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Comparison of Ziehl-Neelsen Smear Microscopy and AFB Culture in a Resource Limited Setting from Various Clinical Samples

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ABSTRACT

Background: Tuberculosis is one of the leading causes of morbidity and mortality in the developing countries. It is considered a great challenge for clinical microbiologists to detect and identify mycobacterium tuberculosis. Ziehl-Neelsen (ZN) smear is a rapid diagnostic tool for TB, Lowenstein Jensen (LJ) medium culture is the golden diagnostic tool for TB in the developing countries.
Objectives: To evaluate the reliability and validity of ZN smear of acid fast bacilli when compared to the culture on LJ medium.
Design and setting:- This study was conducted from 1st April 2016 to 31st march 2019 in the tuberculosis section of Microbiology department MGM Aurangabad, Maharashtra state.
Patients and Methods:- The comparative cross sectional study included patients visiting OPD clinics and wards of MGM medical college and hospital Aurangabad.
A total of 218 samples/specimens from various clinical sites were processed for both direct smear and culture. The direct smears were stained with ZN method [(1%) carbol fuchsin, (25%) sulphuric acid, (0.3%) methylene blue] according to RNTCP. Specimens were observed under 100x oil immersion lens, while the culture were inoculated on LJ medium which were collected aseptically and were expected to have no contaminants. Samples were decontaminated where ever applicable.
Results:-Out of total 218 patients 3(1.376%) were smear positive, while 35 (16.055%) were culture positive Out of the (03) smear positive specimens 1 (33.333%) was found to be growth positive on LJ culture. A total of 34 (15.596%) were negative on the smear but were found to the positive on LJ culture for mycobacterium tuberculosis. A total of 215 (98.623%) specimens were negative on the smear and LJ culture for mycobacterium tuberculosis.
Conclusion:- This study indicated low sensitivity of sputum smear direct microscopy for early diagnosis of TB, while LJ culture remains the golden standard modality.

Keywords: *Tuberculosis, ZN Smear, LJ Culture Golden Standard*

INTRODUCTION

Tuberculosis (TB) remains a public health challenge with nearly 2 billion persons (~29% of the world`s population) exposed to *Mycobacterium tuberculosis* annually and 8 million new cases of TB diagnosed each year , resulting in 2 million deaths (WHO, 2006).[1] Tuberculosis (abbreviated as TB for Tubercle bacillus or Tuberculosis) is common and often deadly infectious disease caused by mycobacteria in humans, mainly by M.tuberculosis.[2] This age old disease, including multidrug resistant (MDR) and extensively drug resistant (XDR)TB ,is no longer restricted to developing regions of the globe. People and the disease are mobile.[3]

Tuberculosis commonly affects lungs but can also be extrapulmonary. Hence microscopic examination of sputum for detection of acid fast bacilli is of utmost importance.[4] Hence for developing

countries with a large number of cases and financial constraints, evaluation of rapid and inexpensive diagnostic methods like demonstration of AFB (acid fast bacilli) in smears is of great importance. No other diagnostic tool offers the affordable as well as efficiency in diagnosis of tuberculosis in public health set up, as sputum microscopy does. In sputum smear microscopy, ZN is the most commonly used technique, because of its simplicity and low cost.[5]

Culture remains the gold standard for the diagnosis of TB, particularly in immunocompromised smear negative patient.[6] Parallel to the progress in automated systems much work has also been published on the development of rapid but economical methods which are equally effective and comparable to automated systems, especially for resource limited countries.[7]

MATERIALS AND METHODS

This comparative cross sectional study was carried out in January 2020 in MGM Medical College and Hospital in Aurangabad where data from 1st April 2016 to 31st March 2019 was collected, a total of 218 samples from various clinical sites was included in this study. Subjects were patients visiting OPD clinics and wards of MGM Medical College and Hospital, Aurangabad.

These samples were Sputum, Broncho Alveolar Lavage, Gastric Lavage, pus, ascitic fluid, Lymph node aspirate, ET secretion, swab, synovial fluid, tissue biopsy, CSF, CVP Tip, pleural fluid, omentum, pericardial fluid, abscess, others. A total of 218 samples were collected aseptically and were collected aseptically and were expected to have no contaminants, inoculated without decontaminations, samples were decontaminated where ever applicable.

