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Original Research Article.

Sputum Concentration Improves Diagnosis of Pulmonary Tuberculosis Cases in Children at a Tertiary Care Centre

Krishna Kumar Mani[1], Ravi Kumar[2*]

[1]Assistant professor, [2*]Tutor, Department of Microbiology,
Vardhman Institute of Medical sciences, Pawapuri, Nalanda, Bihar, India.

ABSTRACT

Background: Bacteriological diagnosis of tuberculosis (TB) is largely dependent on Ziehl-Neelsen (ZN) microscopy. This method has a low sensitivity. In this study we performed a prospective evaluation direct and concentrated smear microscopy on three early morning sputum specimens from patients suspected of having tuberculosis.

Materials & Methods: A total of 100 patients including HIV patients, of all age groups and sex, presenting to the OPD for the first time, with clinical suspicion of pulmonary or extrapulmonary TB was included in the study. All the demographic details of the patients were noted and consent was taken from patients.

Results: A total of 300 sputa were analyzed by direct and concentration methods with culture as a gold standard. In patients under 15 years both methods were different in sensitivity (62.5% vs. 100%, CI= 95%, P<0.05), in patients of 15 years of age and more, both methods had the almost sensitivity (50% vs. 57.14%, CI= 95%, P = 0. 87). Regardless of age groups both methods were different in sensitivity (87.5% vs.77.77%, C.I= 95%, P = 0.001).

Conclusion: We concluded that the sensitivity of the concentration technique was markedly increased in pediatric age (< 15 years), this increase has influenced the overall sensitivity in all patients. Considering the low cost and safety of the technique and greater sensitivity, this method can be of vital importance at least for patients under 15 years of age with negative smears on direct technique.

Keywords: Tuberculosis, Direct Method, Sputum Concentration, Sensitivity.

***Correspondence to:**

Dr. Ravi Kumar,
Tutor, Department of Microbiology,
Vardhman Institute of Medical sciences,
Pawapuri, Nalanda, Bihar, India.

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INTRODUCTION

Tuberculosis (TB) is one of the biggest public health challenges confronting the world today despite the fact that its causative organism *Mycobacterium tuberculosis* was discovered more than a century ago.[1] Out of 8.8 million TB cases that occurred globally in 2010, 59% occurred in Asia, 26% in the African Region, 7% in the Eastern Mediterranean Region, 5% in the European Region, and 3% in the American Region.[2] India is one of the 22 high-burden countries. It bears the share of 26% of global cases with TB incidence of 2.5 million as notified cases in 2011. Though, about 80% of TB patients suffer from pulmonary tuberculosis, the incidence of extra-pulmonary manifestations is also high (1 in 5 patients).[3] India ranks 2nd in the world and accounts for about 10% of the global burden of HIV associated TB.[4]

The microbiological diagnosis of pulmonary TB by direct sputum smear microscopy plays a key role in routine diagnosis of TB and treatment follow up in Tuberculosis Control Programs in India. For a smear to be positive, at least 5000-10,000 bacilli per ml of sputum must be present.[5] The simplicity, inexpensiveness and predictive power of Ziehl – Neelsen (ZN) sputum smear microscopy makes it the applicable laboratory diagnostic tool of choice for tuberculosis in low resource settings but, the sensitivity of this method is low (43-60 %) when compared with that of the cultures.[6] The sensitivity of this technique is further reduced in paediatric and HIV (20-35%) patients because HIV mediated immunosuppression leads to impaired granuloma formation, resulting in both ineffective containment of *M. tuberculosis* bacilli and diminished formation of pulmonary cavities and lower concentrations of bacteria in sputum. Frequent smear negative cases exacerbate the difficulty of detecting HIV associated TB resulting in the death. The sensitivity of direct smear microscopy is low in children because their sputa harbour lower number of acid fast bacilli.[7]

There are several other methods that can be used to improve sensitivity of detection of *M. tuberculosis* such as culture (LJ, MGIT, other liquid media), but these methods are limited by a long processing time and high cost. Newer molecular techniques like

PCR, though rapid, are too expensive to be widely applied in resource limited settings. Mycobacterial culture is the gold standard method for detection of tubercle bacilli with the sensitivity ranging from 70% to 80%. In this study we performed a prospective evaluation direct and concentrated smear microscopy on three early morning sputum specimens from patients suspected of having tuberculosis.

MATERIALS & METHODS

This is a prospective observational study were conducted in the Department of Microbiology, Vardhman Institute of Medical sciences, Pawapuri, Nalanda, Bihar, India.

Inclusion Criteria

A total of 100 patients including HIV patients, of all age groups and sex, presenting to the OPD for the first time, with clinical suspicion of pulmonary or extrapulmonary TB was included in the study. All the demographic details of the patients were noted and consent was taken from patients.

