

## BACTERIOLOGICAL STATUS OF TUBERCULOSIS CASES AND TUBERCULOSIS SYMPTOMS IN HIV INFECTED PERSONS IN KATHMANDU

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### Abstract

**Background:** Clinical presentations of Tuberculosis (TB) vary with in HIV positive and HIV negative individuals. Smear negative tuberculosis is the leading cause of death of HIV patients.

**Objective:** To examine the relationship between bacteriological status of TB cases and TB symptoms in HIV patients.

**Methods:** A cross-sectional analytical study was conducted during January 2004 and August 2005 in a representative sample of 100 HIV infected persons visiting different Voluntary Counseling and Testing Centers (VCT) and HIV/AIDS care centers located in Kathmandu. Laboratory investigation of Tuberculosis was done by AFB staining and culture in Ogawa medium. Data obtained through pre structured questionnaire and laboratory investigation were entered into SPSS 11.5 and analyzed.

**Results:** Twenty three percent prevalence of TB is observed in HIV patients. Eighty one percent of the total TB cases were smear negative cases. Significant relationship was observed between the TB symptoms and Smear positive TB cases ( $\chi^2 = 4.01$ ,  $p < 0.05$ , at 1 degree of freedom) but no significant relationship could be established between TB symptoms and smear negative cases ( $\chi^2 = 0.82$ ,  $p > 0.05$ , at 1 degree of freedom). *Mycobacterium avium* complex (40.9%) was predominant followed by *M. tuberculosis* (27.3%)

**Conclusion:** In HIV patients, the utility of direct microscopy of AFB stained smear is limited because most of these patients were smear negative and are asymptomatic. So, direct microscopy in combination with Culture is recommended for higher case finding of TB in HIV patients.

**Key words:** Asymptomatic, smear negative TB, HIV/AIDS, Kathmandu

### Introduction

Among different HIV related opportunistic infections, tuberculosis is the most common one as suggested by the fact that more than a quarter of the 42 million people infected with HIV world wide are also co-infected with TB.<sup>1</sup>

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HIV associated TB has a marked increased mortality rate: up to 25% of patients with Sputum smear positive results and 40-50 % of those with AFB smear negative results die of TB.<sup>2</sup> As the types of TB in HIV positive patients differs from those occurring in HIV negative individual, particular features must be taken into account in the case detection, diagnosis and treatment of TB in HIV positive patients. Of importance, the bacteriological status is the key issue concerned with the management of HIV related TB. "Smear negative" and "Smear positive" are the most important bacteriological classification of pulmonary TB cases. Smear

positive TB cases means those TB patients with at least two sputum specimen positive for AFB by microscopy or at least one sputum specimen positive for AFB by microscopy, and abnormal chest radiography/Culture positive for *Mycobacterium tuberculosis*. Smear negative TB cases means those TB patients with at least 2 sputum specimen negative for AFB by microscopy and culture positive for *M. tuberculosis*.<sup>3</sup>

Directly observed treatment Short-course (DOTS) is the worldwide accepted TB control strategy which still relies on passive case finding methods. This basic approach in TB control is supposed to be insufficient to control TB in high HIV prevalence region.<sup>4</sup>

Cough is the most common symptom of TB and present in 95% of the smear positive cases. However, it is not a specific sign of TB since it is present in many conditions affecting the lower respiratory tract i.e. most patients with cough do not suffer from TB. Similarly, Cough and smear positive TB is less common in HIV patients. Often a PTB suspected patients ( i.e. PTB suspects ) has one or more of the following symptoms as well as cough:  
Respiratory symptoms: Short ness of breath, chest pain, haemoptysis.

Constitutional symptoms: Weight loss, loss of appetite, fever, night sweat and tiredness.<sup>3</sup>

Although microscopic examination of appropriately stained sputum specimen for tubercle bacilli is the quick and easier method it is less reliable in certain cases because it requires between 5,000–10, 000 tubercle bacilli per milliliter sputum for detection of AFB. So, examination by bacteriological culture provides the definitive diagnosis of tuberculosis because as few as ten viable bacilli per milliliter sputum can be detected. Culture increases the number of TB cases often by 30-50 times and it is essential to distinguish different mycobacterial species.<sup>5</sup> Atypical mycobacterial lung disease, mainly due to *Mycobacterium avium* complex, is most prevalent in HIV patients.<sup>6</sup>

Until now in developing countries diagnosis of smear negative Tuberculosis is rarely done due to concerns regarding the feasibility and cost. However several studies have revealed that smear negative tuberculosis constituted the significant proportion in HIV patients.<sup>3</sup> So far in Nepal, the guidelines and policies concerning the case detection of TB in HIV patients are same as that of normal population. The NTP program primarily relied on DOTS and quality assured microscopy. Culture facility is available only on the basis of the first phase treatment result. So, this study is conducted to fill this existing gap concerning the TB culture to these needy individuals.

