

# Cytodiagnosis of tuberculosis using modified bleach method on lymph node aspirates

Sarah Khan, Hemalata Mahantappa, Anagha A Joshi

Department of Pathology, Kempegowda Institute of Medical Sciences, Bengaluru, Karnataka, India

## Abstract

**Background:** The conventional Ziehl–Neelsen (ZN) staining method for demonstration of acid-fast bacilli (AFB) by direct microscopy plays a key role in the diagnosis of tuberculosis. Its major disadvantage is low sensitivity. **Aims:** The present study was undertaken to emphasize the role of bleach concentration method over conventional ZN smear for detection of AFB in fine-needle aspiration (FNA) material of clinically suspected tuberculous lymphadenitis.

**Settings and Design:** Tertiary care teaching hospital, cross-sectional design.

**Materials and Methods:** FNAs were done on 150 patients with clinical suspicion of tuberculosis presenting with lymphadenopathy. Smears from the aspirates were processed for routine cytology and the conventional ZN method. The remaining material in the needle hub and/or the syringe was used for the bleach method. The significance of the bleach method over the conventional ZN method and cytology was analyzed.

**Results:** Among the 150 aspirates, 57% (85/150) were indicative of tuberculosis on cytology, 37% (55/150) were positive for AFB on conventional ZN method, and the smear positivity for AFB increased to 41% (62/150) on bleach method.

**Conclusion:** The bleach method is simple, safe and inexpensive. It improves the microscopic detection of AFB and can be a useful contribution to routine cytology.

**Keywords:** Acid-fast bacilli, bleach method, lymph node, tuberculosis, Ziehl–Neelsen stain

**Address for correspondence:** Prof. Hemalata Mahantappa, Department of Pathology, Kempegowda Institute of Medical Sciences, Bengaluru - 560 070, Karnataka, India.

E-mail: hemalatajreddy@gmail.com

**Received:** 09.01.2018, **Accepted:** 23.05.2018

## INTRODUCTION

Tuberculosis (TB) is a common public health problem and the second leading cause of infectious disease worldwide. In 2016, the WHO estimated that the disease affected 10.4 million people and led to 1.3 million deaths.<sup>[1]</sup> India has the highest TB burden accounting for one-fifth of global incidence (21%).<sup>[2]</sup> It is the most common infectious cause of death in our country.<sup>[3]</sup> It can involve any organ system and has a wide spectrum of clinical presentation.

Pulmonary TB is the most frequent.<sup>[4]</sup> Lymph nodes are the most common extrapulmonary sites affected.<sup>[5]</sup> The incidence of TB has increased since the onset of HIV and AIDS epidemic.<sup>[4]</sup>

The diagnosis of TB primarily involves clinical examination with subsequent laboratory confirmation by bacteriological examination for *Mycobacterium tuberculosis* bacilli.<sup>[6]</sup> The gold standard for diagnosis is culture on L. J medium, but is

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

**For reprints contact:** reprints@medknow.com

**How to cite this article:** Khan S, Mahantappa H, Joshi AA. Cytodiagnosis of tuberculosis using modified bleach method on lymph node aspirates. J Oral Maxillofac Pathol 2018;22:193-8.

### Access this article online

#### Quick Response Code:



#### Website:

www.jomfp.in

#### DOI:

10.4103/jomfp.JOMFP\_4\_18

time consuming.<sup>[7]</sup> Sensitivity and specificity of serological tests are unsatisfactory.<sup>[8]</sup> Newer molecular techniques such as polymerase chain reaction are rapid and sensitive but expensive for routine use in the developing countries, so detection of acid-fast bacilli (AFB) on direct smear examination still plays an important role in diagnosis.<sup>[9]</sup> Hence, fine-needle aspiration (FNA) plays a pivotal role in diagnosing extrapulmonary tuberculosis.<sup>[10,11]</sup>

