Original Article

Role of Bleach Sedimentation Technique for the Diagnosis of Tuberculosis.

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Abstract

Tuberculosis remains a major threat to world health. The main aim of this study was to improve the microscopic detection of AFB from sputum by Bleach sedimentation technique using household bleach. This was a cross sectional type of study conducted for a period of one year from July 2010 to June 2011 in the Department of Laboratory Services, Khwaja Yunus Ali Medical College Hospital (KYAMCH), Sirajgonj. A total of 115 clinically suspected tuberculosis patients aged between 7-85 years were included in the study. Among them 35 (30.4%) patients had TB positive by routine (Direct microscopy) method; whereas, by bleach concentration method 42 (36.5%) were found positive; diagnosing 07 additional patients. The rise of 16.7% in sputum positivity by bleach sedimentation microscopy over the direct smear microscopy was found to be statistically significant (p= <0.005). Paired samples t test analysis of sputum showed a significant correlation between this two methods (r=0.896, p <0.001). This study suggests that Bleach sedimentation method is better than direct conventional method. So it should be applied to all laboratories across the country because it is cheap early available and also safety to the laboratory staffs.

Key words: *Tuberculosis (TB), Sputum, Bleach, Sedimentation tech, AFB (Acid-fast bacilli).*

Introduction

Mycobacterium tuberculosis causes Tuberculosis, which is a worldwide problem especially in developing countries[1]. Tuberculosis remains as a major cause of morbidity and mortality globally[2]. Franco et. al. mentioned Mycobacterium tuberculosis as the leading cause of death than any other single microbial agent[3]. Approximately one-third of the world's population infected with this organism[1]. World Health Organization (WHO) declared TB as a Global Health Emergency in 1993[4].

In Bangladesh, TB remains a major public health problem and major cause of morbidity and mortality. The country ranks sixth in terms of burden of TB, having an estimated 300,000 new cases and 70,000 deaths annually[5]. The WHO estimated that in 2005, there were approximately 576000 TB cases in the country. The number of new cases occurring in 2005 was estimated at approximately 322000. Of these, approximately 145000 were infectious cases transmitting TB in the community. WHO further estimated that about 67000 TB patients, most of them were not registered, had died of tuberculosis in 2005[6,7].

Diagnosis of tuberculosis depends on clinical history, detection of AFB, culture, tuberculin test, chest X-ray, serology, PCR and histological examination etc. The definitive diagnosis of tuberculosis is the demonstration of tubercle bacilli in clinical specimens.

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The microscopic examination of acid-fast bacilli (AFB) remains the main tool for diagnosis[8,9]. A large number of bacilli, 5,000 to 10,000 per ml of sputum are required to find smear positive for AFB[10,11]. In these countries, limited resources allow only direct microscopy as the available option for tuberculosis diagnosis[12,13] recent years, interest is improving the direct smear microscopy technique in developing countries[14,15].

Digestion of sputum with sodium hypochlorite (NaOCL, 5.025%) give the best recovery of AFB[12] and concentration of bacilli by centrifugation of sputum increased the recovery rate of mycobacteria[16]. After treatment with NaOCl, it might be attributable to changes in surface properties of mycobacteria and for denaturing of sputum constituents leading to flocculation and subsequent increased sedimentation rate of mycobacteria[17].

This study also reported that NaOCl not only increased the sensitivity of sputum but also acts as a potent disinfectant, thus eliminates the risk of transmitting infection especially in laboratories with inadequate safe standards[17]. Miorner et. al. demonstrated that the simple liquefaction and overnight sedimentation of sputum with sodium hypochlorite (NaOCl) would significantly augment smear sensitivity up to 70%[15].

Tuberculosis was the problem in the past, is the problem now, and will be further aggravated in the future if immediate attention is not paid. New diagnostic techniques are urgently needed to replace or facilitate microscopy, especially in low-income countries where the disease is endemic and the incidence is high. The present study was designed to analyse and compare the findings of Bleach treated sputum with direct conventional method.

Materials and methods

A total of 115 patients were involved in this study. This was a cross sectional type study conducted for a period of one year from July 2010 to June 2011 in the Department of Laboratory Services, Khwaja Yunus Ali Medical College Hospital (KYAMCH), Enayetpur, Sirajgonj. After collection, the container labelled with the date and patient's profile. A correctly completed request form must accompany each specimen. Then the specimens were processed for routine examinations by Bleach (Sodium hypochlorite) sedimentation method [1-2 ml sputum was taken to a screw-cap tube of 15-20 ml capacity > equal volume of sodium hypochlorite (household bleach, 5.25 %) solution added and mixed > left at room temperature for 10-15 minutes, shaked at intervals > added about 8 ml of distilled water and mixed > centrifugation at 3000 g for 15 minutes / left to sediment overnight > discarded the supernatant fluid and stained using Ziehl-Neelsen technique] and examined microscopically for acid-fast bacilli (AFB). Patient's age, sex, AFB and other findings were taken as variables. The data were analysed using software statistical program for social sciences (SPSS).

