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Utility of Papanicolaou Stain Induced Autoflorescence in the Cytology of Suspected Tubercular Lymphadenitis

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Abstract

Background: Tuberculosis is one of the major public health problems in India and other developing countries.Tuberculosis is still considered a social disease; it reflects the standard of living in the community. The bacilli in the lymphnode can be detected microscopically by ZN stain and fluorochrome stain and culture methods. *Objectives:* To study the utility of Papanicolaou stain as a screening tool in detection of tubercle bacilli by microscopic method. *Method:*The prospectively study carried out at Department of Cytology, JJM Medical college Davangere, Karnataka between from September 2013 to February 2014. A total 104 cases of the lymph node FNAC were studied and stained by PIF, AO and Ziehl-Neelsen stained smears. Mycobacterial culture was used as the gold standard to compare the results. *Results:* Out of 100 specimens, 30%, 56% and 53 % were found positive by ZN, PIF and AO respectively. PIF was found to be superior to ZN on several aspects. Culture was positive in 19.04%. *Conclusion:* The efficacy of fluorescence microscopy proved to be much higher than conventional light microscopy and comparable to that of culture. Pap-induced fluorescence of lymph node smears is a safe, reliable and rapid method, which can prove as a valuable diagnostic tool for diagnosis of TB.

Keywords: Fluorescence Microscopy; Papanicolaou Stain; Fine Needle Aspiration; Tuberculosis.

Introduction

Tuberculosis is one of the ancient disease known to affect the mankind [1]. Lymphadenopathy is the most common form of extrapulmonary TB [2]. The clinical features in the diagnosis of tuberculosis lymph node are neither specific nor do their absence exclude TB involvement [3,4]. Moreover, the conventional Ziehl–Neelsen (ZN) method for demonstration of acid-fast bacilli (AFB) plays an important role in the diagnosis and monitoring the treatment of TB [5]. ZN stain is commonly used method to diagnosis tuberculosis. But sensitivity of this method is low in comparison with Auramine-Rhodamine(AR) Fluorescence stain [6]. AR flourescent method is well accepted screening tool for AFB but it adds additional smear stained with a special stain.

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Both fluorescence staining with auramine-rhodamine (AR) and Papanicolaou (PAP) have been considered to be superior to routine used ZN staining for demonstration of AFB [7]. Since papanicolaou stain is widely used in cytology, its use to induce fluorescence to demonstrate AFB saves time and material involved in the additional staining. Mycobacterial culture (Lowenstein- Jensen medium) is still the reference method for detection of tubercle bacilli, but it is time consuming and requires specialized safety procedures in laboratories [5].

The Aim of this Study

This study aims to find out the utility of Papanicolaou stain as a screening tool in detection of tubercle bacilli by microscopic method.

Materials and Method

This is a prospectively study carried out at Department of Cytology, JJM Medical college

Davangere, Karnataka between from September 2013 to February 2014. The study population was composed of cases of suspected tubercular lymphadenitis with cytomorphology suggestive of tuberculosis. Other cause of granulomatous reaction, such as actinomycosis,fungal infections, malignancy were excluded. A total of one hundred and four patients were included in the study. A detailed clinical history was observed and relevant investigation details, such as hemogram, Mantoux test, chest radiogram, were reviewed in these patients. Aspiration was perfomed with a 10 ml syringe attached to a 22- gauge needle. A small amount of aspirate was inoculated over Lowenstein-Jensen medium, and remaining material used for air-dried, alcohol and wet fixed smears. Three smears were prepared from each of the FNAC aspirates: one alcohol-fixed wet smear was stained by PAP stain for cytological examination directly, and second and third air-dried smears were stained with AR and ZN stains, respectively. In case of scanty aspirate, only one smear was prepared and examined for cytology followed by both AR and ZN techniques. The evaluation was performed by light and fluorescence microscope using an Olympus Trinocular Fluorescence Research microscope-BX-41 with blue/ green exitation .

The ZN stained smears were examined for a minimum of 15 minutes under an oil immersion lens at 1000X magnification. Acid fast bacilli identified as bright red,straight or slightly curved rods PIF smears and AR stained smears were examined for 10 minutes at 100X, 400X, 1000X using an oil immersion lens.Bacilli in PAP smears -bright yellow-green fluorescent, straight or slightly curved rods proportionately larger than those with ZN stain. Bacilli in AR smears - yellow to orange, slender, rod-shaped bacilli .

For mycobacterium culture lymph node sample were decontaminated and centrifuged by using 4% NaOH, according to modified Petroff method and inoculated into LJ medium. LJ media were incubated at 37°C and left in the slanted position for 7 days for even distribution of sample over the entire surface of the medium. The tubes are then placed upright and incubation at 37°C for 6-8 weeks. The colonies of M. tuberculosis were studied. For identification of M. tuberculosis rate of growth, colonies characters, acid fast bacilli staining and niacin test were used as per manufacture instruction.

