Original Article

The Ratio of Smear-Negative Tuberculosis in Sindh: An insight using Fluorescence Microscopy

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ABSTRACT

BACKGROUND: Sputum smear microscopy is the most useful tool used for the screening of presumptive tuberculosis cases. Conventionally Ziehl-Neelsen (ZN) based staining is used to microscopically screen sputum from the presumptive Tuberculosis Cases. However, Fluorescent microscopy (FM) is now gradually replacing the conventional ZN microscopy because of its higher efficiency and efficacy in detecting *Mycobacterium tuberculosis*. In patients, who do not produce sufficiently high number of bacilli, and are known as Smear-negative, efficacy of both types of microscopy remains questionable as no Mycobacteria are detected in the sputum of these patients and they are falsely labelled as smear-negative.

OBJECTIVE: This retrospective study was conducted on the patient samples received from different parts of Sindh to study the proportions of false-negative tuberculosis using fluorescent microscopy.

MATERIALS AND METHODS: A retrospective cross-sectional study was conducted from June 2014 to June 2016 at the Tuberculosis Laboratories of Dow University of Health Sciences. Data analyzed for 1439 samples received during 24-month period, which had subsequently been confirmed by culture. All laboratory work was performed as per guidelines of the National Program. Statistical analysis was performed on MedCalc® and Open Epi®.

RESULTS: During 24-month period, among the 1439 cases, 832(57.81%) were from males and 499 (34.67%) were smear negative. Culture showed that 72 (14.42%) were false-negative among all-negatives. Overall sensitivity observed was 92.21% [95%CI 90.29%-93.85%] and specificity 82.91% [95%CI 79.38%-86.06%]. The proportion of false negativity among all negatives in females (16.11%) was significantly higher (p=0.002) as compared to males (13.47%). Conclusions: Even with the introduction of Fluorescent Microscopy, smear microscopy cannot be solely relied upon for the diagnosis of tuberculosis, as considerable number of patients producing insufficient number of bacilli to be detected by microscopy.

KEY WORDS: Tuberculosis (TB), Microscopy, Culture, Fluorescence Microscopy (FM)

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INTRODUCTION

Pulmonary tuberculosis (TB) is a major health issue in the developing world[1]. Since it is spread by droplet infection, timely diagnosis, treatment and cure is of utmost importance[2]. Patients who are suspected of having tuberculosis, are referred for sputum smear microscopy to tuberculosis laboratories, where AFB microscopy using either ZN or FM techniques is performed. Despite being a costly alternative to the traditionally performed ZN staining[3], yet FM is gradually replacing ZN due to its higher efficacy and ease in detection.

Unfortunately, microscopy cannot be solely relied upon for the diagnosis as some patients do not produce sufficient number of bacilli in their sputum, which result in negative smear[4,5]. These patients must be seen in the light of clinical picture and further confirmed for the presence of tuberculosis using tuberculous culture or other appropriate tests[6] which is a time-consuming, labor-intensive and requiring good laboratory expertise.

The study was planned to get an insight about the proportion of false negative results in the local population. Data were analyzed for diagnostic sputum samples submitted for Tuberculosis screening through FM using Tuberculosis culture as a gold standard.

MATERIAL AND METHOD

A retrospective cross-sectional study was conducted at the Tuberculosis laboratory, Ojha Institute of Chest Diseases, Dow University of Health Sciences from June 2014 to June 2016. Data were de-identified and gathered using anonymization for all diagnostic 1439 samples received during 24-month period for microscopy and culture from presumptive Tuberculosis

patients belonging to different parts of Sindh Province (Population 42.4 million, Area of 140,914 km^2) [7].
Sputum slides were prepared using the standard World Health Organization (WHO) protocols and stained using Auramine O fluorescent stain. LED Microscopy was performed by expert and trained microbiologists and slides were graded using standard WHO protocols. Tuberculosis Cultures were performed on Löwenstein–Jensen medium, as per the specification of N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) method[11]. Media quality was ensured for Mycobacterial growth using standard American Type Culture Collection (ATCC) strains of H37Rv. Cultures were observed during the initial 48 hours of inoculation and then weekly for duration of eight weeks.
Data evaluated was from diagnostic smears only; any culture with contamination was excluded from the study. Cases with incomplete data, for example age of the patients, were also excluded.
Descriptive data analysis was performed using Microsoft Excel, while statistical testing was performed using online calculators. Sensitivity and specificity were calculated by online Medcalc® calculator using values for true and false positive and negative microscopic samples with 95% confidence interval (CI)[8]. A value of P=0.05 was considered significant. Z-test for comparison of proportions was performed using Medcalc® calculator with values of false negativity among all negatives[8]. Data were stratified for gender and age, and analyzed using chi-square test with Open-Epi® software[9].
Approval for data collection and analysis was taken from the department.

