YIELD OF DIRECT VERSUS CONCENTRATED SPUTUM MICROSCOPY FOR DIAGNOSIS OF PULMONARY TUBERCLOSIS

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ABSTRACT

Objective: To evaluate the yield of Mycobacterium tuberculosis in suspected pulmonary tuberculosis patients by concentrated sputum microscopy against direct sputum microscopy.

Material and Methods: In the present descriptive cross sectional study total seven Hundred and fifty six (n=756) samples were collected from all those patients who were suspected to have pulmonary Tuberculosis and all samples were performed in the TB control laboratory Lady Reading Hospital Peshawar. Direct sputum smear was prepared from all the samples and stained with Z.N stain. In Similar way all samples were parallel processed by concentrated techniques (N-acetyl L-cysteine –NaOH centrifugation) and smear was prepared from all samples and examined under microscope. Yields of both laboratory investigation tools were compared and statistical analysis of the data was analyzed. Chi-square and odd ratio were used for measurement of comparison between variables.

Results: Out of 756 suspected TB patients 459 (60.7%) were male and 297 (39.3%) were females. The ages of the study group ranged between 11 - 100 years and average age being 34 years. In the present study 622 (82.3%) patients were negative and 134 (17.7%) patients were positive for pulmonary TB by direct sputum smear microscopy while 610 (80.7%) patients were negative and 145 (19.1%) patients were positive for TB by concentrated smear microscopy. Result of this study revealed that only 0.9 times improvement by concentrated sputum smear microscopy with N-acetyl L-cysteine –NaOH technique against the result of direct sputum smear microscopy.

Conclusion: Statistical analysis of the study shows that less significant differences were seen in yield of direct sputum smear microscopy versus concentrated smear microscopy. only 0.9 times improvement by concentrated sputum microscopy (N-acetyl L-cysteine –NaOH technique) against the result of direct sputum smear microscopy of the study.

Recommendation: In present study the concentrated sputum smear microscopy with N-acetyl L-cysteine –NaOH techniques was used, however it shows no significant result. It is recommended that other techniques may be studied for a comparative analysis with direct smear microscopy.

Key Words: Pulmonary Tuberculosis (TB), Concentrated (NALC-NaOH) Smear microscopy, Direct Sputum Smear microscopy, Z.N Stain.

INTRODUCTION

Tuberculosis (TB) is very old infectious disease, caused by M. tuberculosis.[1] TB is a major worldwide public health problem and 9 million new cases documented and two millions deaths occurred due to this disease each year[2]. It is the second most frequent infectious disease in the Pakistan affecting about 620,000, people.[3]

Clinical diagnosis of TB includes history of exposure to TB patients, fever, complaint of cough for more than three weeks, chest pain, anorexia, weight loss and hemoptysis.[4] Laboratory investigation of TB is made by multiple diagnostic tools such as direct sputum smear microscopic examination, Fluorescence microscopy, concentrated sputum smear microscopy, TB ICT method, Tuberculin skin test, histopathological diagnosis of TB, TB culture, Mycodot IgM /IgG, T-Spot TB Test and PCR for Mycobacterium tuberculosis (MTB).[5]

Florescent microscopy is used for screening population.[6] Fluorescence microscopy required special type of staining reagents (Auromine and Rhodium) and Fluorescent microscope. TB culture on Lowenstein-Jensen (LJ) medium and this method considered as gold standard method for diagnosis of TB but drawback of this method is that Mycobacterium tuberculosis takes long time up to 12 weeks to grow.[7] In tuberculin skin test, an antigen of TB is injected intra dermis to observe cell-mediated response at 48 to 72 hours.[8] However in immune-deficient individuals this test might give false negative results as occurred in sarcoidosis, miliary TB and Acquired immunodeficiency syndrome (AIDS).[9] Histopathological TB diagnosis of biopsy reveals case-

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ating granulomatous inflammation as similar histological pattern can also observed in other clinical conditions like Syphilis, Brucellosis and Toxoplasmosis. MTB PCR can be used for diagnosis of TB.[10] however there are several drawbacks of this method such as its high cost, requirement of highly skilled technologist to run the test and it is not yet proved to be the gold standard method. T-Spot TB Test and MycodotIgM / IgG has similar drawbacks as that of PCR method.

Sputum by concentrated techniques (N-acetyl L-cysteine –NaOH centrifugation) is commonly used both in the under developed and developed countries. Sputum concentration techniques by homogenization and slide prepared from their sediments, microscopic examination increase the AFB detection sensitivity. Many concentration techniques using different chemicals and physical methods such as sedimentation or centrifugation have been reported.[11] Sedimentation can significantly increase detection of AFB comparatively to direct microscopy.[12]

Several studies were conducted on concentration methods used different concentrated techniques like Bleach homogenization method, Modified Petroff's method, Phenol ammonium sulfate sedimentation method, ReaSLR Method.[13] Many studies results showed that concentration methods can significantly increase diagnosis of AFB compared to direct sputum smear microscopy.