Exclusion Criteria

- Patients already taking antitubercular drugs and/or quinolone.

Verbal and written informed consents were obtained from the participants. They were informed of the main objective of the study and were requested to sign the form if they agreed to participate in the study and were assured of confidentiality of any disclosures.

A total of 300 early morning sputum samples were collected from 100 patients (3 samples for each). The time and irregularity of TB patients prompted us to use a consecutive sampling method where we took all accessible samples during our research data collection. The patients were instructed to produce about eight to ten ml of sputum and collect in a wide mouthed sterile container without soiling it. The samples were transported and processed within 2 hours, in class 2 Biological Safety Cabinet by using Personal Protective Equipments (PPE).

Digestion and decontamination procedures were used in processing sputum for examination and culture of sputum specimens. The concentration technique that was used is sodium hypochlorite 5 % overnight sedimentation method. The supernatant was discarded, the sediment mixed with the remaining fluid and smeared onto a labeled slide and then stained with Ziehl Neelsen technique.[8] The slides were examined under oil immersion (x100 objective). Acid-fast bacteria appear fine red rods against a blue background, and non-acid-fast bacteria (and other organisms and cellular materials) appear blue. The negative slides were considered if there are no acid-fast bacilli in 300 fields.[9] Pellets from direct specimens were inoculated on Lowenstein Jensen medium then the cultures were incubated for eight weeks. They were examined every seven days for possible growth. M. tuberculosis appears as brown granule colonies.[10] The sensitivity and specificity of the direct and concentrated smear microscopy techniques were calculated using culture result as gold standards.[11]

RESULTS

Our study showed that the18 patients under 15 years of age only 2 (2%) patient had positive smear, while 16 (16%) negative smear, and 5 (5%) positive on culture and 13 (13%) negative on culture. In 40 patients between 15 and 40 years old only 8 (8%) had positive smear, 32 (32%) negative smears, 4 (4%) positive on culture and 34 (34%) negative culture. In 41 patients over 40 years old only 6 (6%) had positive smears, 36 (36%) negative smears and 5 (5%) positive on culture and 34 (34%) negative on culture. 5 (5%) samples were characterized by non-mycobacterial colonies; therefore they were considered as contaminated. (Table 1) While the table 2 shows the results of concentrated sputum smear microscopy and culture according to age. In 18 patients under 15 years old only 5 (5%) had positive smear, 13 (13%) negative smear, 5 (5%) positive on culture and 13 (13%) negative on culture. In 40 patients between 15 and 40 years old only 7 (7%) had positive smear, 33 (33%) negative smears, 4 (4%) positive on culture and 34 (34%) negative smears. In 42 patients over 40 years old only 6 (6%) had positive smears, 36 (36%) negative smears and 5 (5%) positive on culture and 34 (34%) negative on culture. 5 (5%) samples were contaminated on culture.

Table 1: Comparison of results between direct smears and culture according to age

Age	Direct technique		Culture		
	Positive	Negative	Positive	Negative	Contaminated
< 15 years	2 (2%)	16 (16%)	5 (5%)	13 (13%)	0 (0%)
15 – 40 years	8 (8%)	32 (32%)	4 (4%)	34 (34%)	2 (2%)
>40 years	6 (6%)	36 (36%)	5 (5%)	34(34%)	3 (3%)
Total (%)	16 (16%)	84 (84%)	14 (14%)	81 (81%)	5 (5%)

Table 2: Comparison of results between concentrated smears and culture according to age

Age	Concentration technique		Culture		
	Positive	Negative	Positive	Negative	Contaminated
< 15 years	5 (5%)	13 (13%)	5 (5%)	13 (13%)	0 (0%)
15 – 40 years	7 (7%)	33 (33%)	4 (4%)	34 (34%)	2 (2%)
>40 years	6 (6%)	36 (36%)	5 (5%)	34(34%)	3 (3%)
Total (%)	18 (18%)	82 (82%)	14 (14%)	81 (81%)	5 (5%)

DISCUSSION

Direct microscopy of sputum is still the backbone for diagnosing pulmonary tuberculosis, the study aimed at increasing the sensitivity of tuberculosis diagnosis by concentration after pre-treatment with sodium hypochlorite which also makes sputum samples safe to be handled by laboratory workers.

In patients under 15 years old, sputum concentration technique showed a difference comparing to the direct smear microscopy (100% vs. 62.5%, C.I = 95%, P < 0.05) this difference is in agreement with the findings found in Kenya[12] where the sensitivity was (26.7%vs 21.7%,CI= 95%, P< 0.05). Our findings are also in agreement with the results found currently in Mindouli Hospital in Republic of Congo[13], where the sensitivity of direct and concentration technique in pediatric age was totally different (47.9%vs 87.5%, 95% CI 6.5-18.6, P = 0.001).