Conventional Ziehl–Neelson (ZN) method for staining AFB plays a key role in diagnosis of tuberculosis but has a low sensitivity ranging from 9% to 46%.<sup>[12]</sup> Hence, many researchers have tried various techniques such as bleach concentration method, which clears the necrotic background, thus improving the sensitivity of detecting AFB. This method is safe, inexpensive, does not require expertise and is easy to perform.<sup>[5,8,13,14]</sup>

The bleach method using sodium hypochlorite solution was first used on sputum samples.<sup>[15–17]</sup> Later, many authors have tried using on other extrapulmonary specimens such as body fluids, FNA cytology (FNAC) aspirates, abscesses and skin scrapings.<sup>[2,3,5,8,11,14]</sup>

The study is undertaken to compare the sensitivity of modified bleach concentration method with the standard ZN staining technique in detecting AFB on FNA smears of clinically suspected tuberculous lymphadenitis cases.

## MATERIALS AND METHODS

The study was carried out on 150 cases with clinical suspicion of tuberculous lymphadenitis referred for FNAC in the department of cytopathology from December 2013 to October 2015. After obtaining informed consent from the patients, data regarding age, sex, relevant clinical history and clinical examination of the lesion such as site, number, size and association with HIV were documented. The procedure was explained to the patient, and FNAC was performed under strict aseptic precautions. The gross appearance of aspirate was noted in each case. Some slides were fixed in 95% ethyl alcohol for H&E and Pap stain. Some were air dried for May–Grunwald–Giemsa (MGG) and ZN stain. The leftover aspirate in the needle was rinsed with 1 ml of 5% sodium hypochlorite solution (NaOCl/bleach). After thorough mixing, the sample was incubated for 15 min at room temperature with frequent mixing at intervals. An equal volume of distilled water was added and mixed thoroughly and then centrifuged at 3000 rpm for 15 min. The supernatant was discarded, and 1–2 smears were prepared using one drop of the sediment, the area was

marked with marking pencil, air dried, heat fixed and stained by Ziehl–Neelsen staining technique.

The H&E, Pap and MGG stained smears were studied for cytomorphological features of tuberculosis. The conventional ZN-stained smears and modified bleach concentration method smears were then examined under oil immersion ( $\times 1000$ ) for the presence of AFB and compared. The data were processed using test of association (Chi-square test).

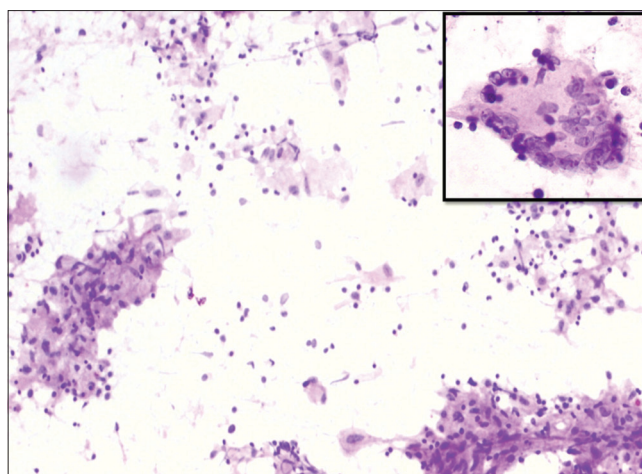
## RESULTS

A total of 150 FNA specimens from lymph nodes were included in the study. Twelve patients were HIV positive. The age ranged from 4 to 88 years, with a mean age of  $32.18 \pm 15.7$  years. A slight female preponderance was noted, accounting for 58% of the cases. The most common lymph node group involved was cervical 66% (99/150), followed by supraclavicular 8.6% (13/150), submandibular 7.3% (11/150), axillary 6.6% (10/150), inguinal 6% (9/150), postauricular 2.6% (4/150) and submental 2.6% (4/150).