## Methodology

This research was approved by Nepal Health Research Council ethics committees and carried out by the central department of microbiology, Tribhuvan University, Kirtipur in collaboration with Tribhuvan University teaching hospital (TUTH), Maharajgunj during January 2004 to August 2005. The cross-sectional study was conducted in a representative sample of 100 HIV infected persons attending different HIV/STI clinics, Voluntary Counseling and Testing Centers and HIV/AIDS care homes located in Kathmandu valley were enrolled in the study. The estimation of sample size was based on the number of People Living with HIV/AIDS (PLWHA) available in different HIV care centres located with in the valley. Major sampling sites included the following: Indoor and out patient department of TUTH (The major sampling site), Nava Kiran Plus HIV/AIDS Care Home, Sparsha Nepal HIV/AIDS Care Home, Karuna Bhavan HIV/AIDS Care Home, Sneha Samaj HIV/AIDS Care Home, Maiti Nepal, Nepal Plus HIV/AIDS Care Home, Vision Plus VCT, SACTS-VCT, Nepal Youth HIV/AIDS Care Home and Blue Diamond Society. Sampling was done by random sampling method. In this process, the name of all the HIV patients residing in the HIV care home / STI clinics were written in separate papers and 50 % of them were selected by simple random technique with out replacement.

After taking informed consent, pre structured questionnaires were filled on the basis of which the patients were identified as symptomatic or asymptomatic. Those patients who self reported cough for about 2 weeks along with chest pain and other constitutional/respiratory symptoms are considered as symptomatic; otherwise asymptomatic. Every attempt was made to reduce bias during filling up of questionnaire. Three sputum specimens were collected as per WHO guidelines. As asymptomatic patients could not produce the sputum readily, they were instructed to inhale 3-5% saline mist for 15 minutes to obtain induced sputum. Diagnosis of tuberculosis by conventional methods such as direct microscopy of AFB stained smear, AFB culture and identification tests in Mycobacteriology Lab. of TUTH which has been providing quality assured microscopy (and culture) for decades. In direct microscopy three sputum specimens i.e. 1<sup>st</sup> spot specimen, early morning specimen and 2<sup>nd</sup> spot specimen were collected, stained by ziehl-Nelsen staining technique and then reporting was done according to WHO/IUATLD positively grading system.<sup>7</sup> In culture technique, early morning specimen was subjected to modified petroff's method for decontamination and then inoculated into 3% Ogawa medium followed by incubation at 37<sup>o</sup>c for 8 weeks. In identification tests, the observation of growth rate and pigmentation, Niacin Test, Nitrate Reductase Test and Catalase Test were performed according to WHO manual, 1998.<sup>5</sup>

The data obtained from questionnaire and laboratory results were entered into SPSS 11.5 and  $\chi^2$  tests and other relevant statistical tools were applied.

## RESULTS

Of the 100 HIV infected persons, 66 (66%) were males and 34 (34%) were females. Majority of them were in the age group 21-30 (60%) followed by 31-40 (31%). The overall prevalence of tuberculosis (including atypical mycobacterial lung disease) was 23%. More

males were co-infected than females ( male:female = 17:6), and the age group of 21-30 were predominantly co-infected as shown in table 1.

