International Journal of Otorhinolaryngology and Head and Neck Surgery
Krishna M et al. Int J Otorhinolaryngol Head Neck Surg. 2016 Apr;2(2):66-69
http://www.ijorl.com
pISSN 2454-5929 | eISSN 2454-5937

Research Article

DOI: http://dx.doi.org/10.18203/issn.2454-5929.ijohns20160956

Tuberculous mycobacteria bacilli fluorescence and compare with Ziehl- Neelsen stain in fine-needle aspiration cytology of tubercular lymphnode

Mani Krishna[1]*, Adesh Kumar[2]

[1]Department of Pathology, [2]Department of Pulmonary Medicine, U.P. RIMS & R Saifai Etawah, Uttar Pradesh, India

Received: 30 December 2015
Accepted: 04 February 2016

***Correspondence:**
Dr. Mani Krishna,
E-mail: drmanikrishna@gmail.com

ABSTRACT

Background: Tuberculosis is infectious disease caused by *Mycobacterium tuberculosis*. There are various methods for diagnosis of tuberculosis such as direct clinical material examination of tubercular bacilli by Ziehl – Neelsen (ZN) staining, demonstration of tubercular bacilli by auramine – rhodamine (AR) staining and autofluorescence (AF).
Methods: Present study was done clinically suspected tubercular patients. All received samples Zn stain, fluorescent stain and PAP stain were applied.
Results: Among the clinically suspected patients 88 was diagnosed with tuberculosis. Female preponderance was noted accounting for 60.23% (53/88) of cases. Of the 88 aspirates, the smear positivity for acid fast bacilli (AFB) on the ZN method was 37.5% (33/88) while the positivity increased to 81.82% (72/88) on the AR fluorescent method and 86.36% (76/88) on AF.
Conclusions: AF staining is more sensitive than the auramine – rhodamine fluorescent and ZN staining in demonstration of mycobacterium bacilli in fine needle aspiration cytology of tubercular lymphnode.

Keywords: Tuberculosis, *Mycobacterium tuberculosis*, AFB, ZN staining, AR staining, AF

INTRODUCTION

Tuberculosis continues to be a major health problem in developing countries. Tuberculosis is one of the oldest diseases known to affect humans and is caused by Mycobacterium tuberculosis. Lymphadenopathy is the most common presentation of extra-pulmonary tuberculosis.[1,2] Tuberculous lymphadenitis can be presumptively diagnosed morphologically on fine-needle aspiration cytology of lymph node. Fine-needle aspiration cytology is now widely utilized as a first line diagnostic procedure in the diagnosis of palpable masses, including peripheral lymphadenopathy. Its value in the diagnosis of mycobacterial lymphadenitis in adults is well documented. Fine-needle aspiration cytology is a simple effective and safe modality for obtaining a representative sample of material from a lymph node and the diagnosis of mycobacterial adenitis can be confirmed utilising a number of different investigations, including cytomorphology, specific stains to identify the organism, culture and polymerase chain reaction. However, culture is essential for obtaining a definitive diagnosis. Unfortunately, culture is time consuming and expensive. Koch first described the tubercle bacilli in 1882 which is now known to *Mycobacterium tuberculosis*. *Mycobacteria* are now known to comprise a large group of acid – fast, alcohol – fast, aerobic or microaerophilic, non – spore forming, non-motile bacilli.[3] However, the Ziel-Neelsen method for acid-fast bacilli plays a key role in the diagnosis and also for the monitoring of treatment in tuberculosis. Its major disadvantage is low sensitivity

ranging from 20% to43%.[4,5] Serological techniques have the disadvantage of lack of sensitivity and specificity.[4] Newer molecular techniques such as polymerase chain reaction, although rapid, are costly to be routinely used in developing countries where most TB cases occur.[6] Newer investigative methods are required. Fluorescent microscopy plays an important role for detection of Mycobacteria because lower magnifications are used as well as less time is required to examine smears. Flurescence microscopy using auramine-rhodamine (AR) or papanicolaou (PAP) staining has been considered to be superior to ZN staining.[7,8] The method is quick and inexpensive. The efficacy of autofluorescence and fluorescence in the diagnosis of tuberculous lymphadenitis was evaluated for this purpose.