The hypothesis of the present study is that concentrated sputum smear microscopy provides better results than the direct sputum smear microscopy in diagnosis of pulmonary tuberculosis. To test the hypothesis the specific objective of the study is to evaluate the yield of mycobacterium tuberculosis in suspected pulmonary tuberculosis patients by direct sputum microscopy against concentrated sputum microscopy. The aim of this study was comparison of yield of direct sputum microscopy and concentrated sputum microscopy by N-acetyl L-cysteine –NaOH centrifugation method for diagnosis of pulmonary tuberculosis.

MATERIAL AND METHODS

The present study was a descriptive cross sectional and conducted in Peshawar for the comparative diagnostic tools of pulmonary tuberculosis through direct versus concentrated sputum smear microscopy techniques. The total period of the present study was sixth months after approval of synopsis and submission of thesis. Total 756 suspected Tuberculosis patients were enrolled in present study. All those patients who have history of previous Anti Tuberculosis Treatment (ATT) or Multi Drug Resistance (MDR) were excluded. More than 5 ml sputum samples were collected in open mouth screw capped, sterile and disposable plastic container from all subjects at TB center Gunj, TB center LRH, and District TB Control Office Peshawar City. Direct sputum smear was prepared from all the collected samples.[14] In similar way all samples were parallel processed by concentrated method (N-acetyl L-cysteine –NaOH centrifugation) smear microscopy method.[15] All slides were stained with Z.N stain and smear was examined under microscope. Interpretational result was compiled according to WHO criteria for AFB as shown in Table 1. All collected data was recorded and analyzed in SPSS-20. Chi-square and odd ratio were used for measurement of comparison between variables. P value is less than 0.05 it was consider as statistical significant.

RESULTS

Total number of 756 (n=756) pulmonary Tuberculosis suspected patients were enrolled in this study among them 459 (60.7%) were male and 297 (39.3%) were females. Pulmonary suspected patient's ages ranged between 11-100 years and average age being 34 years. The study population was further sub-categorized into three age groups.

1. Group A (12-19 years of age)
2. Group B (20-40 years of age)
3. Group C (>= 50 years of age).

In the present study 9.1% patients were group A, 73.1% were group B patients and 17.7% were C patients. Group B was most frequent AFB suspected group of studied population.79.71% of group A patients were AFB positive and 20.29% patients were AFB negative. In group B 80.65% patients were AFB positive and 19.35% patients were AFB negative while in Group C 81.34% Patients was AFB positive and 18.66 patients were AFB negative. No significant differences were seen in prevalence of tuberculosis in different age's group.

In present study 240 females were diagnosed as AFB negative and 57 females were positive for AFB. In

Table 1: WHO reporting criteria for AFB slide after Z.N staining

S. No.	AFB Count	Oil Immersion field	Reporting
1.	No AFB	Per 100 fields	Negative (No AFB/100 fields)
2.	01-09 AFB	Per 100 fields	Record exact figure/100 fields
3.	10-99 AFB	Per 100 fields	Positive (1+) (01-99 AFB/100 fields)
4.	01-10 AFB	Per field	Positive (2+) (01-10/per field) in 50 fields
5.	>10 AFB	Per field	Positive (3+)>10 AFB / field in 20 fields

Table 2: Yield of concentrated smear microscopy verses direct sputum smear microscopy

		Results		Total
		Negative.	Positive	
Concentrated smear microscopy	Count	610	146	756
	% Results	(49.5)	(52.1)	(50.0)
Direct smear microscopy	Count	622	134	756
		(50.5)	(47.9)	(50.0)

Table 3: Degree of AFB positivity and subtypes in different age groups

Age Categories		Results (Groups)					Total
		Negative	1-9	1+	2+	3+	
12-19 years age	Numbers	55	2	2	9	1	69
	% within Result	(9.0)	(16.7)	(3.6)	(20.9)	(2.9)	(9.1)
20-49 years age	Numbers	446	7	44	29	27	553
	% within Result	(73.1)	(58.3)	(78.6)	(67.4)	(77.1)	(73.1)
=/> 50 years age	Numbers	109	3	10	5	7	134
	% within Result	(17.9)	(25.0)	(17.9)	(11.6)	(20.0)	(17.7)
Total	Numbers	610	12	56	43	35	756
	% within Result	(100)	(100)	(100)	(100)	(100)	(100)

male group 370 were AFB negative and 89 were positive. There was no significant difference in prevalence of tuberculosis among male and female tuberculosis suspected patients with P value 0.512 (Chi-square test).

Comparative analysis

In the present study 622 (82.3%) patients were negative for AFB and 134 (17.7%) patients were positive by direct sputum smear microscopy and 610 (80.7%) patients were negative for AFB and 145 (19.1%) patients were positive by concentrated smear microscopy as shown in Table 2. Data Analysis shows that less significant differences were seen between this two diagnostic tools for pulmonary Tuberculosis. Only 0.9 times improvement by concentrated sputum microscopy (N-acetyl L-cysteine –NaOH technique) against the result of direct sputum smears microscopy of the study. (P value= 0.233, Chi-square test). The positive AFB results of both techniques were further sub divided into four subgroups.

