Smear microscopy and conventional culture on Lowenstein – Jensen media in cases of Tuberculosis - A comparative study

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Abstract

Introduction: To compare the smear stained by ZN stain and culture on Lowenstein Jensen media for the diagnosis of tuberculosis from various clinical samples. It is a laboratory based retrospective study done at Viswabharathi Medical College, Kurnool from February 2015 to February 2016.

Materials and Method: A total of 604 samples were collected from patients suspected of tuberculosis clinically. Samples were processed by ZN smear staining and culture on Lowenstein Jensen media.

Results: A total of 604 samples were processed out of which 565 were sputum samples and 39 were extra pulmonary samples which included pus, lymph node aspirates, synovial fluid, and endometrial curetting. Pulmonary samples yielded 18.76% smear positivity and 20.88% culture positivity .Extra pulmonary samples were 7.69% smear positive and 15.38% culture positive. Overall smear and culture positivity was slightly higher in males than in females.21-40 years age group was found to be affected the most.

Conclusion: In our study we conclude that though Culture on Lowenstein-Jensen media is time consuming but it still remains gold standard for detection of tuberculosis. In some centres, more so in developing countries where advanced investigative techniques are not available smear and culture can be very useful in tuberculosis diagnosis.

Keywords: Mycobacterium tuberculosis, Ziehl Neelsen (ZN) staining, Lowenstein Jensen (LJ) media, Pulmonary tuberculosis, Extra pulmonary tuberculosis.

Introduction

Tuberculosis is disease which could have been controlled but still remains a public health problem globally even though effective anti-tubercular drugs are available. It has been an important cause of morbidity and mortality especially in developing countries, among which India is one of the countries with highest burden of tuberculosis.(1) Global estimation of new cases of tuberculosis was found to be around 8.8 million out which 3.9 million are sputum smear positive which indicates high infectivity.(2) Global mortality due to tuberculosis ranges from 1.6 to 2.2 million lives per year.(3) World-wide prevalence of tuberculosis including in India is found to be higher in males when compared to females.(4) Tuberculosis was controlled to a large extent in developed countries but it has been rising in recent times. It could be due to emergence of drug resistant strains or association with immune suppression condition like AIDS. A new target was made to eradicate tuberculosis by 2030 as a part of post 2015 global TB strategy. To meet this target, early diagnosis of the disease and timely treatment are required. Delay in diagnosis may aggravate the disease, enhances the transmission and increases the mortality rate.(5) A presumptive diagnosis is essential to limit the spread of infection, for treatment guidance and to know the activity of the disease.(6)

Newer methods like BACTEC and molecular methods (Gene X pert) are very effective and give rapid results but they may not be accessible to many laboratories due to their high cost and advanced technology.(7) In centres where more specific investigations like BACTEC, Gene X pert are not available, smear microscopy and conventional culture methods can be used for screening, diagnosis and treatment of tuberculosis.

Smear microscopy can give rapid results but requires at least 10,000 bacilli/ml in the specimen to be seen. Growth from culture on Lowenstein Jensen media takes longer time (6-8 weeks) but still it can be considered as gold standard.(8) In developing countries where the prevalence of tuberculosis is maximum, productive age group responsible for generation of income is most affected contributing to the low socio economic status which again is the predisposing factor for tuberculosis.

Materials and Method

This retrospective study was conducted in a tertiary care hospital, Viswabharathi Medical College, Kurnool district, AP, India. The study group included clinically suspected cases of pulmonary tuberculosis attending both outpatient clinic and inpatients of pulmonary medicine department during the period February 2015 to February 2016.Symptoms seen in clinically suspected patients included prolonged cough for more than 2 weeks, night sweats, moderate fever, anorexia, haemoptysis and weight loss. Different specimens collected were sputum, pus, lymph node aspirate, synovial fluid, endometrial curetting. Sputum samples were processed by Petroff's method for decontamination and concentration.

Petroff's method: Sputum was mixed thoroughly with equal volumes of 4% sodium hydroxide, centrifuged and the sediment was neutralized with 8% hydrochloric acid.

Smears were made from all the samples and stained with Ziehl - Neelsen (ZN) stain. Primary staining was done with strong carbol fuchsin for 5 minutes. Slide was heated intermittently till fumes appear. Decolourisation was done with 20% sulphuric acid and counterstain with 0.3% Loefflers methylene blue for 1 minute. Under oil immersion objective Mycobacterium tuberculosis is seen as long, slender, beaded red coloured acid fast bacilli.

Culture: All the samples were inoculated on Lowenstein Jensen media, inspected for growth regularly for about 8 weeks. Mycobacterium tuberculosis produces rough, tough and buff colonies.

Results

A total of 604 samples were processed during the period February 2015 to February 2016. Out of the 604 samples processed 565 were pulmonary samples, remaining 39 were extra pulmonary samples. All the samples were subjected to smear examination by ZN staining and culture on L-J medium.