METHODS

This study was conducted in department of Rural Institute of Medical Sciences and research Saifai Etawah (U.P), India from January to July 2015. A total of 212 patients having peripheral lymphadenopathy were referred for FNAC to cytology lab of the department of pathology. Four smears were made from each aspirate: three air dried smears were stained with Giemsa, ZN, and AR stains and one was wet fixed for PAP stain for autofluorescence. AR and PAP stained slides were examined under fluorescent microscope. By ZN stain AFB stain pink curve or straight beaded rods against blue background and by AR stain AFB appears as bright reddish-yellow fluorescing rods against a dark background and by PAP stain AFB stained mycobacteria fluoresce as brilliant yellow bacilli, thin and slightly curved.

RESULTS

A total of 212 fine-needle aspirated specimens from lymph nodes were included in the study. Out of 212 cases, 88 aspirates were reported as cytomorphology suggestive of tuberculous lymphadenitis. The age ranged from 1 to 70 years, with the mean age of 35.5 years. Female preponderance was noted accounting for 60.23% (53/88) of cases (Table 1). 68.18% (60/88) of the cases with suggestive cytomorphology of tubercular lymphadenitis were in the range of 11-30 years of age. Depending upon cytomorphological features, granuloma 75 (85.23%), necrosis 70 (79.55%), acute inflammation 25 (28.41%), lymphoid background 21 (23.86%), giant cell 15 (17.05%) were found in tuberculous lymphadenitis (Table 3). In present study, the most common site of involved lymph nodes was of the cervical region in 64.77% (57/88) of the cases (Table-2). Of the 88 aspirates, the smear positivity for AFB on the ZN method was 37.5% (33/88) while the positivity increased to 81.82% (72/88) on the AR fluorescent method and 86.36% (76/88) on AF (Table-4).

Table 1: Age and sex distribution of tubercular lymphadenitis.

Age	Male	Female	Total
0-10	2	3	5
11-20	9	17	27
21-30	15	18	33
31-40	3	8	11
41-50	3	4	7
51-60	2	2	4
61-70	1	1	2
Total	35 (39.77%)	53 (60.23%)	88

Table 2: Site distribution of tubercular lymph node.

Site	Number (%)
Cervical lymph node	57 (64.77%)
Axillary lymph node	8 (9.09%)
Supraclavicular lymph node	4 (4.55%)
Cheek	1 (1.14%)
Submandibular	7 (7.95%)
Submental	2 (2.27%)
Chest	4 (4.55%)
Inguinal lymph node	1(1.14%)
Other	4 (4.55%)
Total	88

Table 3: Cytomorphological patterns.

Cytomorphological patterns	Number	Percentage
Granuloma	75	85.23%
Necrosis	70	79.55%
Lymphoid background	21	23.86%
Acute inflammation	25	28.41%
Giant cell	15	17.05%
Non documented	4	4.55%

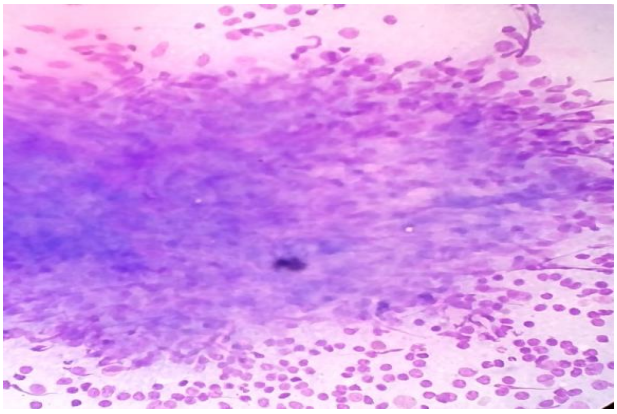

Figure 1: Well-formed granuloma in Giemsa stain 40x.

