Original Research Article

Comparative Study of Ziehl-Neelsen Stain versus Fluorescent Microscopy in Diagnosis of Tuberculous Lymphadenitis on FNAC at A Tertiary Care Centre

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Abstract

Introduction: Worldwide, TB is one of the top 10 causes of death and the leading cause from a single infectious agent. Lymph nodes tuberculosis is considered the most common form of extrapulmonary tuberculosis and most common cause of lymphadenopathy in developing countries like India.Conventional Ziehl-Neelsen (ZN) method & Fluorescent microscopy (FM) plays an important role for detection of Acid fast bacilli (AFB). **Objectives:** This study is an attempt to find out cost effective, rapid and sensitive technique for early diagnosis of tuberculous lymphadenitis. To study incidence, age, sex and site wise distribution of tuberculous lymphadenitis in this area. **Material Methods:** The prospective observational study was carried out in the Department of Pathology, S.R.T.R. Govt. Medical College, Ambajogai in year 2017. All 247 aspirated samples from Lymph node swellings were subjected to ZN stain, Fluorescent stain, MGG & PAP stain and 52 cases of tuberculous lymphadenitis were subjected for further analysis, **Results:** Out of 247 samples aspirated from lymph node lesions 52 were of tuberculous lymphadenitis (21.05%). For tuberculous lymphadenitis, age ranged from 7 months to 77 years. Female predominance was noted with Female to male ratio 1.17:1. Half of the cases were in the range of 21-40 years of age. Cervical region was the commonest siteinvolved with 51.92%. Of 52 aspirates, smear positivity of AFB on ZN stain method was 78.84%, while positivity of Auramine fluorescent stain method was 90.38%. **Conclusion:** In developing countries with high prevalence of tuberculosis, Fine needle aspiration cytology (FNAC) coupled with fluorescent stain & ZN stain could distinctively improve diagnosis of tuberculous lymphadenitis in patients presenting with superficial lymphadenopathy.

Keywords: Comparative, Fluorescent, FNAC, Lymphadenitis, Tertiary, Ziehl-Neelsen

Introduction

Worldwide, TB is one of the top 10 causes of death and the leading cause from a single infectious agent. Globally the best estimate is that 10.0 million people developed TB disease in 2017. In 2017 TB caused an estimated 1.6 million deaths. Diagnosis and successful treatment of people with TB averts millions of deaths each year, but there are still large and persistent gaps in detection and treatment. Gaps between the estimated number of new casesand the number actually reported are due to a mixture of underreporting of detected cases, and under diagnosis (either because people do not access health care or because they are not diagnosed when they do). Worldwide India accounts for 27% of global burden of tuberculosis and accounts for 32% of TB deaths among HIV negative people and 27% of combine death of HIV negative and positive people [1]. Lymph nodes tuberculosis is considered the most common form of extrapulmonary tuberculosis and most common cause of lymphadenopathy in developing countries like India. Lymph nodes may be affected by tuberculosis, in the primary tuberculosis – primary lymph node tuberculosis – as a result of the primary complex development in the pharyngeal-cervical territory; in the secondary tuberculosis – secondary lymph node tuberculosis – resulting from the secondary location of the tuberculosis in the peripheral lymph nodes.

It occurs between six and nine months after the initial infection. It could be said that lymph node involvement is always secondary to the tuberculosis development in

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their tributary organ, or, in other words, tuberculous lymphadenitis can be considered a local manifestation of a systemic disease [2]. FNAC is now widely utilized as a first line diagnostic procedure in the diagnosis of palpable masses, including peripheral lymphadeno-pathy. Tuberculous lymphadenitis can be presumptively diagnosed morphologically on fine-needle aspiration cytology of lymph node.

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 [3].

The cytological criteria for the diagnosis of possible tubercular lymphadenitis have been clearly defined as epithelioid cell granulomas with or without multinucleated giant cells and caseation necrosis [4]. Culture is essential for obtaining a definitive diagnosis. Unfortunately, culture is time consuming and expensive also has lower sensitivity in paucibacillary conditions.

Conventional Ziehl-Neelsen (ZN) method for acid fast bacilli (AFB) plays a key role in the diagnosis and the monitoring of treatment in tuberculosis. Its major disadvantage is low sensitivity ranging from 20% to 43%. Serological techniques have the disadvantage of lack of sensitivity and specificity.