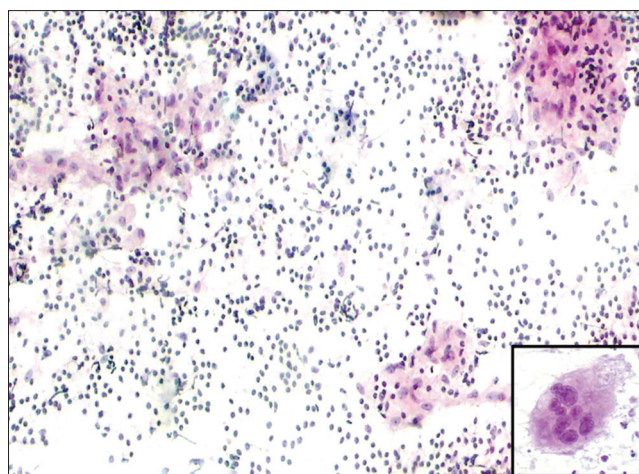
Isolated lymphadenopathy was the most common mode of presentation seen in 61% (92/150) cases. Fever, cough, loss of weight and appetite were the other associated symptoms. On examination, 82% (113/150) cases showed discrete nodal enlargement and 18% (27/150) cases showed matted lymph nodes. Sinus scars were noted in 4.5% (7/150) cases. The aspirate was gray-white in 66% (99/150) cases, followed by purulent aspirate in 21% (32/150) cases.

Out of 150 cases, 57% (85/150) cases were cytomorphologically diagnosed as tuberculosis, 32% (48/150) reactive lymphadenitis and 11% (17/150) suppurative lymphadenitis. The cases were diagnosed as reactive lymphadenitis on the basis of a polymorphous population of lymphoid cells comprising of small lymphocytes admixed with centrocytes, centroblasts, immunoblasts and tingible body macrophages. The diagnosis of acute suppurative lymphadenitis was based on the aspirated purulent material showing abundant neutrophils and nuclear debris in a necrotic background. The cytomorphological diagnosis of tuberculous lymphadenitis was made based on the presence of epithelioid cell granuloma, caseous necrosis and Langhans giant cells. These were further categorized into three patterns:

- Pattern 1: Epithelioid granulomas,  $\pm$  Langhans giant cells with caseous necrosis [Figure 1]
- Pattern 2: Epithelioid granulomas,  $\pm$  Langhans giant cells without caseous necrosis [Figure 2]



**Figure 1:** Tuberculous lymphadenitis - Pattern 1 showing epithelioid granuloma with caseous necrosis (H&E, x100). Inset shows Langhans giant cell (H&E, x400)



**Figure 2:** Tuberculous lymphadenitis - Pattern 2 showing epithelioid granuloma without caseous necrosis (Pap, x100). Inset shows multinucleate giant cell (Pap, x400)

- Pattern 3: Necrosis with or without scattered epithelioid cells [Figure 3].

Pattern 1 was seen in 40% of cases (34/85), pattern 2 in 31% of cases (26/85) and pattern 3 in 29% of cases (25/85).

In pattern 1, 26/34 (74%) were positive for AFB by conventional ZN, while bleach method detected AFB in 28/34 (82%) cases. Hence, two cases missed by the conventional ZN were picked up by the bleach method. In pattern 2, 4/26 (15%) were positive for AFB by conventional ZN, while, the bleach method detected AFB in 5/26 (19%) cases. One case missed by the conventional ZN was picked up by the bleach method. In Pattern 3, 25/25 (100%) showed positivity for AFB with both the conventional ZN method and bleach method.

All the cases cytomorphologically diagnosed as reactive lymphadenitis were negative for AFB by both the methods. In suppurative lymphadenitis, 4/17 (24%) cases were positive for AFB by bleach method, while, none were positive by conventional ZN. There was a statistically significant correlation ( $\chi^2 = 19.99, P < 0.05$ ) between cytomorphological diagnosis and AFB positivity by the conventional ZN and bleach method [Table 1].