RESULTS

During 24-month period, among the 1439 samples, 832(57.81%) were from males and 607 (42.18%) were from females. Among all, 499 (34.67%) were smear negative (319 Males and 180 Females). Culture showed that 72 (14.42%) out of 499 were false-negative. Overall sensitivity was 92.21% [95%CI 90.29%-93.85%] and specificity 82.91% [95%CI 79.38%-86.06%]. On stratification with respect to gender showed that the proportion of false negative smear in females (16.11%) was significantly higher (p=0.002) as compared to males (13.47%).
A chi-square test of independence was performed to

TABLE I: GENDER-WISE DISTRIBUTION OF SMEAR AND CULTURE ANALYSIS OF PRESUMPTIVE TB PATIENTS

Culture Outcome	Male	Female	Total	X^2	*df*	*p*
True Positive	474	378	852	-	-	-
True Negative	276	151	427	16.49	3	0.000
False Positive	39	49	88	-	-	-
False Negative	43	29	72	-	-	-
	832	607	1439	-	-	-

True Positive= Smear +VE Culture +VE, False Negative= Smear -VE Culture +VE False Positive= Smear +VE Culture -VE, True Negative = Smear -VE Culture -VE
Chi square test performed using Open Epi ® , p= 0.05 taken as significant

TABLE II: AGE-WISE COMPARISON OF DIFFERENT GROUPS OF PATIENTS

	Males							Females						
Culture Outcome	<18	19-45	>46	Total	X^2	*df*	*p*	<18	19-45	>46	Total	X^2	*df*	*p*
True Positive	48	301	125	474	-	-	-	71	257	50	378	-	-	-
True Negative	20	139	117	276	24.39	6	0.000	35	72	44	151	34.67	6	0.000
False Positive	6	17	16	39	-	-	-	6	30	13	49	-	-	-
False Negative	4	24	15	43	-	-	-	12	13	4	29	-	-	-
	78	481	273	832	-	-	-	124	372	111	607	-	-	-

True Positive= Smear +ve Culture +ve, False Negative= Smear -ve Culture +ve , False Positive= Smear +ve Culture -ve, True Negative = Smear -ve Culture -ve ,
Chi square test performed using Open Epi ® , p= 0.05 taken as significant

examine the relationship between microscopy outcomes and gender of the patients. It was observed that microscopy outcomes were significantly associated with the gender of the patients (p=0.000) (Table I). On age and gender wise stratification of the data, chi-square test of independence performed to assess the relationship of microscopy outcomes with the patient age-group, results were statistically significant in both males (p=0.00) and females (p=0.000) (Table II).

DISCUSSION

Our results with fluorescent microscopy showed significant false-negativity among the both genders, with an overall false-negativity of 14.42% (Males: 13.47%; Females: 16.11%) with a higher ratio of false-negativity in females. This signifies that for every 100 patients seen in the clinic and microscopically labeled as smear negative, up to 15 patients could be Tuberculosis patients. Missing these patients could pose a serious public health hazard, as even low-AFB producers in the sputum have been reported to spread the disease. In case of high clinical suspicion, molecular tools such as PCR should be employed to confirm the diagnosis.

The sensitivity we observed was 92.21% [95% CI 90.29%-93.85%], which is identical to a study with ZN microscopy[10], which showed a sensitivity of 92.38%, though specificity was slightly less when compared to ZN microscopy, which could be explained by the fact that there are higher chances of labeling artifacts' as mycobacteria in fluorescent microscopy (82.91% Vs 88.26)[10]. Published studies performed in other settings show identical trends of comparable accuracy among the two methods.[11,12] though some of them have reported slight diagnostic advantage of FM over ZN microscopy[3,13], though FM has a distinct advantage of ease and time over ZN[12] and WHO recommends LED FM over classical ZN due to "qualitative, operational and cost advantages"[14].

Our results showed that microscopy outcomes are significantly associated with the gender and age of the patients, and being a male or a female or from a specific age group is associated with the capacity to be a producer of bacilli in the sputum, enough to be detected by microscopy. Although it had been pointed out earlier that males has higher odds ratio to be detected as smear positive when compared to female[15] yet not plausible explanation can be put forth and finding warrants further studies to probe the matter. Tuberculosis is considered a male-dominant disease in the world, with a higher diagnostic ratio in males as compared to the females to an extent that male gender was labeled as a risk factor for being diagnosed as a Tuberculosis patient[16,17].

CONCLUSION

Even with the introduction of Fluorescent Microscopy, smear microscopy cannot be solely relied upon for diagnosis of tuberculosis and the results should be carefully evaluated in the light of clinical picture due to considerable number of patients producing insufficient number of bacilli to be detected by microscopy.

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