Newer molecular techniques such as polymerase chain reaction, although rapid, are costly to be routinely used in developing countries where most TB cases occur. Fluorescent microscopy (FM) plays an important role for detection of Mycobacteria as lower magnifications are used as well as less time is required to examine smears [3, 4].

This study is an attempt to find out cost effective, rapid and sensitive technique which can be used routinely for early diagnosis of tuberculous lymphadenitis, also to study incidence, age, sex and site wise distribution of tuberculous lymphadenitis in this area.

Currently there is scant data regarding comparative study of ZN and fluorescent staining on pulmonary and/ or extra pulmonary samples from this region.

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Material and Methods

Setting- The study was conducted at Pathology Department of SRTR Govt. Medical College Ambajogai, a tertiary care centre located in a rural area.

Type of study- Prospective observational study

Sampling method- Palpable lymph node lesions were aspirated for cytological evaluation using 22–23 gauge needle and 10 ml plastic syringe with a detachable syringe holder. In few patients ultrasonography guided fine needle aspiration cytology was performed.

Four smears were made from each aspirate three air dried smears were stained respectively with May Grunwald- Giemsa, Auramine fluorescent(AF) and ZN Stain, and one was wet fixed with alcohol and stained with Papanicolaou stain.

Sample collection, staining of samples with the help of laboratory staff and data collection was done by Junior Resident.

Diagnosis of samples, data analysis, result formation and preparation of manuscript was done by Associate professor and Assistant Professor.

Review of diagnosis and manuscript was done by Professor and Head of the Department

Duration of study- Over a period of 12 months

Sample Size-52 cases of tuberculosis lymphadenitis diagnosed morphologically on fine-needle aspiration cytology of palpable lymph node lesions.

Inclusion Criteria- patients with palpable lymph lesions were selected and were subjected for fine needle aspiration cytology sampling, out of which patients diagnosed as tuberculous lymphadenitis on fine needle aspiration cytology, were included in the study.

Exclusion Criteria- Patients with diagnosis other than tuberculous lymphadenitis were excluded from the study.

Ethical consideration- Informed consent was taken and FNAC procedure was explained to the patients.

Results

A total of 52 (21.05%) cases were diagnosed as tuberculous lymphadenitis out of 247 cases of palpable lymph node lesions. Out of 52 cases, 26 cases (50%) were in the range of 21-40 years of age with lowest age observed was 7 months and highest age observed was 77 years (Table 1).

Table-1: Age Wise Distribution of Tuberculosis Lymphadenitis.

Age In Years	Frequency	Percentages (%)
0-10	4	7.69
11-20	6	11.54
21-30	15	28.85
31-40	11	21.15
41-50	7	13.46
51-60	4	7.69
61-70	3	5.77
>70	2	3.85
Total	**52**	**100.00**

Table-2: Sex Wise Distribution of Tuberculous Lymphadenitis.

Sex	Frequency	Percentages (%)
Male	24	46.15
Female	28	53.84

Female preponderance was noted accounting for 53.84 %, With Female to male ratio was 1.17:1 (Table 2).

Table-3: Site Wise Distribution.

Site	Frequency	Percentages (%)
Cervical lymph node	27	51.92
Axillary lymph node	6	11.53
Supraclavicular lymph node	9	17.31
Submandibular	3	5.77
Submental	1	1.92
Inguinal lymph node	4	7.69
Others	2	3.85
Total	**52**	**100.00**

The most common site of involvement was cervical region with 27 cases out of 52 cases (51.92%) followed by supraclavicular region (17.31%) and axillary region (11.53%) (Table 3).

Table-4: Comparison of ZN Staining with Fluorescent Staining.

Total no. Of AFB positive cases	ZN positive Cases	Fluorescent positive cases	ZN positive &Fluorescent Negative cases	ZN negative &Fluorescent positive cases
52	41	47	5	11

Of total 52 cases of tuberculous aspirates, 41 showed smear positivity for AFB on ZN stain method (78.84%) and 47 cases showed positivity on Auramine fluorescent stain method (90.38%),while 36 out of 52 cases were both ZN & fluorescent positive. 5 cases were ZN positive but fluorescent negative and 11 cases were Fluorescent positive but ZN negative.