Smear Microscopy (Z-N Stain): (1%) Carbol fuchsin, (25%) sulphuric acid,(0.3%) methylene blue was used according to RNTCP[4].A minimum of 100 oil immersion fields was observed to declare negative smear. More than 3AFB in observed 100 oil immersion fields were considered as positive for this study. Culture: Culture on Lowenstein Jensen medium was done .The culture are incubated at 37 degree Celsius in the dark and the light. The tubercle bacilli usually grow in 2-8 weeks. Results: Statistical analysis was applied to find sensitivity, specificity, positive predictive value, negative predictive value. This study was carried out after approval of the institutional ethical committee.

RESULTS

Table No. 1

Year	ZN Smear		L J Culture	
1st April 2016 to 31st March 2017 Specimens	Positive	Negative	Positive	Negative
Pulmonary Specimens (20)	00	20	09	11
Extra pulmonary Specimens (38)	01	37	10	28
1st April 2017 to 31st March 2018	Positive	Negative	Positive	Negative
Pulmonary Specimens (24)	01	23	02	22
Extra pulmonary Specimens (34)	00	34	02	32
1st April 2018 to 31st March 2019	Positive	Negative	Positive	Negative
Pulmonary Specimens (30)	01	29	02	28
Extra pulmonary Specimens (72)	00	72	10	62

A total of 218 specimens were processed in our tuberculosis laboratory in which 74 (33.944%) were pulmonary specimens and 144 (66.055%) were extrapulmonary specimens.

Table No. 2

Number of Patients	(Sex)	Z N Smear Positive	Z N Smear Negative	Culture Positive	Culture Negative
135	Male	02	133	19	116
83	Female	01	82	16	67

In the present study males were found to be more prone to tuberculosis as compared to females.

Table No. 3

AGE GROUP	ZN smear POSITIVE	ZN smear NEGATIVE	CULTURE POSITIVE	CULTURE NEGATIVE	TOTAL NUMBER OF PATIENTS Tested
0-10	0	7	0	7	7
11-20	0	27	6	21	27
21-30	1	42	6	37	43
31-40	2	33	4	31	35
41-50	0	25	4	21	25
51-60	0	34	5	29	34
61-70	0	30	6	24	30
71-80 and above	0	17	4	13	17

In the present study T. B. was not found to be diagnosed in the age group below 10 years of age as there is unavailability of purulent sputum in this age group. In our study we found that T. B. was present maximum in older age groups and also almost comparably followed by in the young, middle aged, economically most productive age groups.

Table No. 4

Serial No.	Type of Specimens/ Samples	Total number of samples	ZN Smear Positive	ZN Smear Negative	L J culture Positive	L J culture Negative
1	Pus	65	00	65	14	51
2	Sputum	59	02	57	13	46
3	Ascitic fluid	05	00	05	00	05
4	L N aspirate	04	01	03	02	02
5	ET Secretion	06	00	06	01	05
6	Swab	04	00	04	01	03
7	Synovial fluid	05	00	05	00	05
8	Brain tissue	01	00	01	00	01
9	Biopsy	05	00	05	00	05
10	Tissue	13	00	13	01	12
11	CSF	04	00	04	00	04
12	Bone	01	00	01	00	01
13	BAL	09	00	09	00	09
14	CVP Tip	01	00	01	00	01
15	Pleural fluid	26	00	26	02	24
16	Abscess	03	00	03	00	03
17	Vertebral lamina	01	00	01	00	01
18	Vertebral body	01	00	01	01	00
19	Omentum	02	00	02	00	02
20	Pericardial fluid	01	00	01	00	01
21	Synovium	01	00	01	00	01
22	Body fluid	01	00	01	00	01

Out of the total number of samples processed only 1.376% was Z N Stain positive distributed in various samples (i.e. pulmonary and extrapulmonary) of them 2 (2.702%) were sputum (pulmonary) samples and 1 (0.694%) extrapulmonary sample. A total of 35 LJ positive (specimens) where 14(18.918%) were pulmonary samples and 21(14.583%) were extrapulmonary samples.

