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APPLICATION OF MODIFIED BLEACH METHOD IN DETECTION OF TUBERCULOSIS IN FINE NEEDLE ASPIRATES

Pathology

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ABSTRACT

Background: The Mycobacterial culture is the reference method for the diagnosis of Tuberculosis (TB), but requires specialized safety procedures and is time consuming. Newer molecular techniques like polymerase chain reaction, are costly for routine use. Therefore, in developing countries like India, the key role in diagnosis is played by the conventional Ziehl-Neelsen (ZN) staining for demonstration of acid-fast bacilli (AFB) by direct microscopy. The major disadvantage of this method is low sensitivity and if the sensitivity could be improved using methods such as bleach concentration, it is quite useful. **Methods:** The prospective study was done in the Department of Pathology, GMC, Amritsar in which 100 patients, with clinical suspicion of extra-pulmonary tuberculosis sent for FNAC were examined for cytomorphological features. The conventional ZN stain and modified-bleach concentration method smears were then examined for the presence of Acid-fast bacilli and were compared. **Results:** The AFB positivity by conventional ZN method was 34% (34/100) and AFB positivity by bleach concentration method was 47% (47/100). **Conclusions:** The detection rate of the bleach method is better (13% more) than the routine ZN stain. Thus, this can be a useful contributor to the diagnosis of tuberculosis, if combined with routine cytology.

KEYWORDS

bleach concentration, fine needle aspiration cytology, tuberculosis, conventional ZN stain

INTRODUCTION:

Tuberculosis is an infectious disease and a prevalent public health concern worldwide. The causative agent is Mycobacterium tuberculosis that transmits mainly from humans to humans by airborne route. According to WHO estimates (2016), the disease claimed the lives of 1.3 million individuals and impacted 10.4 million people [1]. The most common manifestation is pulmonary tuberculosis; while extrapulmonary tuberculosis is less frequent. The most often afflicted extrapulmonary locations are lymph nodes [2]. The primary method of diagnosing TB is clinical examination, which is then confirmed in a laboratory setting [3]. Culture on L. J medium is the gold standard investigation for the diagnosis, although it takes a lot of time [4]. Since more recent molecular methods, including polymerase chain reaction, are costly and time-consuming. In developing nations, AFB identification via direct smear examination remains a crucial diagnostic tool. Various studies have documented the importance of FNA in extrapulmonary lesions [5]. The nature of FN aspirates from tuberculous lesions has been described as blood admixed, cheesy, and purulent [6]. On cytology, tuberculosis shows three major cytomorphological patterns: Pattern 1, Pattern 2, Pattern 3 [7]. The traditional ZN staining technique for AFB is essential for tuberculosis diagnosis; however, its sensitivity is just 9% to 46% [8]. As a result, numerous researchers have experimented with a variety of approaches, to increase ZN method's sensitivity such as the bleach concentration method, which offers several advantages over traditional methods which is very simple to use [9]. This method significantly increases the number of bacilli visible under the microscope, also creating a clearer background that makes them easier to spot leading to faster analysis time [10].

Aims And Objectives:

1. To compare standard Ziehl-Neelsen (ZN) staining technique with bleach method of concentration for detecting tubercular bacilli in suspected extrapulmonary tuberculous lesions
2. To correlate cytomorphological features with AFB positivity (Direct and Concentrated smear).

MATERIALS AND METHOD:

The prospective study was done in the Department of Pathology, Government Medical College, Amritsar in which 100 patients, with clinical suspicion of tuberculosis underwent FNAC after fulfilling all the inclusion and exclusion criteria.

- **Inclusion Criteria:** All clinically suspected cases of tuberculous lesions referred for FNAC
- **Exclusion Criteria:** Inadequate samples and patients on antitubercular therapy.

Procedure: FNA was done in each case. The gross appearance of aspirate was noted. The smears were stained with H & E (Haematoxylin & Eosin); and Giemsa stains for routine cytology and were then examined for cytomorphological features under microscope.