Among 5 smear positive cases, 4 cases ( 80%) presented TB symptoms (Both respiratory and constitutional) showing significant relationship between TB symptoms and smear positive tuberculosis ( $\chi^2 =4.01$  ,  $p<0.05$  at 1 degree of freedom ) as shown in table 2. In contrast to this, only 38.8% ( 7 out of 18 ) smear negative TB cases presented both types of TB symptoms and no significant relationship could be established between TB symptoms and smear negative TB ( $\chi^2 =0.82$  ,  $p>0.05$  at 1 degree of freedom ) as shown in table 3. Analysis of individual symptoms presented by Smear positive, smear negative and Non TB cases reveals that as high as 80% of Smear positive TB cases presented all symptoms of TB where as 38.8% to 55.5% smear negative TB cases presented different TB symptoms. Cough, the major symptom presented by both the TB cases, is less common (only 28.5%) in Non TB cases (Table 4). Although culture detected higher number of TB cases in comparison to direct microscopy of AFB stained smear, one case was detected only by direct microscopy (Table 5). Although one case was culture negative, there was no doubt in smear positive result because the morphology (shape and size) of the mycobacteria is exactly same as that of positive control AFB slide; and positivity grading result of the case was 2+. Furthermore it was known that the patients were under DOTS treatment and hence dead bacilli might have been seen in direct microscopy but not in culture (which require live bacilli to produce colony). Among 22 culture positive isolates, the predominant species was *M. avium complex* (40.9%) followed by *M. tuberculosis* (27.3 %) as shown in table 6. At the time of study, only very few cases have done CD4 count and hence staging was done on the basis of sign and symptoms rather than CD4 status (Table 7).

**Table 1 Distribution of HIV patients by TB status, age group and gender**

Age group	TB positive				TB negative				Total			
	Male		Female		Male		Female		Male		Female	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
11-20	0	0.0	0	0.0	1	2.0	1	3.6	1	1.5	1	3.0
21-30	12	70.6	3	50.0	25	51.1	20	71.4	37	56.1	23	67.7
31-40	3	17.6	3	50.0	20	40.8	5	17.8	23	34.8	8	23.5
41-50	1	5.9	0	0.0	2	4.1	2	7.2	3	4.5	2	5.8
51-60	1	5.9	0	0.0	1	2.0	0	0.0	2	3.1	0	0.0
<b>Total</b>	<b>17</b>	<b>100</b>	<b>6</b>	<b>100</b>	<b>49</b>	<b>100</b>	<b>28</b>	<b>100</b>	<b>66</b>	<b>100</b>	<b>34</b>	<b>100</b>

**Table 2 Relationship between TB symptoms and Smear Positive TB in HIV cases**

Smear positive TB	TB symptoms			$\chi^2$ , 1 degree of freedom
	Yes	No	Total	
Yes	4	1	5	4.01 (p<0.05)
No	26	69	95	
<b>Total</b>	<b>30</b>	<b>70</b>	<b>100</b>	

**Table 3 Relationship between TB symptoms and Smear negative TB in HIV cases**

Smear negative TB	TB symptoms			$\chi^2$ , 1 degree of freedom
	Yes	No	Total	
Yes	7	11	18	0.82 (p>0.05)
No	23	59	82	
<b>Total</b>	<b>30</b>	<b>70</b>	<b>100</b>	

Note: Although 10 smear negative TB cases reported cough, only 7 of them reported chest pain along with cough. So, smear negative TB cases with TB symptoms (including chest pain) would be 7.

**Table 4 Relationship between clinical symptoms of TB and bacteriological status of TB in HIV Positive patients**

Symptoms	Smear +ve TB cases n=5		Smear -ve TB cases n=18		TB negative cases n=77		Total n=100	
	No.	%	No.	%	No.	%	No.	%
Fever	4	80.0	7	38.8	29	37.6	40	40.0
Cough	4	80.0	10	55.5	22	28.5	36	36.0
Chest pain/shortness of breath	4	80	8	44.4	31	40.2	43	43
Night sweat	4	80.0	7	38.8	22	28.5	33	33.0
Weight loss	4	80.0	10	55.5	39	50.6	53	53.0

Note- Actually 7 cases are chest pain and one case is shortness of breath (but not chest pain). So if consider only those persons exhibiting cough for 2 weeks along with chest pain also, as the TB symptomatic, we should consider only seven cases as TB symptomatic. This is because we are considering those patients complaining merely shortness of breath (but not chest pain) as TB asymptomatic, whether or not they complain cough.