Results

The present study includes 104 fine-needle aspirated specimens from lymph nodes. Of these, 100 specimens were evaluated and the remaining 4 were eliminated because aspirates identified malignancy. The age ranged from 2 to 65 years, with the mean age of 21.4 ± 12.5 years. Male preponderance was noted accounting for 60% (60/100) of cases. Among the 100 lymph nodes studied, aspirates were from cervical (n = 72), inguinal (n = 09), and axillary (n = 19) groups. The aspirates obtained were of 3 types: haemorrhagic, chessy and purulent. Based on cytomorphology, the tuberculous lymph node, was diagnosed on following criteria: Group I epitheloid granulomas without caseous necrosis (Group II) epitheloid granulomas with caseous necrosis (Group III) caseation or acute inflammatory exudate only (Group IV)occasional epitheloid cells without necrosis/giant cells. Out of 100 cases the clinically diagnosed tubercular patients, AFB positivity by ZN staining was 30 cases(30%), while it was 56 cases(56%) with PIF in 53 cases(53%) with AR staining. The highest AFB positivity was seen group III, while the lowest seen in group II(Table 1).

Table 1: Comparison between ZN,PIF and AR Positivity of various cytomorphological pattern in lymph nodes

Cytomorphological pattern	Number of cases	AFB Positivity ZN	PIF	AR
Group I	22(22%)	05(22.7%)	11(50 %)	10(45.4%)
Group II	51(51%)	15(29.4%)	25 (49.01%)	24(47 %)
Group III	24(24%)	09(37.5%)	18(75%)	17(70.8%)
Group IV	03(3%)	01(33.3%)	02(66.6%)	02(66.6%)
Total	100	30(30%)	56(56%)	53(53%)

Table 2: Correlation between ZN staining, PIF and AR staining with culture

Culture	Total number of cases	ZN		PIF		AR	
		Positive	Negative	Positive	Negative	Positive	Negative
Positive	08	08	0	6	2	7	1
Negative	34	12	22	15	19	16	18
Total	42	20	22	21	21	23	19

Table 3: Comparison of the conventional ZN method with the AR stains of AFB

Conventional ZN method	Auramine-Rhodamine		Total
	Positive	Negative	
Positive	30(75%)	10(25%)	40
Negative	20(34%)	40(66%)	60
Total	50	50	100

Table 4: Comparison of the conventional ZN method with Papanicolaou induced flourescence for AFB

Conventional ZN method	Papanicolaou induced flourescence		Total
	Positive	Negative	
Positive	38(95%)	02(5%)	40
Negative	36(60%)	24(40%)	60
Total	74	26	100

Culture was done in 42 cases, with overall culture positivity of 8 cases(19.04%).There was a good correlation between ZN staining PIF and AR when compared to the gold standard of culture (Table 2).

Of the 100 aspirates, the smear positivity for acid fast bacilli on the conventional ZN method was 40% (40/100) while the positivity increased with AR stain 75%(30/40) and Papanicolaou induced flourescence 95% (38/40) (Table 3 & 4).

The sensitivity and specificity of the ZN staining were 94% and 68.4%, while those of PIF were 95% and 75.2% and AR method were 92 % and 75% respectively thus correlation between ZN staining and PIF, AR showed that PIF detected more bacilli than the ZN staining.

Discussion

Diagnostic tools currently available for rapid diagnosis of tuberculosis have remained largely the same since the discovery of the tubercle bacillus more than have a century ago [8]. For developing countries with a large number of cases and financial impairment, evaluating the rapid and inexpensive diagnostic methods like demonstration of acid fast bacilli (AFB) in smears has great importance [1].

In the early 1940s, the comparison of the fluorescent method with the conventional ZN method on smears was implemented to increase the smear positivity for the detection of AFB. The use of a fluorochrome on acid-fast bacilli staining , such as AR, is recommended because of its increased sensitivity and ease of interpretation compared with the ZN method [9].

In ourstudy the overall AFB positivity by PIF (56%) and AR(53%) was more than that of ZN staining (30%). The advantage of fluorescent staining is superior than that of ZN staining in the presence of a less bacterial load as seen in smears with diagnosis of cytomorphologial features of tuberculosis, in problem areas like AIE (acute inflammatory exudates) alone or with occasional granuloma, AFB positivity by ZN staining is nearly as better as the fluorescent method because bacterial load is high [11,12]. The fluorescent technique is economical in both time and expense and recommended for laboratories handling large number of sputum specimens [10].

