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HOW EFFECTIVE IS ZIEHL-NEELSON STAINING IN COMPARISON WITH LOWENSTEIN JENSEN CULTURE FOR DETECTING ACID FAST BACILLI?

[1]Dr. Muhammad Ali, [2]Dr. Nouman Anees, [3]Dr. Mariam Ilyas

[1]Student of MCPS (HCSM), College of Physicians and Surgeons Karachi, Pakistan.

[2]Indus Hospital, Manawan, Lahore

[3]DHQ Hospital Narowal

Abstract:

Objective: *To evaluate false negativity rate of Ziehl-Neelson smear microscopy.*

Methodology: *This descriptive study was performed at Mycobacteriology laboratory of Holy Family Hospital, Rawalpindi during June 2017 to March 2018. 3951 was study sample. Out of these 3951 cases, 2773 cases had pulmonary tuberculosis while 1178 were extra pulmonary tuberculosis cases. Study population was selected from suspected cases of tuberculosis referred to laboratories from the outdoor clinic of pulmonology as well as internal medicine department and neighboring referral health facilities. Follow up cases were not included in study sample. LJ media was considered the standard to test the false negativity rate of ZN smear microscopy.*

Results: *Most common sample was sputum 48.5%, followed by pleural fluid 12% and pus 8.3%, respectively. 23.1% samples were false negative out of which pulmonary samples were 19.6% and extra pulmonary samples were 29.2%. P value was statistically significant <0.001. False negativity rate of following samples was most common; pericardial (40%), synovial (38%), pleural fluids (33%) and pus (32%), respectively.*

Conclusion: *ZN staining technique has high false negativity rate, especially in patients with low mycobacterial load. National TB control program should adopt more efficient diagnostic methods for diagnosing tuberculosis instead of ZN staining.*

Keywords: *Tuberculosis, Ziehl-Neelson Smear, False negative, Microscopy.*

Corresponding author:

Dr. Muhammad Ali,

Student of MCPS (HCSM),

College of Physicians and Surgeons,

Karachi, Pakistan

QR code

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INTRODUCTION:

Tuberculosis is one of the major problems in subcontinent. During the year 2013, around 2 million deaths were estimated due to tuberculosis [1]. The causative agent *Mycobacterium tuberculi,* is an acid fast bacillus. Disease involves many organs and systems of body. The most common form of tuberculosis is pulmonary tuberculosis which spreads through droplet infection. Due to its easy transmissibility to the contacts this form of tuberculosis is the most common. Other forms of tuberculosis involve bones, lymph nodes, intestine, meninges etc.

In countries with higher tuberculosis burden, there is need for rapid and effective detection of mycobacteria from respiratory samples and other body secretions like pus, BAL, urine, CSF. A computer aided whole smear screening system has been launched in other countries which provides real time images and diagnostic grades from fluorescence microscopy as well as bright light microscopy, for detection of acid fast bacilli. This new computer based detection system has high sensitivity. In a research conducted by Law YN, et al 288 out of 488 patients were detected positive by using this technique. It also helped in reducing laboratory technicians' workload by reducing manual work [2].

A retrospective analytic cross sectional study was conducted in Tripoli- Libya by Shoukrie A, et al in 2018, in which sensitivity of ZN smear microscopy was compared with LJ media and it was concluded that ZN staining has very low sensitivity in detecting acid fast bacilli, LJ culture is the gold standard for detection of acid fast bacilli [3, 7].
Due to cost effectiveness and less time consumption, ZN smear microscopy is widely used in Pakistan for detection of acid fast bacilli. So, a need was felt to study the sensitivity of ZN smear microscopy. Pakistan has high tuberculosis burden, there is a need to launch more sensitive diagnostic techniques which can reduce the false negativity rate and help in accurate diagnosis of tuberculosis patients.

MATERIALS AND METHODS:

The study was conducted over the period of ten months in Holy Family Hospital, Rawalpindi, Pakistan. The objective and study details were approved by ethical review board of hospital research department. 3951 patients, who were referred for diagnosis of tuberculosis to mycobacteriology laboratory, from pulmonolgy and internal medicine departments and also from surrounding referral health centers were included in the study. Out of 3951 patients, 2773 were suspected cases of pulmonary tuberculosis while 1178 patients had extra-pulmonary tuberculosis. Pulmonary tuberculosis suspects presented with sputum, broncho-alveolar lavage samples, while extra-pulmonary tuberculosis suspects presented with pus, CSF or pleural fluid, ascetic fluid, pericardial fluid, synovial fluid and urine. Only those samples were enrolled which were referred by a registered medical practitioner, as strong tuberculosis suspect. The strong tuberculosis suspect was defined as productive cough for more than 3 weeks, with or without evening hyperthermia for more than 2 weeks, weight loss, chest x-ray findings suggestive of tuberculosis. Follow up cases, old TB cases who were under-treatment were excluded from study. Specimen collection was done under expert supervision following WHO protocols. ZN staining and LJ staining was performed on each specimen. After LJ staining, specimens were observed for 48 hours. Mycobacterial growth was observed after every week for 8 weeks.