**Table 5 Comparative evaluation of direct microscopy with cultural technique for TB diagnosis in HIV Positive patients**

AFB culture	AFB staining			Inference
	AFB found	AFB not found	Total	
Culture positive	4	18	22	Direct microscopy in combination with culture is appropriate technique for TB case finding in HIV patients.
Culture negative	1	77	78	
<b>Total</b>	<b>5</b>	<b>95</b>	<b>100</b>	

**Table 6 Distribution of *Mycobacterium* species in HIV Positive patients**

Mycobacterial Species	Number of isolates	%
<i>M. avium</i> complex (MAC)	9	40.9
<i>M. tuberculosis</i>	6	27.3
<i>M. kansasii</i>	4	18.2
<i>M. fortuitum</i>	2	9.1
<i>M. chelonae</i>	1	4.5
Total	22*	100

\*One species could not be identified because it was culture negative

**Table 7 Periodic prevalence of TB in HIV Positive patients**

Period	Sampling site	Sample size	WHO staging of HIV	TB prevalence
Jan –May 2004	TUTH bed/OPD	25	II-IV	8/25 (32%)
June –Aug 2004	Nava Kiran Plus	25	II- IV	5/25 ( 20%)
Sept- Nov 2004	Sparsha Nepal	20	I-III	4/20 (20%)
Dec 04- Feb 05	SS+MN+KV*	14	I-II	2/14 (14.3%)
Mar – May 2005	NP+VP+SVCT*	10	I-II	3/10 ( 30%)
June- Aug 2005	NY+BDS	6	I-II	1/6 ( 16.6%)

\*SS= Sneha Samaj, MN = Maiti Nepal, KV= Karuna Bhavan, NP = Nepal Plus, VP= Vision Plus, SVCT= SAACT VCT, NY = Nepal Youth, BDS= Blue Diamond Society

## Discussion

In this study the prevalence of tuberculosis (including atypical mycobacterial lung infection) is found to be 23 % in HIV positive patients of Kathmandu which is in concordance with WHO/UNAIDS report stating one third of HIV/AIDS patients co-infected with tuberculosis.<sup>8</sup> In context to Nepal, it was observed that during 1991-2000, 66% of AIDS cases were co-infected with TB.<sup>9</sup> Comparatively Lesser prevalence in our study may be due to inclusion of both HIV as well as AIDS cases. Studies done in United Mission Hospital, Tansen showed that TB prevalence in HIV cases increased from 10.8% in 2002 to 39.5% in 2004.<sup>10,11</sup> These studies shows that high variation of TB prevalence in HIV patients depending on the nature of surveillance. If sampling is done in patients visiting HIV/STI clinics/ hospital bed, the prevalence will be obviously high. As our study is also primarily hospital based, this may be one of the reasons for observing high TB rate.

One of the important findings of this study is that HIV patients mostly suffers from smear negative tuberculosis (as high as 81.8 % of the total TB cases) and are usually asymptomatic. Other studies have also shown that the usual symptoms of TB are less common in this group

of immunocompromised persons.<sup>3</sup> So, they require bacteriological investigation of through culture which is found to be more than 4 times effective than direct microscopy. It has been observed that in general, culture increases the case finding rate by 30 – 40%.<sup>5</sup> Furthermore, several comparative evaluation of different diagnostic technique for tuberculosis have concluded that case detection rate of direct microscopy is very low although it is simplest and cost effective.<sup>13</sup> This study suggests that unlike the case finding strategy for general population, the TB case finding strategy for HIV patients needs to adopt different approach / policies because higher number of asymptomatic cases were found to be positive for tuberculosis. Another important finding of this study is the documentation of alarmingly higher rate of atypical mycobacterial lung disease (mainly due to *Mycobacterium avium* complex). It was documented that as high as 50 % of HIV/AIDS patients of western countries were co-infected with *Mycobacterium avium* complex.<sup>12</sup> This can be justified that the HIV patients being highly immunocompromised, even these less virulent mycobacteria (which are abundantly found in environment) can cause serious lung disease.

Diagnosis of smear negative tuberculosis is a difficult task. In developing countries, the majority

of these cases has been treated only on the basis of clinical and chest radiographic findings. Without a standardized clinical work up, the misdiagnosis rates have been estimated as high as 35% to 52%.<sup>14</sup> So, it is recommended that the NTP should adopt policies concerning the sputum culture, wherever possible.

## Conclusion

This study has demonstrated that significantly higher number of asymptomatic HIV patients suffer from smear negative TB. The disease is mainly due to atypical mycobacteria which are rarely detected in direct microscopy. Hence culture is recommended to detect higher number of TB cases in HIV patients.

## Limitation of the study

As the sampling was done in hospitals, HIV/STI clinics and HIV/AIDS care home located in Kathmandu, the species prevalence of mycobacteria and bacteriological status of TB obtained in our study can not be generalized as national scenario.

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