The smear positivity for AFB on conventional ZN method was 37% (55/150) while the positivity increased to 41% (62/150) when the bleach method was used. Thus, the bleach method detected AFB in additional seven cases, which was statistically significant ( $\chi^2 = 123.25, P < 0.05$ ) [Table 2].

In all the cases, bleach concentration method not only provided a clean background with easy visibility of

**Table 1: Correlation of cytomorphological diagnosis with the bleach method and conventional Ziehl-Neelsen method in detecting acid-fast bacilli**

Cytomorphological diagnosis	AFB				Total (%)
	Conventional ZN		Bleach method		
	Positive	Negative	Positive	Negative	
Reactive LN	0	48	0	48	48 (32)
Suppurative LN	0	17	4	13	17 (11)
TB Pattern 1	26	10	28	8	34 (23)
TB Pattern 2	4	20	5	19	26 (17)
TB Pattern 3	25	0	25	0	25 (17)
Total cases <sup>†</sup>	55	95	62	88	150 (100)

<sup>†</sup>Correlation of significance:  $\chi^2 = 19.99, df = 3, P < 0.05$ . AFB: Acid-fast bacilli, TB: Tuberculosis, LN: Lymph nodes, ZN: Ziehl-Neelsen

**Table 2: Comparison of the conventional Ziehl-Neelsen method with the bleach method for detection of acid-fast bacilli**

Conventional ZN method	Bleach method		Total (%)
	Positive	Negative	
Positive	55	0	55 (37)
Negative	7	88	95 (63)
Total <sup>†</sup> (%)	62 (41)	88 (59)	150 (100)

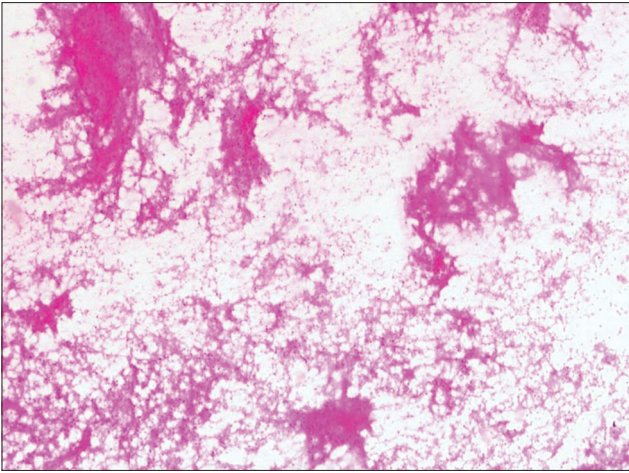
<sup>†</sup>Correlation of significance:  $\chi^2 = 123.25, df = 1, P < 0.05$ . AFB: Acid-fast bacilli, ZN: Ziehl-Neelsen

AFB, but it also increased the number of bacilli per field [Figure 4].

## DISCUSSION

Tuberculosis has remained a worldwide health burden, claims over 2 million lives and causes an economic drain of over 3 billion rupees a year in India. The primary obstacle faced in control of TB is poor case finding, which in India is 61.3%.<sup>[15]</sup> Early diagnosis and initiation of treatment will enable cure and also limit the spread. Thus, significantly reducing morbidity and mortality associated with the disease.<sup>[8]</sup>