Results

The results of this study are shown below in the following tables and figures. Ages of the patients were between 7-85 years with mean age 36.71 and SD 15.83. As shown in Table 1

Table 1: Age distribution of the patients (n=115)

Age in years	No. of subject	Percentage (%)	Mean age (M ±SD)
<20	16	13.9	36.71 ± 15.83
21 -30	34	29.6	
31 -40	20	17.4	
41 -50	21	18.3	
51 -60	15	13.0	
61 -70	5	4.3	
71 -80	3	2.6	
>80	1	.9	

Among the 115 cases, 64 were male (55.65%) and 51 (44.35%) are female shown in Figure 1.

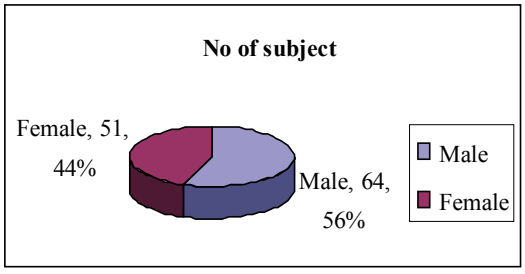

Figure 1. Pie diagram showing the sex distribution of study population.

Table 2. ESR values of the study cases (n=115)

ESR	No. of subject	Percentage (%)	Mean ESR (M ±SD)
<30	18	15.7	57.09 ± 26.74
31 -60	48	41.7	
61 -90	36	31.3	
91 -120	12	10.4	
>120	1	0.9	

Table 3. MT values of the study population (n=115)

MT value	No. of subject	Percentage (%)	Mean MT (M ±SD)
< 5	3	2.6	14.62 ± 4.64
6-10	19	16.5	
11 -15	41	35.7	
16 -20	42	36.5	
21 -25	9	7.8	
> 25	1	0.9	

The findings showed that 35 (30.4%) cases were found TB positive on direct method, 42 (36.5%) cases TB positive by Bleach method which is shown in Figure 2.

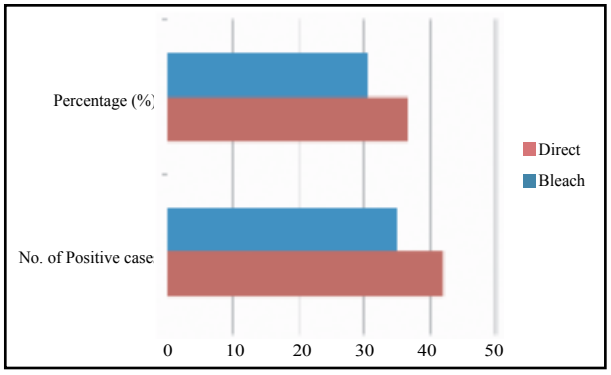

Figure 2. Bar diagram showing number of TB cases.

Table 4. Comparative study between Direct smear microscopy and Bleach sedimentation microscopy (n=115).

Specimen	Microscopy			
Sputum	Direct smear microscopy (n=115)		Bleach sedimentation microscopy (n=115)	
	No. of positive specimens (Percentage positive)		No. of positive specimens (Percentage positive)	
	35 (30.4%)		42 (36.5%)	
	Day -1	Day -2	Day -1	Day -2
	27 (23.4%)	35 (30.4%)	34 (29.6%)	42 (36.5%)
Note: No positive smear found on third day that were not found on 1st and 2nd day.				

Common clinical features of the study population were mainly cough, low-grade evening rise of temperature, weight loss, lymph node swelling, haemoptysis etc. as shown in Figure 3.

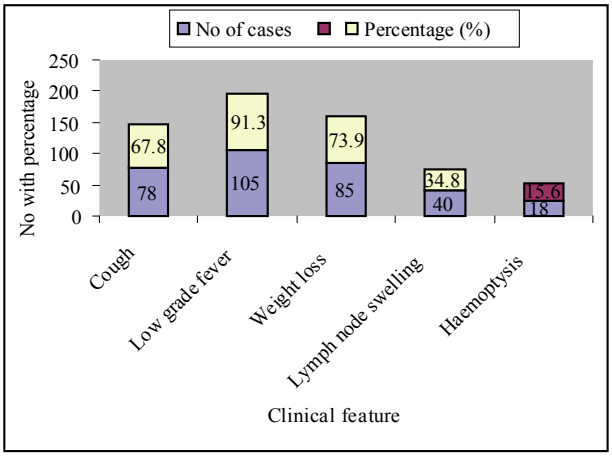

Figure 3. Bar diagram showing the clinical features.