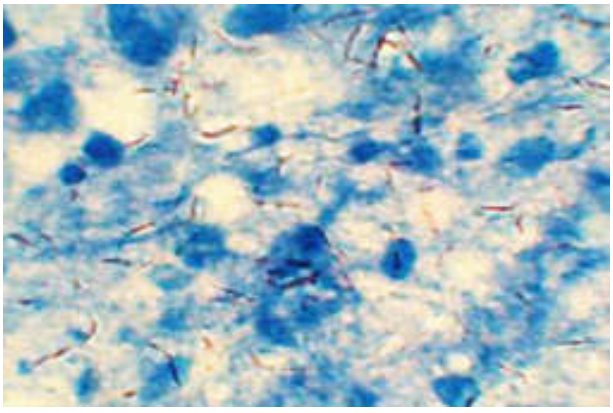

Fig. 1: Smear showing acid fast bacilli under ZN bright red,straight, slightly curved rods x40

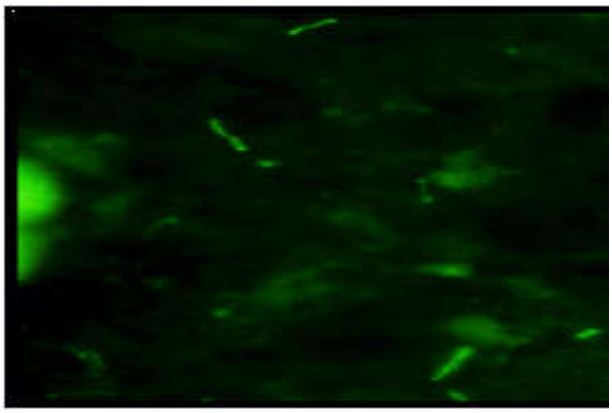

Fig. 2: Smear showing acid fast bacilli typically fluorescing as Bright yellow-green,straight, slightly curved rod shaped under Papx1000

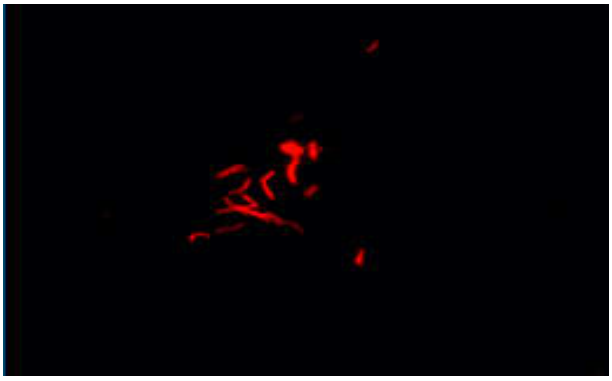

Fig. 3: Smear showing acid fast bacilli typically fluorescing as orange, slender, rod-shaped bacilli under ARx1000

Using AR staining, the tubercle bacilli examined under ultra violet light , the bacilli appeared as a yellow to orange slender rod shaped, but they may appear curved or bent against a dark back ground. As there was a contrast, the bacilli were readily seen and therefore in very less time large area could be examined, superior, involves toxic and carcinogenic substances [13].

The sensitivity and specificity of both methods were calculated for 42 cases in which cultures were done. The lowest values for specificity (75.2%, 75% and 68.4% for PIF, AR and ZN respectively and much higher values for sensitivity(95.2%,95% and 94%)respectively were not due to false positivity or false negativity but were due to very low culture positivity (19.04%) in our study. The possible reason for low culture positivity was most of the patients treated with antitubercular drugs, lack of good quality culture facilities to maintain temperature.

In the present study on lymph node aspirates in cytology, the positivity rates were 40% for the ZN method and 95% for PAP , 75% for the AR stain and 19.04% for culture . Similar results are obtained Githui W et al [14] sputum smears are positivity rates for both the ZN and AR methods as 65% and 80%, respectively. In Laifangbam S, Singh HL, Singh NB, Devi KM, Singh NT [15] in their study of pulmonary tuberculosis patients, found that ZN and AR methods as 44.1%, 71.6% which is closely correlated with our study, where AR was found to be 55% more effective than ZN staining and thus shows that fluorochrome staining of lymph node smears in comparison to that of ZN staining is a better method of microscopy. Jungare A et al [8] shows positivity rates for both the ZN and PIP methods as 30.8% and 40.6% respectively which correlates with our study of positivity rates for both the ZN and PIP methods as 40% and 95%.

In this study, cultures were positive in 19.04% cases. On evaluation of the microscopic techniques by comparing them with the gold standard culture method, we found that in case of ZN stain there was agreement in 63% cases and disagreement in 37% whereas for AR stain there was agreement in 95% cases and disagreement in 5% cases. This proves that AR stain is a best technique for its close comparability to the gold standard method.

A comparison between sensitivity and specificity of PIF microscopy is difficult, as pointed out in the literature, because the material used is different like bronchial secretions and pleural fluid samples.

We concluded that AR and PAP fluorescent staining is more sensitive than the conventional ZN staining for demonstration of Acid Fast Bacilli. Since screening is done under low power of magnification (10X), fluorescence has been found to be less time consuming method compared with ZN method (100X) in the diagnosis of tuberculosis. Hence, it could be beneficial when the fluorescent method is used as an adjuvant along with clinical parameters and cytological features in lymph node aspirates. Culture examination is gold standard technique , no doubt, is more reliable but is time consuming, expensive and requires trained technical hands. The efficacy of fluorescence microscopy proved to be much higher than conventional light microscopy and comparable to that of culture. Both staining methods are easy and cheap and may be used effectively instead of doing difficult, expensive and time consuming.

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