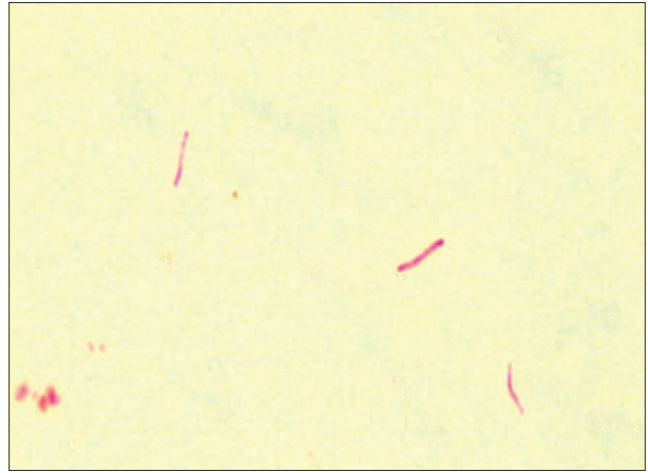


**Figure 3:** Tuberculous lymphadenitis - Pattern 3 showing caseous necrosis (H&E, x100)

In developing countries like ours, the diagnosis for TB conventionally relies on FNA cytology and direct smear microscopy. However, FNAC alone has limited specificity because the morphologic spectrum in tuberculous lymphadenitis varies widely depending on the stage of the disease and the immunity of the host. The presence of epithelioid cell granulomas, caseous necrosis and Langhans giant cells is the first step in the diagnosis. Although similar features can be seen in lesions other than TB such as in fungal infections, other inflammatory causes and sarcoidosis.<sup>[14]</sup> Problems also arise when the aspirate shows abundant neutrophils and necrosis with occasional epithelioid cells and absence of typical Langhans giant cells or caseous necrosis. Hence, definitive diagnosis most often requires demonstration of AFB by ZN stain.

In the late 1940s, sputum liquefaction with NaOCl (readily available at low cost as household bleach) and then concentration by centrifugation before ZN stain was implemented to improve the smear positivity for detection of AFB. Recently, modified bleach concentration technique has been applied in cytology on FNA material from lymph nodes, abscesses and body fluids.<sup>[5]</sup> The studies demonstrated an increase in the smear positivity for AFB by the modified bleach method in comparison with the conventional ZN method.<sup>[2,3,14]</sup>

In the present study, 150 aspirations were performed on lymph nodes of patients clinically suspected of tuberculous lymphadenitis. Most patients in our study were in the age group of 21–30 years (30.5%). Mean age was  $32.18 \pm 15.7$  years. Female preponderance was seen in our study accounting for 58% (87/150) cases. Similar findings were noted by Patel *et al.*<sup>[2]</sup> However, Annam *et al.* reported male predominance.<sup>[8]</sup>



**Figure 4:** Acid-fast bacilli positivity by bleach method with a clear background in a case cytologically diagnosed as tuberculous lymphadenitis - Pattern 1 (Bleach ZN, x1000)

Isolated lymphadenopathy was the most common mode of the presentation seen in 61% (92/150) cases, which is slightly higher compared to other studies.<sup>[3,11]</sup> Fever, cough, loss of weight and appetite were the other associated symptoms. Cervical group of lymph nodes was most commonly affected, seen in 66% of patients followed by supraclavicular in 8.6%; these findings are similar with other studies.<sup>[8,14,16]</sup> On examination, discrete nodal enlargement was noted in 82% (113/150) cases and 18% (27/150) showed matted lymph nodes, similar to previous studies.<sup>[3,11]</sup> The aspirate was gray-white in 66% (99/150) cases constituting the major group followed by purulent aspirate in 21% (32/150) cases.

The cytomorphological features were analyzed based on the nature of aspirate and microscopy. They were categorized as reactive lymphadenitis, suppurative lymphadenitis and tuberculous lymphadenitis. Routine ZN stain and modified bleach method were done in all the cases, the AFB positivity and cytomorphology were compared.