Among the 115 study cases of the present study, 49 were poor (42.6%), 52 were from middle class family (45.2%) and 14 (12.2%) rich people as shown in Figure 3. The study demonstrated that the incidence of tuberculosis was higher among the poor and middle class family.

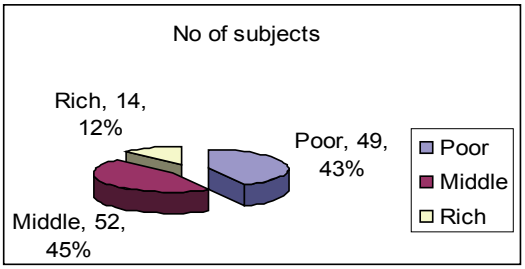

Figure 4. Pie diagram showing the social status of study population.

Microscopic findings revealed that maximum AFB positive patients 14 (33.33%) were found as (++) followed by (+) (28.57%), (+++) (19.05%) and (++++) (2.38%) by direct smear preparation. When using Bleach sedimentation technique, 21 (50.00%) sputum positive patients were detected as (++), 14 cases detected as (+++), 4 cases detected as (++++) and only 3 sputum positive cases detected as (+) shown in Table 5.

Table 5. Data of significance of AFB findings between bleach and direct method.

Method	Number of patient according to AFB positivity				Total positive
	(+)	(++)	(+++)	(++++)	
Direct	12 (28.57%)	14 (33.33%)	08 (19.05%)	01 (2.38%)	35 (83.33%)
Bleach	03 (7.14 %)	21 (50.00%)	14 (33.33%)	04 (9.52%)	42 (100.0%)

T-test for direct and bleach method:

T-test was done for sputum examination between Direct and Bleach method. For convenience of the calculation '+' was count as 1, '++' was count as 2, '+++' as 3 and '++++' as 4 and mean was calculated accordingly as shown in the below tables.

Table 6 (a). Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair	Direct	.59	115	1.008	.094
	Bleach	.90	115	1.273	.119

Table 6 (b). Paired Samples Correlations

		N	Correlation	Sig.
Pair	Direct -Bleach	115	.896	< 0.001

Table 6 (c). Paired Samples Test: Differences between Direct and Bleach method.

Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference		*t*	df	*p* value
			Lower	Upper			
-.304	.580	.054	-.411	-.197	-5.627	114	< 0.001

This paired samples t test analysis indicates that for the 115 subjects. The mean score on the bleach method (M=1.273) was significantly greater at the p <0.001 level than mean score on the direct method. These results also indicates that a significant correlation exists between this two methods (r=0.896, p <0.001).

Discussion

Despite all advances made in the treatment and management of tuberculosis, it continues to be a major public health problem in Bangladesh as well as in other developing countries. This study showed that Bleach sedimentation method is better than direct method. 07 additional cases were found to be positive which were missed by direct microscopic method. Previous studies have shown that concentration and liquefaction of sputum significantly improves the sensitivity of direct microscopy[12]. In the study by Habeenzu et al, the use of NaOCl was found to increase the smear sensitivity from 43.4% to 76.3% with the specificity of 100%[14].

Saxena et al reported that sputum samples were studied by direct staining and after sodium hypochlorite treatment and centrifugation. Use of sodium hypochlorite method increased the number of positive samples from 52 to 96[18]. The purpose of the current study was to evaluate the performance and feasibility of tuberculosis diagnosis by sputum microscopy after bleach sedimentation, compared to conventional direct smear microscopy. We have compared microscopy of smear made directly from sputum with microscopy of sputum made after liquefaction and concentration of sputum with household bleach (5.25%, NaOCl).

In our study 35 (30.4%) patients were found to be TB positive by routine (Direct microscopy) method whereas, by bleach concentration method 42 (36.5%) were found positive; diagnosing 07 additional patients. The rise of 16.7% in sputum positivity by Bleach sedimentation microscopy over direct smear microscopy was found to be statistically significant (p= <0.005).