Out of the total 565 pulmonary samples 106 (18.76%) were smear positive, 118 (20.88%) were culture positive (Table 1). 39 extra pulmonary samples were smear positive in 3(7.69%) and culture positive in 6 (15.38%) samples (Table 1). ZN Smear sensitivity, specificity, positive predictive value and negative predictive value for pulmonary and extra pulmonary samples were calculated, and were found to be high for pulmonary samples. (Table 2)

In the present study we observed that smear and culture positivity were slightly higher in males when compared with females in both pulmonary and extra pulmonary samples. The age group which was observed to show maximum positivity was 21-40 years both in males and females (Tables 3 and 4).

Extra pulmonary samples included pus, lymph node aspirates, synovial fluid, and endometrial curetting. From the pus samples, 2(10%) were smear positive, 5(25%) were culture positive. From lymph node aspirate 1(9.09%) sample was positive by smear and culture. Over all positivity in extra pulmonary samples was 3 (7.69%) and 6(15.38%) by smear and culture respectively.(Table 5)

Table 1: Frequency of AFB from smear and culture

Sample type	Total samples	Smear positive	Culture positive	Smear positive/culture positive	Smear negative	Culture negative	Smear negative/culture positive
Pulmonary	565	106	118	106(18.76%)	459	447	12(2.12)
Extra pulmonary	39	3	6	3(7.69%)	36	33	3(7.69%)
Total	604	109	124	109	495	480	15

Table 2: Sensitivity, specificity, PPV, NPV of AFB smear microscopy for pulmonary and extra pulmonary specimens

Specimen	Sensitivity	Specificity	PPV	NPV
Pulmonary	89%	100%	100%	97%
Extra pulmonary	50%	100%	100%	91.6%

Table 3: Distribution of smear by ZN staining according to age and gender

Age in years	Samples				Positive			
	Pulmonary		Extra pulmonary		Pulmonary		Extra pulmonary	
	Male	Female	Male	Female	Male	Female	Male	Female
<20	24	24	6	5	2	-	-	-
21-40	68	62	7	5	33	21	2	-
41-60	184	89	6	5	28	12	1	-
>60	92	22	4	1	7	3	-	-
Total	368	197	23	16	70 (19.02%)	36 (18.27%)	3 (13.04%)	-
Grand total	604				106		3	

Table 4: Distribution of culture according to age and gender

Age in years	Samples				Positive			
	Pulmonary		Extra pulmonary		Pulmonary		Extra pulmonary	
	Male	Female	Male	Female	Male	Female	Male	Female
<20	24	24	6	5	2	-	-	-
21-40	68	62	7	5	35	24	3	-
41-60	184	89	6	5	31	13	1	1
>60	92	22	4	1	9	4	1	-
Total	368	197	23	16	77 (20.92%)	41 (20.81%)	5 (21.73%)	1 (6.25%)
Grand total	604				118		6	

Table 5: Distribution of smear and culture in extra pulmonary samples

Sample	Total Number	Smear positive	Culture positive
Pus	20	2(10%)	5(25%)
Lymph node aspirate	11	1(9.09%)	1(9.09%)
Synovial fluid	6	-	-
Endometrial curetting	2	-	-
Total	39	3(7.69%)	6(15.38%)

Discussion

Tuberculosis continues to be a major health problem with high mortality even today. Therefore it calls for a huge concern with respect to early diagnosis and treatment from the grass root level.

In this study 565 pulmonary samples were processed of which 18.76% were smear positive which coincides with studies conducted by Mk Munir[9] (16.91%), Ravish Kumar Muddaiah[10] (16%), Jain et al[11] (18.6%), Claude Mambo Muvunyi[18] (17.3%). But this study is not in agreement and has slightly higher result than in study by Roohi Aftab[3] (10.3%) and Arsalan Ahmad Salam[12] (11.33%).

Out of the 565 pulmonary samples 20.88% were culture positive which is comparable with studies conducted by Sajjad Iqbal[13] (25.84%), Mustafa Ulukaligil[14] (23.1%), Peter Daley P[15] (20.2%). But our results were not in coincidence with studies by MK Munir[9] (66.23%) and Roohi Aftab[3] (10.3%).

Smear positivity from extra pulmonary samples (7.69%) can be compared with study by R.C. Kesarwani[16] (11.1%). But it is slightly more than in the study by M.K. Munir[9] (3.97%). Culture from extra pulmonary samples can be compared with study by M.K Munir[9] (21.16%), but it is less than in study by R. C. Kesarwani (33%). In both pulmonary and extra pulmonary samples prevalence of tuberculosis was found to be more in males than females which is similar to the observation in study by Raja Rao.[4] Age group most commonly affected was 21-40 years which is the maximum income generating period.[17,18]

Conclusion

The present study concludes that AFB smear examination is cost effective, technical staff can be trained easily, gives rapid results and can be very useful for screening. Though time taking, culture on Lowenstein Jensen medium can still be considered gold standard and should be used for confirmation of the diagnosis where ever facilities are available. Advanced investigations like Gene X pert can detect drug resistance also but require huge investment and highly trained staff. This may lead to delay in diagnosis. Early detection has the advantage of preventing spread in the community. Especially in developing countries in centres where advanced investigations may not be available, smear and culture may be key to diagnosis and treatment of tuberculosis without delay. Early diagnosis and treatment may contribute in reducing global burden of tuberculosis.

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