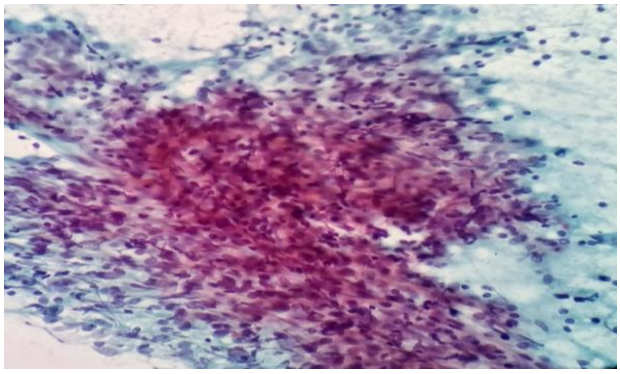

Figure 2: Well-formed granuloma in PAP stain 40x.

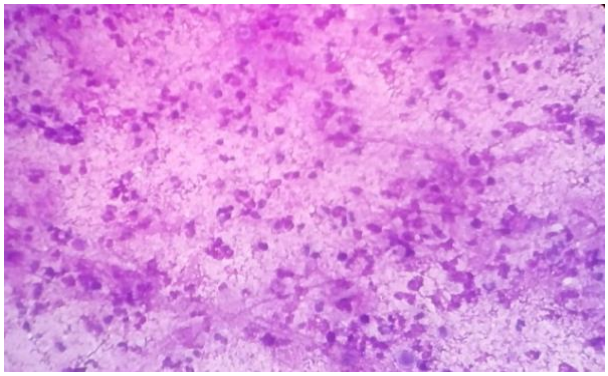

Figure 3: Acute inflammation with necrosis in Giemsa stain 40x.

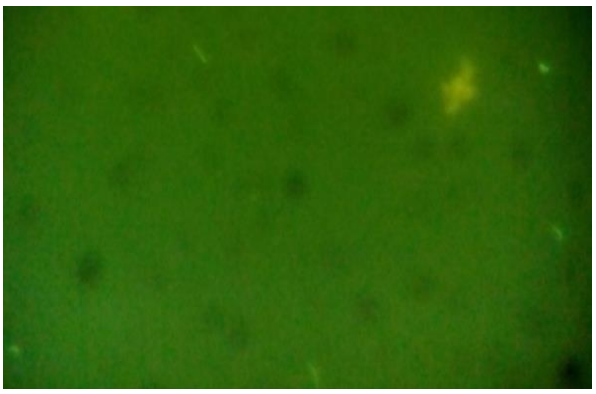

Figure 4: Demonstration of bacilli on auramine-rhodamine stained smear under fluorescent microscopy using blue excitation filter 40x.

Table 4: Comparesion of detection of AFB by Ziehl-Neelsen stain, auramine-rhodamine stain and autofluorescence.

Cytomorphology tubercular lymphadenitis	Zn+	AR+	AF+
88	33 (37.5%)	72 (81.82%)	76(86.36%)

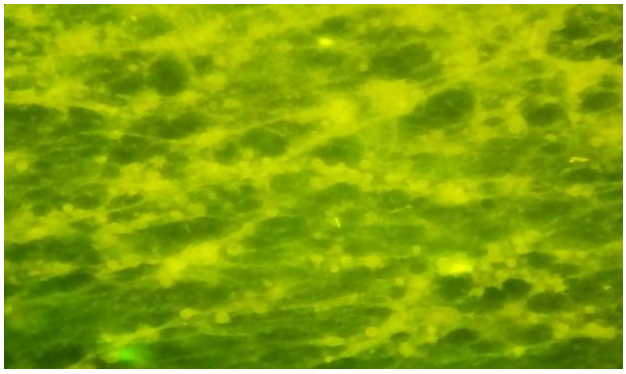

Figure 5: Demonstration of bacilli through autofluorescence technique on papanicolaou stained smears (PAP) 40x.