The diagnosis was made based on the following cytomorphological features:

A. Reactive lymphadenitis: on the basis of polymorphous population of lymphoid cells comprising of small lymphocytes, centrocytes, centroblasts, immunoblasts and macrophages.
B. Suppurative lymphadenitis: on the basis of aspirated purulent material and abundant neutrophils and nuclear debris in a necrotic background.
C. Tuberculous lymphadenitis and Tuberculous abscess: on the basis of presence of epithelioid cell granulomas, caseous necrosis and multinucleated giant cells.

The cases diagnosed as tuberculous lymphadenitis and tuberculous abscess were further categorized as 3 major patterns:
Pattern 1: epithelioid granulomas, with caseous necrosis, ± giant cells.
Pattern 2: epithelioid granulomas without necrosis, ± giant cells.
Pattern 3: necrosis with or without scattered epithelioid cells.

Smears were air dried, and stained for routine ZN stain. The remaining aspirate in the needle was rinsed with 2ml of 5% NaOCl/bleach (sodium hypochlorite). After mixing properly, the sample was incubated in a sterile test tube for 15 minutes at room temperature with frequent mixing at intervals. 2ml of distilled-water was added and mixed thoroughly and centrifuged at 3000 rpm for 15 min. The supernatant was discarded and smears were made with a drop of sediment, air-dried, heat-fixed, and stained by routine ZN staining technique. The conventional ZN-stained smears and modified-bleach concentration method smears were then examined for the presence of AFB and were compared.

The data obtained was analyzed according to the standard statistical methods to reach a conclusion.

RESULTS:

In the present study, majority of the patients belonged to the age group 11-20 years (33%). Females were predominant (66%). The most common presenting symptom was lymphadenopathy seen in 98% of cases and out of that cervical lymphadenopathy was the commonest (66%) followed by submandibular lymph nodes (11%). One case each of back swelling and chest wall swelling was also noted.

Upon aspiration, 56% of patients had blood admixed aspirate, followed by purulent aspirate in 26% of cases and cheesy white aspirate in 18% of cases. On cytomorphology, out of 100 cases, 65% of patients were diagnosed as Tuberculous lymphadenitis, 2% diagnosed as Tuberculous abscess, adding up to total of 67% cases of extrapulmonary tuberculosis; 17% were diagnosed as Suppurative lymphadenitis (Fig.1), and 16% were diagnosed as Reactive lymphadenitis (Fig. 2).

Out of 67 diagnosed cases of tuberculosis, 43 (64%) patients showed cytomorphological pattern 1, 14 cases (21%) showed pattern 2, and 10 cases (15%) showed pattern 3 as shown in figures 3, 4 and 5 respectively.

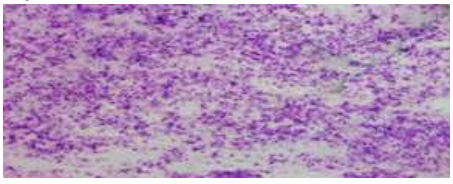

Figure 1: Suppurative Lymphadenitis (Giemsa; X100).

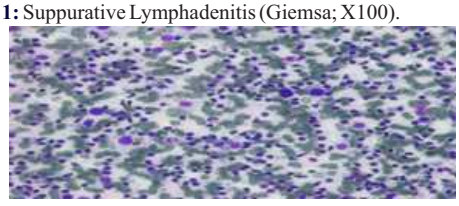

Figure 2: Reactive Lymphadenitis (Giemsa; X100).

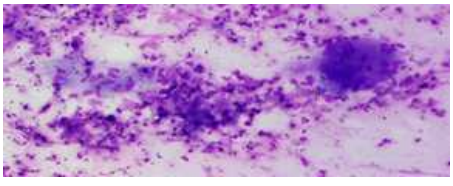

Figure 3: Tuberculous Lymphadenitis – Pattern 1 (Giemsa; X400).