A. 1-09 acid fast bacilli/ per 100 fields.

B. (+) 10-99/ per 100 fields.

C. (++) 1-10/ per field.

D. (+++) >10/ per field.

In all AFB positive patients subtypes A: was less prevalent type only 12 cases reported while subtype B: were high prevalent 56 cases were reported. Subtype C & D were 43 and 35 positive cases were reported respectively. AFB positive subtypes in different age groups were shown in Table 3.

DISCUSSION

In under developed countries like Pakistan with high tuberculosis burden, pulmonary TB diagnosis is based on direct sputum smear microscopy after Z.N staining instead of TB culture due to high cost and time consumable. Direct sputum smear microscopy have some limitations one of them is that when acid-fast bacilli exist < 5000–10000 / ml of sputum cannot be detectable.[16] Previous study suggested that pulmonary TB suspected patients must expectorate proper sputum from deep of lungs to obtain the high-AFB density sputum in the submitted specimen.[17] To enhance AFB diagnosis by direct sputum smear microscopy technique, the only option for TB diagnosis in rural health centers in Pakistan, it is needed that this method of diagnosis may be improved.

Multi diagnostic approaches have been suggested to enhance AFB diagnostic sensitivity by direct sputum smear microscopy including, serial sputum specimen examinations, sputum visual assessment and early morning sputum specimen examination. These all diagnostic approaches may be helpful to enhance the TB diagnosis by direct smear microscopy.[18]

Visual assessment of sputum specimen by highly trained technologists is very useful and easy than microscopic grading for screening of specimens. Sputum specimen visual assessment rejected only 0.3% AFB positive specimens while microscopic grading criteria (squamous epithelial cell count) rejected only 30-66% AFB suspected specimen that contained 6-12% AFB positive samples. Serial sputum examination enhances

AFB diagnostic sensitivity 2% and 5% in second and third sample. Serial sputum examination is helpful in TB control programs, and AFB diagnosis.[19] Direct sputum smear microscopy can also be enhanced by fluorescence microscopy and concentrated sputum techniques. Fluorescence microscopy is more preferable method comparative to direct smear microscopy because it has greater sensitivity than conventional microscopy and has similar specificity.[20]

To modify and improve the direct sputum microscopy techniques is interesting for scholars/researchers and can be introduced easily in rural TB health centers of Pakistan. Recently several concentration techniques are used like NALC–NaOH sedimentation method, NALC–NaOH centrifugation method, Phenol ammonium sulfate sedimentation method, Bleach homogenization method, Modified Petroff's method 13 etc.

Results of the present study differs from the results of Uddin et al (2013) study who found that NALC–NaOH centrifugation method enhance up to 12% AFB diagnostic sensitivity by using TB culture as the gold standard method.[21]

Cattamanchi et al (2010) research results are same with the results of present study which shows that N-acetyl L-cysteine –NaOH centrifugation technique against direct smear sputum microscopy enhances only 1% AFB diagnostic sensitivity in those TB patients who were co-infected with HIV.[22]

Sharma et al (2012) study result revealed that Petroff's and modified Petroff's method has no significant interferences on AFB diagnostic sensitivity but valuable in TB culture comparatively culture from direct samples.[23]

Pulmonary Tuberculosis (TB) laboratory diagnosis can be enhanced by using ReaSLR technique. Sheetal et al (2013) carried out a study on improving AFB diagnostic sensitivity by ReaSLR technique. A total of 150 pulmonary tuberculosis suspected patients were enrolled in the study. Sputum samples were processed by ReaSR technique and modified petroff method and TB culture by L.J media as gold stander. The study results revealed that 12% samples were AFB positive detected by modified Petroff technique while 31.33% were AFB positive with the ReaSLR technique, and this differences was statistically significant with $P < 0.001$. The ReaSLR technique and modified Petroff technique results were also compared with direct smear microscopy that results revealed that increase AFB diagnostic sensitivity and specificity of 90.47% and 91.6% respectively. Modified Petroff method increases sensitivity and specificity of 40.47% and 99.07%, respectively using TB culture as the gold standard method. The Sheetal et al concluded that ReaSLR technique is more sensitive than the conventional method for sputum smear microscopy. Further more studies are needed to determine other aspects of this technique. ReaSLR kit is not available at rural TB centers due to cost effects and Kit supply services.[13]

CONCLUSION

Analysis shows that less significant differences were seen in yield of direct sputum smear microscopy versus concentrated smear microscopy. 0.9 times improvement by concentrated sputum microscopy (N-acetyl L-cysteine –NaOH) technique against the result of direct sputum smear microscopy of the study. The 0.9 times increase is evenly distributed between 11-100 year age samples.

RECOMMENDATION

In present study the concentrated sputum smear microscopy with N-acetyl L-cysteine –NaOH techniques was used, however it shows no significant result. It is recommended that other techniques may be studied for a comparative analysis with direct microscopy.

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