These above results from two studies are similar to our findings because their participants were in same age groups (pediatric age < 15 years) and we used the same concentration method. Generally regardless of age groups sputum concentration technique is more sensitive than direct (90.9% vs.80%, difference =10.9%, C.I= 95%, P = 0.001). Our findings are in accordance with the study done in India [10] where their results in both methods were (13.02% vs. 23.13%, difference = 7, 11%, CI=95%, P=0.001021). This similarity is explained by the use of the same concentration method (using 5% Sodium hypochlorite) and the smears were read by two observers separately to avoid observer's bias. Our findings are also in accordance with the study done in Ethiopia/Adiss Abbaba.[9] About the difference in sensitivity of direct and concentration technique, their results in both methods were (25%vs 34% , difference = 11% C.I= 95%,P=< 0.001) whereas our results in both method concentration and direct techniques were (77.77% vs.87.5%, difference =10.9%, C.I= 95%, P = 0.001). This similarity is explained by the use of the same sampling method (consecutive method) where we all used the available patients in the research period and we used also the same sampling criteria where the inclusion criteria were all sputa collected in early morning and 3 samples were required at the same time and needed to fulfill the requirements of good sputum such as purulent sputum.

CONCLUSION

We concluded that the sensitivity of the concentration technique was markedly increased in pediatric age (< 15 years), this increase has influenced the overall sensitivity in all patients. Considering the low cost and safety of the technique and greater sensitivity, this method can be of vital importance at least for patients under 15 years of age with negative smears on direct technique.

REFERENCES

1. Vaidya R. Tuberculosis. In: RajVirBhalwar, Chief Editor. Text Book of Public Health and Community Medicine, 2nded. Published by Department of Community Medicine, Armed Forces Medical College Pune in Collaboration with WHO, India Office, New Delhi; 2009:1107-6.

2. Mukhopadhyay B, Ganguly NK. Tuberculosis research in India. Current Science. 2013;105:594–6.

3. Sachdeva KS, Kumar A, Dewan P, Kumar A, Satyanarayana S. New vision for Revised National Tuberculosis Control Programme (RNTCP): Universal Access-"reaching the un-reached.".Indian journal of medical research. 2012;135:690-4

4. Central TB Division, Directorate General of Health Services, New Delhi. Tuberculosis Epidemiology-India. In: TB India 2012, Revised National Tuberculosis Control Programme, Annual Status Report. p. 7-11.

5. Kashyap B,et al. Validation of bleach optimization for smear microscopy in pulmonary tuberculosis in resource-constrained settings. Journal of pharmaceutical and biomedical sciences.2012;24:21-5.

6. Tadesse M, Abebe G, Abdissa K, Bekele A, Bezabih M, Apers L et al. Concentration of Lymph Node Aspirate Improves the Sensitivity of Acid Fast Smear Microscopy for the Diagnosis of Tuberculous Lymphadenitis in Jimma, Southwest Ethiopia. PLOS ONE. 2014;9:e106726.

7. Klautau GB, Kuschnaroff TM. Clinical forms and outcome of tuberculosis in HIV-infected patients in a tertiary hospital in Sao Paulo - Brazil. Braz J Infect Dis.2005;9:464–78.

8. Angeby KA, Hoffner SE, and Diwan VK. Should the 'bleach microscopy method' be recommended for improved case detection of tuberculosis? Literature review and key person analysis, Int J Tuberc Lung Dis, 2008, 8:806.

9. Merid Y, Yassin MA, Yamuah L, Kumar R, Engers H, Aseffa A. Validation of bleach-treated smears for the diagnosis of pulmonary tuberculosis in Ethiopia, Int J Tuberc Lung Dis, 2009, 13(1):136.

10. World Health Organization. "Epidemiology". Global tuberculosis control: epidemiology, strategy, financing. 2009.

11. World Health Organization: Global tuberculosis control 2008: surveillance, planning, financing. Geneva: WHO; 2008.

12. Maryline Bonnet, Andrew Ramsay, Willie Githui4, Laramie Gagnidze, Francis Varaine, and Philippe J. Guerin: Bleach Sedimentation as An opportunity to optimize Smear Microscopy for Tuberculosis Diagnosis in Settings of High Prevalence of HIV, Clinical Infectious Diseases, 2008, 46:1710–6.

13. Hepple P, Nguele P, Greig J, Bonnet M, Sizaire V. Direct microscopy versus sputum cytology analysis and bleach sedimentation for diagnosis of tuberculosis: a prospective diagnostic study, BMC Infect Dis, 2010, 21;10:276.

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