DISCUSSION

In many low and middle income countries with limited resources, the diagnosis of TB is still based on poorly validated symptom-based algorithms, often not resulting in a definitive diagnosis. The clinical presentation of tuberculosis is usually fever, night sweat, weight loss, anorexia. But some time delay in diagnosis has often been attributed to atypical clinical presentation and radiological presentation. The diagnosis of tuberculosis by cytomorphology is not new. It is a necrotizing granulomatous infection, which cytologically demonstrates the microscopic equivalent of caseous necrosis, granular-appearing necrotic background, together with mature lymphocytes, epithelioid histiocytes and multinucleated langhans type histiocytes. Several conditions, including mycosis, bacterial and viral adenitis can present the same cytology as does mycobacterium tubercular adenitis does. Laboratory tests may be essential to establish the cause of such adenopathy correctly, because treatment and prognosis may differ. Demonstration of *Mycobacterium tuberculosis* in fine needle aspirates becomes necessary for an early and accurate treatment. Fine needle aspiration cytology provides a rapid and definitive tissue diagnosis in the superficial lymphadenopathy. This study demonstrates that it also permits confirmation of the presence of mycobacteria with AFB stain in microscopy. The diagnosis of tuberculosis is confirmed by the demonstration of tubecular bacilli. *Mycobacteria* are slender rod shaped, non-motile, non-sporing, aerobic bacterium measuring 2 to 10 um in length. It has lipid coat which makes it difficult to stain but once stained cannot be decolourised with alcohol. Thus, termed as acid fast bacilli (AFB) as they retain carbon fuschin staining (AFB stain or ZN stain) even after washing with acid alcohol. Flourscence staining utilize same approach as ZN staining but carbon fuschin is replaced by flourscent dye as auramine which as primary stain followed by counter stain (potassium permanganate) employed to highlight stain organism for easier recognition for the diagnosis of tubercular bacilli in the samples examined. A comparison between ZN, AR and AF was done in the

present study. It was found that autofluorescence was more sensitive for identifying the bacilli. There were only two cases which showed ARS positivity on smear but were negative by AF. In comparison to both, AF on PAP stained smears provides a safe, inexpensive and exposure free diagnostic procedure along with more positive results. Even this technique does not require any addition to the standard PAP stain. In other studies on FNA smears of lymph nodes, autofluorescence was found to be more sensitive than AR and ZN staining.[9-11]

Autofluorescence is simple, sensitive and inexpensive, it is not widely used. It requires a fluorescent microscope, which may not be readily available, but has the advantage of not requiring additional stains and is therefore inexpensive and rapid.[8,9] If the cytomorphology is consistent with mycobacterial infection, and the organism is identified by ZN staining, AR or AF, the probability of a false positive diagnosis is small, and patients may safely commence therapy. FNAC of superficial lymph nodes is an outpatient procedure and requires little infrastructure and equipment and is therefore ideal for resource limited countries. The basic diagnostic modalities of cytomorphology and subsequent morphological identification of the organism are readily available and relatively inexpensive.

CONCLUSION

AF staining is more sensitive than the auramine–rhodamine fluorescent and ZN staining in demonstration of mycobacterium bacilli in fine needle aspiration cytology of tubercular lymphnode.

ACKNOWLEDGMENTS

Authors would like to thank the pulmonary medicine department.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Krishna M, Kumar A. Tuberculous mycobacteria bacilli fluorescence and compare with Ziehl- Neelsen stain in fine-needle aspiration cytology of tubercular lymphnode. Int J Otorhinolaryngol Head Neck Surg 2016;2:66-9.