Discussion

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.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 [4]. The gold standard" for the diagnosis of tuberculosis (TB) is still the demonstration of acid fast Bacilli (AFB) by microscopic examination of smear or bacteriological confirmation by culture method. [5]. In

spite of newer modalities for diagnosis and treatment of TB, unfortunately, millions of people are still suffering and dying from this disease. If left untreated, the mortality rate with this disease is over 50%. Major challenges to control TB in India include poor primary health-care infrastructure in rural areas of many states. Existing diagnostic laboratories need to be strengthened for better utilization of already scarce resources. Better diagnostic tests for quick screening of this disease at field level should be developed and made available at the grass-root level [6]. The FNAC is simple, safe and cost effective and less time consuming procedure. The FNAC can be done in outpatient department without anaesthesia and there will be no disfigurement or scar on the skin [7].

The present study showed that tuberculous lymphadenitis may be present in any of the age ranging widespread from 7 months to 77 years. Gupta et al [8] Mitra et al [9] Paliwal et al [10] observed similar results in their studies. Paliwal et al reported the youngest patient aged 4 years oldest was 63 years old, Gupta et al. reported the youngest patient aged 5 months old and oldest was 95 years old, whereas Mitra et al reported the youngest patient aged 6 months and the oldest 69 years old.

Cervical lymph node was found to be the most common site involved (51.9%) in the present study, Paliwal N et al. in 2011 [90%] [10] and Bezabih M et al. in 2002 [74%] [11] observed similar results in their studies. Out of the total 52 cases, maximum number of cases 26 (50.00%) were seen in the 20–39 years age group and Bodal et al observed similar results in their study [12].

Our study shows out of total 52 cases of tuberculous lymphadenitis, 41 showed smear positivity for acid fast bacilli on ZN stain method and 47 cases showed positivity on Auramine fluorescent stain method. 36 out of 52 cases were both ZN & fluorescent positive, 5 cases were ZN positive but fluorescent negative and 11 cases were Fluorescent positive but ZN negative. Hense Auramine fluorescence staining technique (FM) (90.38%) is more sensitive in detection of AFB as compared to ZN stain (78.84%). Githui et al [13] in 1993 observed FM (80%) & ZN (65%), Ulukanligil et al [14] in 2000 observed FM (85.2%) and ZN (67.6%) , S J Murry et al [15] in 2003 observed FM(93%) and ZN(73%), Jain et al [16] in 2002 observed FM (41%) and ZN(32%).

Our study showed slight female predominance with female to male ratio of 1.17:1. Fazal et al.in 2011 [1.6:1], [17] Bhatta S et al. in 2018 [1.1:1] [18] and Ruchira Wadhwa et al. in 2017 [1.3:1] [19] found similar results in their studies.

The diagnosis of tuberculosis is confirmed by the demonstration of tubercular bacilli. Mycobacteria are slender rod shaped, non-motile aerobic bacterium measuring 2 to 10 um in length. It has lipid coat which makes it difficult to stain but once stained it cannot be decolourised by acid alcohol. Ziehl-Neelson is the most extensively used procedure for the demonstration of mycobacterium tuberculosis in smear [18, 19]. The requisites for the staining procedures are; basic fuchsin, phenol, absolute alcohol; sulphuric acid and methylene blue. Microscopic examination under oil immersion objective reveals mycobacterium as red bacilli. Thus, termed as acid fast bacilli (AFB) as they retain carbon fuschin staining (AFB stain or ZN stain) even after washing with acid alcohol. Fluorescent staining by Auramine is other methods of staining. In this a smear is made from thespecimen and stained with fluorescent stain called auramine. The auramine stain enters the wall of Mycobacterium tuberculosis bacterial cell and makes them glow against dark background under UV light Flourscence staining utilize fluorescent dye in place of carbon fushin, as auramine which as primary stain followed by counter stain (potassium permanganate) employed to highlight stained organism for easier recognition for the diagnosis of tubercular bacilli in the samples examined. Using fluorescent microscopy, the tubercle bacilli when examined under ultra violet light, the bacilli appeared as a bright rod against a dark back ground. Since there is a contrast, the bacilli are readily seen and therefore in very less time large area could be examined.While in ZN staining acid fast bacilli appeared bright red rods in blue background [20].