Table No. 5

Z N Smear	LJ Culture Positive	LJ Culture Negative	Total
Positive	a 1	b 2	3 a +b
Negative	c 34	d 181	215 c+d
Total	a+c 35	b+d 183	218

Sensitivity = 2.857%, Specificity = 98.907%, Positive predictive Value = 33.333%, Negative Predictive Value = 84.186% for mycobacterium tuberculosis specimens.

DISCUSSION

In the present study males were found to be more prone to tuberculosis as compared to females. This is in accordance with other studies.[8]

In the present study T. B. was not found to be diagnosed in the age group below 10 years of age as there is

unavailability of purulent sputum in this age group. This is in accordance with other studies.[8] In our study we found that T. B. was present maximum in older age groups and also almost comparably followed by in the young, middle aged, economically most productive age groups.

Presumptive diagnosis of pulmonary tuberculosis is done by Acid Fast/ZN Stain Microscopy the most practical and fastest technique .The sensitivity and specificity of ZN staining and LJ culture varies depending upon the specimen's nature, its content of bacteria, quality, quantity and viable organisms extent.

Over all AFB smear positive for pulmonary specimens in the present study is 2.702% and it is low as compared to (IUATLD) International union against tuberculosis and lung disease.[9]In the pulmonary specimens AFB smear positivity is 2.702% and this study is not is agreement study with the study reporting the positivity of smears as 11.33% and 20.25% in pulmonary specimens.[9]

Positivity of smears of pulmonary specimens 2.702% is significantly high as in comparison to the extra pulmonary specimens 0.694% in this study and is almost comparable with the studies reporting the positivity of smears of 3.9% in extra pulmonary specimens.[10] This present study is not in agreement with the study that reported the positivity of smears as 20.25% in extra pulmonary specimens .In the extra pulmonary specimens the factors responsible for low smear positivity are its paucibacillary nature, inadequacy of samples and samples apportioning for various diagnostics tests results, non uniform distributions of micro-organisms.[10]

In our laboratory finding the ZN smear positivity number is low as compared to the other studies so we could find the sensitivity, specificity, positive predictive value and negative predictive value of the pulmonary and extrapulmonary specimens (as a whole) in toto and not individually. The sensitivity was calculated taking culture as the golden standard modality which was found to be 2.857% and specificity 98.907%. The positive predictive value and the negative predictive value being 33.33% and 84.186%.

The sensitivity is low as compared to N.A.M.Al. Rashidi et al with 95.2%[8], with M.K Munir et al as of pulmonary specimens as 66.23% & extrapulmonary specimens as 21.16%[10], with Shoukrie A et al as 47.09%[11], Marrie Y.C et al where various studies showed it to be even 50% and as low as 15% in the Mendoza et al and Kothadin et al respectively[12]. In K.P.Rao et al have stated that the sputum smear microscopy is far less sensitive and specific than culture[13].One reason being that the main problem faced with the extra pulmonary specimens is that they yield very few bacilli and so have low sensitivity of acid fast bacillus (AFB) smear and culture (S.Chakravorty et al).[14]

Whereas the specificity ,positive predictive value, negative predictive value are the same or comparable to the other studies.[8,9,10,11]LJ medium which is used for culture in the diagnosis of tuberculosis has been the gold standard in the developing countries for many years.[11]

In the present study an overall AFB culture positivity is 16.055% which is in agreement with 15.47% in Salam .A. A et al[8].They have also mentioned other studies with values of 12.3 % a little lower and a few others with that of 48.91% and 47.1% also.

Positivity of cultures in this study is significantly high in comparision to microscopy of AFB smears to yield positive results by AFB smear microscopy as about 5000 to 10000 AFB/ml of specimens is needed while about the culture on L J medium the advantage is that it is very specific having the sensitivity of 80-85% and can detect as few as 10 bacteria per mililitre of specimens[15,16]. Our study concludes that the sensitivity is low although AFB smear is rapid, cheap, specific test. Culture on L J medium is documented as gold standard and it is evident that it is more sensitive. It is less

expensive than radiometric and molecular methods and therefore can be a useful tool for developing countries. It is also suggested that facilities for carrying out culture of mycobacterium tuberculosis should be made available at individual level hospitals, especially (PHC) Primary Health Centers in India where cartridge based nucleic acid amplification Technique (CBNAAT) is not available, as CBNAAT is available at tertiary care centers only.

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