T-test (paired samples t test) between Direct smear method and Bleach sedimentation method showed that the mean score on the bleach method (M=1.273) was significantly greater at the p <0.001 level than the direct method. These results also indicated a significant correlation between this two methods (r=0.896, p <0.001). The findings are almost similar with the above studies[12,14,18]. In the present study, the highest prevalence of tuberculosis was noticed among patients belonging to the age group of 20- 40 years. This finding of ours is almost similar to that reported by WHO regional office

for South East Asia where most tuberculosis cases occured in the age group of 15-54 years. (http://www.searo.who.int/en/Section10/Section2097/Section 2100_10639.htm). However, our finding differs from a study conducted by Zaman et al in our country on the prevalence of smear-positive tuberculosis in persons aged =15 years; where prevalence of TB was found to be highest in the 55-64 years age group (201/100 000) and lowest in 15-24 years age group (43·0/100 000)[19].

The results of sex-wise incidence of tuberculosis of our study showed that out of the 115 subjects; 64 (55.65%) were male and 51 (44.35%) were female. The prevalence of tuberculosis therefore, was higher among males than the females i.e. males are more sufferer of tuberculosis than females. This finding of ours agree in good agreement with the findings of Zaman et al who worked on the prevalence of sputum smear-positive tuberculosis in Matlab, a rural area in Bangladesh. In their study, they reported that population-based rate of smear-positive TB was almost six times higher in males than females[19].

Another study in Bangladesh conducted by Salim (2004) also reported about three times higher sputum-positive cases in males compared to females (35.4 vs. 12 . 3/100 000)[20]. The male predominance for persistent cough and for AFB-positive sputum is consistent with data from other countries and could reflect occupational, behavioral or immunological contributions to risk[21,22,23]. These studies are quite similar to our findings. The normal Erythrocyte Sedimentation Rate (ESR or Sed-Rate) for male is 1 - 13 mm/hr and for Female 1 - 20 mm/hr. In tuberculosis very high ESR levels (up to 100+mm/h or higher) are noticed ESR values of the Tuberculosis positive patients of the study subjects were also found to be elevated than the normal values and it ranged between 06-140 mm in 1st hour and the mean ESR being 57.09.

Chowdhary et al in their epldemiologlcal investigation of pulmonary tuberculosis reported that people belonging to the lower socio-economic group were the worst sufferers[24]. Similarly Tiwari et al in Mali Village (Lucknow, India) found that those belonging to social lower middle class, poor and very poor class suffered maximum from tuberculosis[25]. Our study also showed almost similar findings, as here tuberculosis was higher among the poor and middle class.

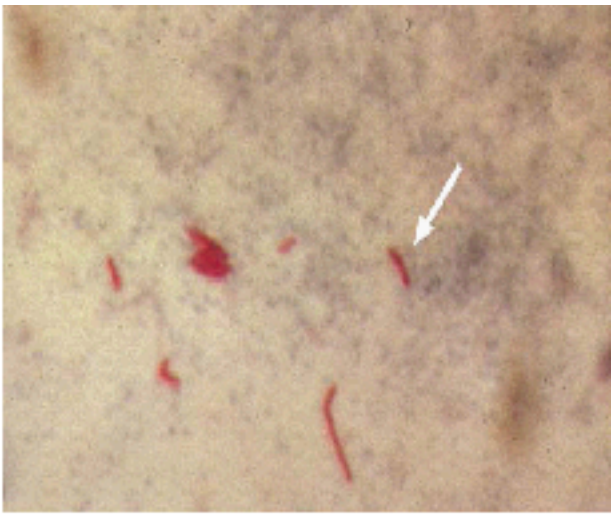

Figure 5. Ziehl-Neelsen Stain showing red-staining AFB (Oil immersion field).

Conclusion

This study suggests that digestion and liquefaction of sputum with bleach and concentration by centrifugation/ left to overnight increases more diagnostic accuracy than direct conventional method. Sodium hypochlorite (bleach) is also easily available, cheaper and it kills Mycobacterium tuberculosis, making the handling of specimens safer for the laboratory staffs. This method is simple and can be used in all diagnostic laboratories across the country.

References

1. Vinay Kumar, Abul K. Abbas, Nelson Fausto. 2010. Robbins and Cotran's Pathologic Basis of Disease. WB Saunders Limited, Philadelphia, USA. 8th ed. pp. 366-372.

2. Warren Levinson. 2007. Review of Medical Microbiology and Immunology. 9th ed. Lange Medical Books/ McGraw-Hill. PP. 161-162.

3. Franco J, Camarena JJ, Nogueira JM. 2001. Serological response (Western blot) to fractions of Mycobacterium tuberculosis sonicate antigen in tuberculosis patients and contacts. Int J Tuberc Lung Dis 5(10):958-962.