In the present study, reactive lymphadenitis was diagnosed in 48/150 lymph nodes, all these cases were negative for AFB by both routine ZN stain and bleach method. Whereas other studies have detected AFB in few cases of reactive lymphadenitis by the bleach method which were initially negative for AFB by the conventional ZN.<sup>[2,3,11]</sup>

Cytomorphologically diagnosed four cases of suppurative lymphadenitis were later diagnosed as tuberculous lymphadenitis based on the detection of AFB in bleach method. Cytological picture showing numerous neutrophils in a necrotic background may pose difficulty in distinguishing an acute suppurative process from a

tuberculous process. Scattered epithelioid cells if present may be difficult to pick up. Hence, differentiation between these two depends on the presence or absence of AFB.<sup>[16]</sup> Conventional ZN stain for AFB was negative in all 17 cases. However, the bleach method picked up AFB in four cases which were missed by conventional ZN method. Similarly, other studies also showed increased AFB positivity by the modified bleach method.<sup>[2,3,8,16]</sup> This could be attributed to the presence of abundant neutrophils and debris which probably obscured the visibility of AFB in the routine ZN smear. Therefore, the digestion of neutrophils and cellular debris by the bleach method resulted in clear background with easy visibility of AFB. The similar observations were noted by others.<sup>[16]</sup>

Features suggestive of tuberculosis were seen in 85 (57%) lymph nodes. Cytomorphologic features of TB were further categorized under 3 patterns as shown in Table 1. Of these, 55 (65%) cases showed AFB positivity by routine ZN smear and 58 (68%) cases were positive for AFB by bleach method.

The cytomorphological pattern of TB and AFB positivity by conventional ZN and bleach method were compared. Pattern 1 showing epithelioid granulomas with necrosis was seen in 34 cases, of which 26 cases were positive for AFB by routine ZN stain and 28 cases were positive for AFB by the bleach method. Two cases which were initially negative for AFB by the routine ZN smear were positive by the bleach method. This increase in positivity could be due to digestion of cellular elements by the bleach and concentration of AFB providing easy visibility. This is in concordance with the study done by Bharadwaj *et al.*<sup>[16]</sup> Pattern 2 showing epithelioid granulomas without necrosis was seen in 26 lymph nodes. Out of these, four cases were positive for AFB by routine ZN stain and five cases were positive by modified bleach method. One case which was missed by the conventional ZN method was picked up by the bleach method. Similar findings were observed by other studies.<sup>[16]</sup> The overall positivity for AFB by both conventional ZN and bleach method was quite low in the above pattern, this has been attributed to an inverse relationship found between the presence of granuloma and of AFB, which is due to the ability of epithelioid cells (activated macrophages) to phagocytose and kill the microorganism.<sup>[17]</sup>

Pattern 3 showing caseous necrosis only with or without scattered epithelioid cells was seen in 25 lymph nodes. All cases were positive for AFB by both routine ZN stain and modified bleach method. AFB positivity rate was high in this pattern and has been explained on the basis of the fact

that the central necrotic portion of the tubercle contains more bacilli.<sup>[7]</sup>

Twelve cases were HIV positive, on cytomorphology nine cases showed pattern 3 and three cases showed pattern 1. All the cases were positive for AFB by both conventional ZN method and bleach.

In our study of 150 cases clinically suspected of tuberculous lymphadenitis, cytology was suggestive of TB in 57% (85/150) cases; of these 37% (55/150) cases were positive for AFB by routine ZN smear. The bleach method for AFB was positive in 41% (62/150) cases.

The bleach method picked up AFB in seven cases which were initially negative by conventional ZN method. None of the cases which were positive by the conventional ZN method were missed by the bleach method. There was a statistically significant difference between cytomorphological diagnosis, conventional ZN method and bleach method ( $\chi^2 = 19.99, P < 0.05$ ).

The increase in positivity for AFB by the bleach method in comparison with the conventional ZN was in agreement to studies done earlier.<sup>[2,3,8,16]</sup> Although we observed higher positivity for AFB by the conventional ZN method compared to others.

Our results showed that majority of the positive cases on routine ZN staining had few AFB and searching for them was time-consuming and tedious. Whereas, the bleach concentration method increased the number of bacilli per field and also gave a clean background making the bacilli easily visible thereby reducing the screening time. The morphology of AFB also appeared to be better preserved, and they were thicker and longer than the routine ZN smears. This could probably be due to swelling of bacilli in the solution. The above-mentioned observations were noted in other studies as well.<sup>[8,11,14]</sup>

## CONCLUSION

The bleach method for AFB is simple, safe and cost-effective. Bleach concentration method has been shown to improve the microscopic detection of AFB. This can be useful when combined with routine cytology and benefit the patients by correct diagnosis and in turn to receive an early and effective treatment.

## Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

### REFERENCES

- World Health organization. Global Tuberculosis Report 2017. World Health Organization; 2017 Available from: <https://www.scribd.com/document/364626690/Global-TB-Report-2017>. [Last accessed on 2018 Jan 03].
- Patel MM, Patel K, Italiya SL, Kaptan KR. Improved diagnosis of tuberculosis in lymph node cytology by bleach method for detection of acid fast bacilli in comparison to conventional Ziehl Neelsen staining method. *Int J Med Sci Public Health* 2013;2:931-5.
- Dwivedi G, Mathur C. Modified bleach method—improving microscopic detection of acid fast bacilli in fine needle aspiration smears of lymph nodes. *J Med Sci Clin Res* 2013;1:176-83.
- Park K. Park's Textbook of Preventive and Social Medicine. 21<sup>st</sup> ed. India: Banarsidas Bhanot Publishers; 2011. p. 164-5.
- Khubnani H, Munjal K. Application of bleach method in diagnosis of extra-pulmonary tuberculosis. *Indian J Pathol Microbiol* 2005;48:546-50.
- Bhatia AS, Kumar S, Harinath BC. Immunodiagnosis of tuberculosis: An update. *Indian J Clin Biochem* 2003;18:1-5.
- Tadesse M, Abebe G, Abdissa K, Bekele A, Bezabih M, Apers L, *et al.* Concentration of lymph node aspirate improves the sensitivity of acid fast smear microscopy for the diagnosis of tuberculous lymphadenitis in Jimma, Southwest Ethiopia. *PLoS One* 2014;9:e106726.
- Annam V, Karigoudar MH, Yelikar BR. Improved microscopical detection of acid-fast bacilli by the modified bleach method in lymphnode aspirates. *Indian J Pathol Microbiol* 2009;52:349-52.
- Savić B, Sjöbring U, Alugupalli S, Larsson L, Mörner H. Evaluation of polymerase chain reaction, tuberculostearic acid analysis, and direct microscopy for the detection of mycobacterium tuberculosis in sputum. *J Infect Dis* 1992;166:1177-80.
- Metre MS, Jayaram G. Acid-fast bacilli in aspiration smears from tuberculous lymph nodes. An analysis of 255 cases. *Acta Cytol* 1987;31:17-9.
- Arora B, Arora DR. Fine needle aspiration cytology in diagnosis of tuberculous lymphadenitis. *Indian J Med Res* 1990;91:189-92.
- Handa U, Mohan H, Bal A. Role of fine needle aspiration cytology in evaluation of paediatric lymphadenopathy. *Cytopathology* 2003;14:66-9.
- Gangane N, Anshu, Singh R. Role of modified bleach method in staining of acid-fast bacilli in lymph node aspirates. *Acta Cytol* 2008;52:325-8.
- Chandrasekhar B, Prayaga AK. Utility of concentration method by modified bleach technique for the demonstration of acid-fast bacilli in the diagnosis of tuberculous lymphadenopathy. *J Cytol* 2012;29:165-8.
- Srikanth P, Kamesh S, Daley P. Bleach optimization of sputum smear microscopy for pulmonary tuberculosis. *Indian J Tuberc* 2009;56:174-84.
- Bharadwaj S, Kashyap I, Kumari R. Comparative evaluation of two staining techniques for detection of Tubercular bacilli in lymphnodal aspirates. *Int J Health Sci Res* 2015;5:115-21.
- Prasoon D. Acid-fast bacilli in fine needle aspiration smears from tuberculous lymph nodes. Where to look for them. *Acta Cytol* 2000;44:297-300.