda V, Kantenga T, Mwansa J, Maslow JN (2007). Tuberculosis and multidrug resistance in Zambian prisons, 2000-2001. Int. J. Tuberc. Lung Dis. 11: 1216-20.
- Harries AD, Mphasa NB, Mundy C, Banerjee A, Kwanjana JH, Salaniponi FM (2000). Screening tuberculosis suspects using two sputum smears. Int. J. Tuberc. Lung Dis. 4: 36-40.
- Holmes CB, Hausler H, Nunn P (1998). A review of sex differences in the epidemiology of tuberculosis. Int. J. Tuberc. Lung Dis. 2: 96-104.
- Katamba A, Laticevschi D, Rieder HL (2007). Efficiency of a third serial sputum smear examination in the diagnosis of tuberculosis in Moldova and Uganda. Int. J. Tuberc. Lung Dis. 11: 659-64.
- Kolawole TM, Onadeko EO, Sofowora EO, Esan GF (1975). Radiological patterns of pulmonary tuberculosis in Nigeria. Trop. Geogr. Med. 27: 339-50.
- Leonard MK, Osterholt D, Kourbatova EV, Del Rio C, Wang W, Blumberg HM (2005). How many sputum specimens are necessary to diagnose pulmonary tuberculosis? Am. J. Infect. Control. 33: 58-61.
- Mathew P, Kuo YH, Vazirani B, Eng RH, Weinstein MP (2002). Are three sputum acid-fast bacillus smears necessary for discontinuing tuberculosis isolation? J. Clin. Microbiol. 40: 3482-4.
- Onadeko BO, Dickinson R, Sofowora EO (1975). Miliary tuberculosis of the lung in Nigerian adults. East Afr. Med. J. 52: 390-5.
- Ozkutuk A, Terek G, Coban H, Esen N (2007). Is it valuable to examine more than one sputum smear per patient for the diagnosis of pulmonary tuberculosis? Jpn. J. Infect. Dis. 60: 73-5.
- Van Deun A, Salim AH, Cooreman E, Hossain MA, Rema A, Chambugonj N, Hye MA, Kawria A, Declercq E (2002). Optimal tuberculosis case detection by direct sputum smear microscopy: how much better is more? Int. J. Tuberc. Lung Dis. 6: 222-30.
- World Health Organisation (1998). Laboratory services in tuberculosis control. Part I: organisation and management. Geneva: WHO.
- World Health Organisation (2005). Global tuberculosis control: surveillance, planning, financing. Geneva. (WHO/HTM/TB/2005.349)