The advantage of Auramine techniques is that slides can be examined at a lower magnification and allows the examination of much larger area per unit of time. In fluorescence microscopy the same area that needs examination for 10 min with a light microscope can be examined in 2 minutes [21]. The smears stained by ZN method can detect bacilli when the concentration of bacilli is 10^5/mL of sputum, whereas a more sensitive staining technique like FM stain detects the bacilli when the bacillary load is 10^4/mL of sputum [22]. The conventional microscope uses visible light (400–700 nm) to illuminate and produce a magnified image of a sample [Figure 3]. A fluorescent microscope, on the other hand, uses a much higher intensity (ultra violet) light source that excites a fluorescent species in a sample of interest. This fluorescent species in turn emits a lower energy light of a longer wavelength that produces the magnified image instead of the original light source [Figure 4]. The number of organisms observed is 3.65 times than with the ZN stain [23].

Figure-1: Cervical Lymph Nodes

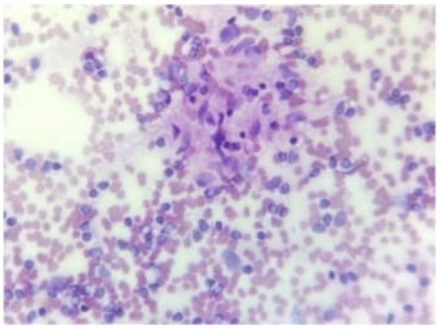

Figure-2: Epitheloid Granuloma (40X)

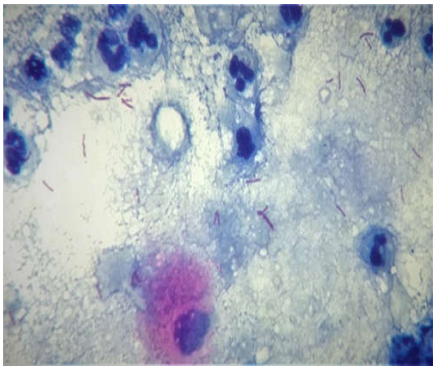

Figure-3: ZN-Stain (100X)

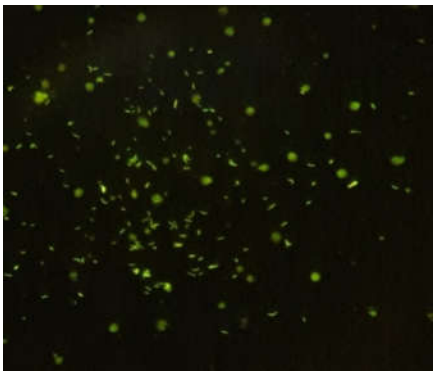

Figure-4: Fluorescent Stain (40X)

Conclusion

In developing countries like India with high prevalence of tuberculosis,sensitivity of smear microscopy for diagnosis of tuberculosis can be enhanced by use of fluorescence microscopy over light microscopy using ZN stain. FNAC coupled with fluorescent stain & ZN stain could distinctively improve diagnosis of tuberculous lymphadenitis in patients presenting with superficial lymphadenopathy.

Although fluorescent microscopy may not be available at every placebut is quite economical in terms of both time and expense also it uses low power magnification (40X), the procedure is less time consuming, and the fluorescing bacilli are easily identifiable and cause less eye-strain as compared to ZN method (100 X) in the diagnosis of tuberculosis. Use of fluorescent microscopy in diagnosis of tuberculous lymphadenitis results in increased detection rate of tuberculosis cases which further will improve number of cases treated and cured.

What this study adds to existing knowledge- The present study reveals that employing fluorescent method along with traditional method of FNAC and ZN staining, remarkably increases detection rate of tuberculosis in superficial lymphadenopathies, this is in particular very significant in countries like India with high prevalence of tuberculosis. Also present study puts forward the data depicting the age and sex wise distribution of tuberculous lymphadenitis in this region which could be useful for ensuing studies.

Findings: Nil; **Conflict of Interest**: None initiated
Permission from IRB: Yes

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