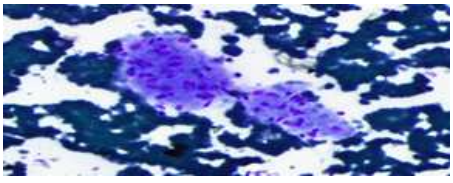

Figure 4: Tuberculous Lymphadenitis- Pattern 2 (Giemsa; X400).

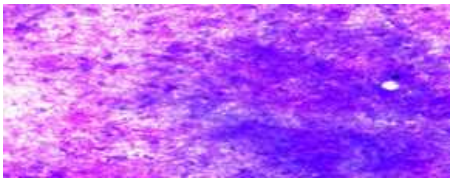

Figure 5: Tuberculous Lymphadenitis- Pattern 3 (Giemsa; X400).

Table 1: Comparison Of Conventional ZN Stain With Modified Bleach ZN Stain For AFB

		Modified Bleach ZN Stain for AFB		
		Negative	Positive	Total
Conventional ZN stain for AFB	Negative	53	13	66
	Positive	0	34	34
Total		53	47	100

p-value = 0.000, kappa = 0.735

As shown in Table 1, it was observed that 53 cases were negative for AFB on both the stains, 34 cases were positive for AFB on conventional ZN stain, and 47 cases (additional 13) were positive for AFB on modified bleach ZN stain. When both the staining techniques were compared with each other, a highly significant correlation (p-value = 0.000), and a substantial level of agreement (kappa = 0.735) between the two techniques was observed.

Table 2: Correlation Of Cytomorphological Diagnosis With The Conventional ZN Stain And Modified Bleach ZN Stain

Cytomorphological Daignosis	Conventional ZN Stain		Modified Bleach ZN Stain		
	Negative	Positive	Negative	Positive	Total
Reactive lymphadenitis	16	0	16	0	16 (16%)
Suppurative lymphadenitis	17	0	12	5	17 (17%)
Tuberculous lymphadenitis	33	32	25	40	65 (65%)
Tuberculous abscess	0	2	0	2	2 (2%)
Total	66 66.0%	34 34.0%	53 53.0%	47 47.0%	100 100.0%

Among 16 cases (16%) diagnosed as reactive lymphadenitis, all were negative for AFB by both the conventional and modified bleach ZN stain. 5/17 (29.4 %) cases diagnosed as suppurative lymphadenitis showed positivity for AFB by bleach method but were missed by the conventional ZN method; 34/67 (50.7%) cases diagnosed as tuberculosis showed acid-fast positivity by routine ZN stain whereas, 42/67 (62.7%) cases were positive by bleach method.

Table 3: Correlation Of Cytomorphological Pattern With Conventional ZN Stain And Modified Bleach ZN Stain

Cytomorphological Pattern	Conventional ZN Stain		Modified Bleach ZN Stain		
	Negative	Positive	Negative	Positive	Total
Pattern 1	18 42%	25 58%	11 26%	32 74%	43 100.0%
Pattern 2	12 86%	2 14%	11 79%	3 21%	14 100.0%
Pattern 3	3 30.0%	7 70.0%	3 30.0%	7 70.0%	10 100.0%
Total	33 50%	34 50%	25 37%	42 63%	67 100.0%

25/43 (58%) cases of pattern 1 showed acid fast positivity by routine ZN stain and 32/43 (74%) cases showed positivity for AFB by the bleach concentration method. 2/14 (14%) cases of pattern 2 were positive for acid fast bacilli by conventional ZN method, whereas the bleach detected AFB in 3/14 (21%) cases. 7/10 (70%) cases of pattern 3 showed positivity for AFB by both methods.