Control slides of ZN stain were made from every batch of ZN. Each specimen was tested twice by two laboratory technicians under light microscope. Slide preparation was done according to WHO guidelines. Few random positive and negative slides and doubtful slides were counter-checked by expert microbiologist in order to reduce the error and to improve result quality. Culture media quality was tested by using ATCC (American Type Culture Collection) strains of H37rv.

Data analysis was done on SPSS version 21. False negativity rate of ZN smear was confirmed by using LJ culture media. Chi square test was applied. P-value <0.05 was considered statistically significant.

RESULTS:

Most common sample was sputum 48.5%, followed by pleural fluid 12% and pus 8.3%, respectively. 23.1% samples were false negative out of which pulmonary samples were 19.6% and extra pulmonary samples were 29.2% [table:1]. P value was statistically significant <0.001. False negativity rate of following samples was most common; pericardial (40%), synovial (38%), pleural fluids (33%) and pus (32%), respectively [table: 2].
The results of study are presented in the form of tables.

Table:1 False negativity rate of ZN staining.

Specimens			LJ positive	LJ negative	Total	Fp	Fn	Significance
Extra-pulmonary	ZN	Positive Negative Total	118 310 428	1 749 750	119 1059 1178	.8%	29.2%	Chi square 289.9 P value .0001
Pulmonary	ZN	Positive Negative Total	868 378 1241	8 1524 1532	876 1897 2773	.9%	19.6%	
Total	ZN	Positive Negative Total	986 683 1669	9 2273 2282	995 2956 3951	.9%	23.1%	

Table:2 ZN smear false negativity of each specimen.

Specimen				LJ positive	LJ negative	Total	Fp	Fn
Pulmonary	Sputum	ZN	Positive Negative Total	708 281 989	5 921 926	713 1202 1915	.7%	23.3%
	Broncho-alveolae lavage	ZN	Positive Negative Total	160 92 252	3 603 606	163 695 858	1.8%	13.2%
Extra-pulmonary	Urine	ZN	Positive Negative Total	3 12 15	0 37 37	3 49 50	0%	24.4%
	Synovial fluid	ZN	Positive Negative Total	1 13 14	0 21 21	1 34 35	0%	38.2%
	Pericardial fluid	ZN	Positive Negative Total	5 14 19	0 21 21	5 35 40	0%	40%
	Ascetic fluid	ZN	Positive Negative Total	7 20 27	0 108 108	7 128 135	0%	15.6%
	CSF	ZN	Positive Negative Total	11 17 28	0 85 85	11 102 113	0%	16.6%
	Pus	ZN	Positive Negative Total	26 97 123	0 206 206	26 303 329	0%	32%
	Pleural fluid	ZN	Positive Negative Total	65 137 202	1 273 274	66 410 476	1.5%	33.4%

DISCUSSION:

Per year almost 10.4 million new cases of tuberculosis appear across the globe and almost 1.4 million deaths occur due to it [4]. According to a survey conducted in 2017, 2 to 10% of population of Pakistan is suffering from tuberculosis, which means every 10 persons out of 100 has the tuberculosis and are a threat to spread the disease to contacts. The risk factors for tuberculosis are poverty, low socioeconomic status, over-crowding, smoking, immuno-compromised state, less effective use and practice of vaccination, less effective provision of health care to patients living in far flung areas [7,10].

A comparison between sensitivity of ZN smear and fluorescence microscopy in detection of AFBs was done by *Gupta S, et al.* and the conclusion was in favor of fluorescence microscopy, in regard to sensitivity [5]. Similar comparison was studied by *Laifangban S, et al.* in which false negativity rate of ZN staining was 40.8% while AO staining had only 2.3% false negativity rate [6]. Fluorescence

microscopy reduces the mycobacterium detection time to half in comparison with ZN staining. In addition, its sensitivity is better than ZN smear microscopy [8,9].

Scientists are looking for more efficient and less time consuming diagnostic techniques for detection of AFBs. Direct microscopy results were compared with the bleach sedimentation technique in a research study at Nigeria, in which sensitivity rates were 28.8% and 30.3% for both techniques, respectively. Hence it was proved that bleach sedimentation has better results than direct microscopy [11].

CONCLUSION:

ZN staining technique has high false negativity rate, especially in patients with low mycobacterium load. National TB control program should adopt more efficient diagnostic methods for diagnosing tuberculosis instead of ZN staining.

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