4. WHO. 2005. WHO, Bangladesh, TB. (Online), Available at: http://www.whoban.org/communicable dis.tb.html

5. Hossain Md. Yunus, Arifeen SE, Abdullah Mahmud, Begum V, Akramul Islam, Baqui AH, Luby SP. 2007.

Tuberculosis in Bangladesh: A 40-year Review. ICDDRB, GPO Box 128, Dhaka 1000, Bangladesh.

6. WHO. 2007. National Tuberculosis Control Programme, Directorate General of Health Services, Mohakhali, Dhaka 1212. Global Tuberculosis Control.

7. Fourth Joint National Tuberculosis Control Programme (NTP) Review, Bangladesh (2007).

8. Aber VR, Allen B W, Mitchison D A, Ayuma P, Edwards EA, Keyes AB. 1980. Quality control in tuberculosis bacteriology.1. Laboratory studies on isolated positive cultures and the efficiency of direct smear examination. Tubercle 61: 123-133.

9. Huebner R E, Good RC, Tokars JI. 1993. Current practice in mycobacteriology: International Union against Tuberculosis and Lung Diseases. Tuberculosis guide for low-income countries, 4th ed. International Union against Tuberculosis and Lung Diseases, Paris, France.

10. David HL. 1985. Bacteriology of Mycobacterioses. In: "Kent PT, Kubica GP. Public Health Mycobacteriology: A guide for the level-III laboratory." US department of health and human services, Public health service, Center for Disease control, Atlanta, Georgia. pp. 1-206.

11. Nicholas A. Boon, Nicki R. Colledge, Brain R. Walker, John A. A. Hunter. 2006. Davidson's Principles & Practice of Medicine. 20th ed. 19: 695-703.

12. Corper HJ, Nelson CR.1949. Methods for concentrating acid-fast bacilli. Am J Clin Pathol 19: 269-273.

13. Kent PT, Kubica GP. 1985. Public health microbiology: A guide for the level III laboratory. US department of health and human services, Public health service, Center for Disease control, Atlanta, Georgia. pp. 1- 206.

14. Habeenzu CD, Lubasi D, Fleming AF. 1998. Improved sensitivity of direct microscopy for detection of acid-fast bacilli in sputum in developing countries. Trans R Soc Trop Med Hyg 92: 415-416.

15. Miorner H, Ganlov G, Yohannes Z, Adane Y. 1996. Improved sensitivity of direct microscopy for acid-fast bacilli: Sedimentation as an alternative to centrifugation for concentration of tubercle bacilli. J Clin Microbiol 34: 3206-3207.

16. Ratnam S, March SB. 1986. Effect of relative centrifugal force and centrifugation time on sedimentation of mycobacteria in clinical specimens. J Clin Microbiol 23: 582-585.

17. Gebre N, Karlsson U, Jonsson G. 1995. Improved microscopical diagnosis of pulmonary tuberculosis in developing countries. Trans R Soc Trop Med Hyg 89: 191-193.

18. Saxena S, Mathur M, Talwar VK. 2001. Detection of tubercle bacilli in sputum: Application of sodium hypochlorite concentration method. J Common Dis 33(4): 241-244.

19. Zaman K, Hossain S, Banu S, Quaiyum MA, Barua PC, Hamid MA, Salim, Begum V, Akramul Islam M, Ahmed J, Rifat M, Cooreman E, Van der Werf MJ, Borgdorff M, Van Leth F. 2007-2009. Prevalence of smear-positive tuberculosis in persons aged =15 years in Bangladesh: results from a national survey.

20. Salim MAH. 2004. Gender differences in tuberculosis : a prevalence survey done in Bangladesh. Int J Tuberc Lung Dis 8: 952- 957.

21. Hudelson P. 1996. Gender differentials in tuberculosis: the role of socio-economic and cultural factors. Tubercle Lung Disease 77: 391-400.

22. Borgdorff MW. 2000. Gender and tuberculosis: a comparison of prevalence surveys with notification data to explore sex differences in case detection. Int J Tuberc Lung Dis 4: 123-132.

23. Yamasaki-Nakagawa M. 2001. Gender difference in delays to diagnosis and health care seeking behaviour in a rural area of Nepal. Int J Tuberc Lung Dis 5: 24-31.

24. Chowdhary D, Chatterjee SN, Banerjee AK. 1967. Epidemiological investigations and early diagnosis of pulmonary tuberculosis. J. Ind. Med. Assoc 48: 591-592.

25. Tiwari KN, Jain PC, Prasad BC. 1969. A medico-social study of pulmonary tuberculosis in Mall village, Lucknow. Ind J Med Res 57: 2283.