DISCUSSION:

As tabulated, 34% (34/100) cases were acid-fast positive by routine ZN staining. The bleach method for AFB was positive in 47% (47/100) cases. All positive conventional ZN stain cases were positive by the bleach method and in addition of 13 cases which were initially missed by conventional ZN stain. The increase in positivity for acid fast bacilli by the bleach concentration method in comparison with the conventional ZN method was in agreement with the studies done by Bhardwaj S et al. (2015) [10] ($k=0.7383$, $p<0.001$), and Mandal P et al. (2021) [11] ($p=0.000$).

In the present study, 16/16 cases of reactive lymphadenitis, were found to be negative for AFB by both the conventional and modified bleach ZN stain which is in accordance with the findings of Khan S et al. (2018) [12]. Whereas, other studies conducted by Annam V et al. (2009) [13], and Mandal P et al. (2021) [11] had found AFB in a few cases of reactive lymphadenitis by the bleach-method. This may be on the basis of loss of scattered epithelioid cells against the background of polymorphous lymphoid cells.

5/17 cases of suppurative lymphadenitis showed positivity for acid fast bacilli by bleach concentration method, whereas no case showed positivity by conventional ZN stain. This may be attributed to the

presence of abundant polymorphs and debris obscuring the visibility of AFB in routine ZN smears. The digestion of neutrophils and cellular debris by the bleach resulted in a clear background and thus easy screening of AFB. Similarly, other studies conducted by Annam V et al. (2009) [13] and Mandal P et. Al (2021) [11] also showed increased acid-fast positivity by the modified bleach method.

Out of the 67 cases of tuberculosis, 34 (50.7%) showed positivity for AFB by conventional ZN stain and 42 (62.7%) showed positivity for acid-fast bacilli by bleach concentration method. Similarly, other studies conducted by Khan S et al. (2018) [12], and Mandal P et al. (2021) [11] also showed increased acid-fast positivity by the modified bleach method.

On analyzing all the cytomorphological patterns (1,2,3). Overall, bleach method was able to detect AFB in more number of cases. This finding is in concordance with the studies done by Bhardwaj S. et al. (2015) [10] and Khan S et al [12].The overall positivity by both the conventional ZN stain and bleach concentration method was quite less in pattern 2 , due to an inverse relationship found between the presence of granuloma and acid-fast positivity, which is due to the ability of epithelioid cells (activated macrophages) to phagocytose and kill the microorganisms as observed by Prasoon D et al. (2003) [14].

Majority of the cases detected by the routine ZN method had few acid-fast bacilli. Whereas, by applying the bleach concentration method, the number of AFB per field increased. The background was much clearer making the bacilli easily visible thereby reducing the screening time. The similar findings were observed in other studies done by Annam V et al. (2009) [13] and Khan S. et al. (2018) [12].

CONCLUSIONS:

The routine ZN staining method showed AFB in less cases in comparison to the bleach method which was able to detect more number of bacilli as the background was clearer making the screening easier, faster, and less tedious. Moreover, bleach method is simple and cost-effective. We were able to establish higher AFB positivity by bleach concentration method as compared to conventional ZN method. Thus, this can be a useful contributor to the diagnosis of tuberculosis, if the bleach method is combined with routine cytology.

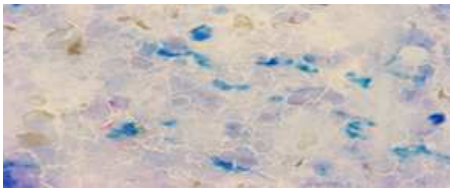

Figure 6: Tuberculous Lymphadenitis Showing AFB Positivity On Conventional ZN Stain (ZN; X1000).

Figure 7: Tuberculous Lymphadenitis Showing AFB Positivity On Modified Bleach ZN Stain (BLEACH ZN; X1000).

Figure 8: Suppurative Lymphadenitis Showing AFB Positivity By Modified Bleach ZN Stain (BLEACH ZN; X1000).

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