"CORRELATION OF AUTOFLUORESCENCE METHOD WITH CONVENTIONAL ZIEHL-NEELSEN METHOD IN DETECTION OF ACID FAST BACILLI IN LYMPH NODE ASPIRATES."

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ABSTRACT

Background and Objectives:

The conventional Ziehl-Neelsen staining method for acid fast bacilli (AFB) plays a key role in the diagnosis of tuberculosis by direct microscopy. Its major disadvantage is low sensitivity. The present study was undertaken to correlate autofluorescence (AF) method on Papanicolaou stain (PAP) with conventional Ziehl-Neelsenstain(ZN) methodin detection of AFB in lymph node aspirates as it is simple, rapid, cost effective & most frequently utilized stain for cytological specimens.

Methods

Fine needle aspirations (FNA) were performed on 153 patients with clinical suspicion of tubercular lymphadenitis. A detailed history, complete general physical examination, and informed consent was taken from the patients before the FNAC procedure. Smears from aspirate were processed for routine cytology for H&E, Giemsa, PAP, ZN and AR staining. PAP stained smears were examined for AFB for their AF under fluorescent microscope using the blue excitation filter. ZN stained smears were examined for AFB under oil immersion of compound microscope. The efficacy of the AF method on Pap stain in detection of acid fast bacilli (AFB) over the conventional ZN stain was analyzed. The results were evaluated by taking AuramineRhodamine(AR) stain as a gold standard method in detection of AFB.

Results

By taking AR as a gold standard test for detection of AFB, the sensitivity of ZN was 41.67% and specificity was 99.15% and the sensitivity of AF was 61.11% and the

specificity was 71.79%. Further the positive predictive value of ZN was 93.75% and negative predictive value was 84.67% and similarly the positive predictive value of AF was 40.00% and negative predictive value was 85.71%.

Interpretation and Conclusion

The AF method is simple inexpensive and novel method in detection of AFB. It is more sensitive than ZN staining in detection of AFB on fine needle aspirates. As it is a novel study it is advisable that multi-institutional studies should be designed for further validation.

Keywords: Acid fast bacilli, ZN stain, PAP, Autofluorescence, Lymph node, Tuberculosis.

LIST OF ABBREVATIONS USED

AFB	Acid Fast Bacilli
AF	Autofluorescence
AR	Auramine-Rhodamine
FNA	Fine Needle Aspiration
LAM	Lipoarabinomannan
MR	Mannose Receptor
MAI	Mycobacterium aviumintracellulare
MAC	Mycobacterium avium complex
PAP	Papanicolaou
WHO	World Health Organization
ZN	Ziehl-Neelsen

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INTRODUCTION

Tuberculosis is a major health problem in developing countries and is common infectious disease caused by various strain of Mycobacteria, usually Mycobacterium Tuberculosis in human beings.[1]

It is airborne disease and most commonly affects lungs where it is called pulmonary tuberculosis. One third of the world's population is currently infected and more than 1.5 million people die each year due to tuberculosis. Among these about 15 -20% of cases are reported as having extra pulmonary involvement. Lymphadenopathy is the most common presentation of extra pulmonary tuberculosis and is responsible for 43% cases of peripheral lymphadenopathy in developing countries [1-4.]

The precise cause of peripheral lymphadenopathy is often difficult to establish by history, physical examination, and radiographic studies alone. Fine needle aspiration cytology (FNAC) has assumed an important role in evaluation of these cases as a possible alternative to excisional biopsy [1]

ZN staining is the most widely used procedure for the demonstration of Mycobacterium tuberculosis in FNAC smear, but it has low sensitivity and the screening is tedious. Fluorescent microscopy with fluorochrome dyes has high sensitivity and specificity but is more expensive. Culture sensitivity test for tuberculosis is diagnostic but less sensitive and takes 3 to 6 weeks to get report.[3]

Hence as per the need, the present study wasundertaken to correlate AF method on PAP stained smears with conventional ZN stain method in detection of AFB in lymph node aspirates as it is simple, rapid, cost effective & most frequently utilized stain for cytological specimens.

AIMS & OBJECTIVES OF STUDY

To analyze the efficacy of autofluorescence method in detection of acid fast bacilli (AFB) by correlating with conventional Ziehl-Neelsen stain method in lymph node aspirates of clinically suspected cases of tuberculosis.

REVIEW OF LITERATURE

Tuberculosis (TB) was described with different names as Pott's disease, Phthisis, Rajyakshma and Tapedic in olden days. Bones of prehistoric man dating back 8000 BC have shown typical changes of TB. In Rigveda which is dated 2000 BC, it has been described as Yakshma. Sushrutha described the disease and observed that it was difficult to treat. In Greek literature dating back around 460-377 BCHippocrates described scrofula on the skin of phthisic pigs.[5]

In the previous era, TB was aptly named as 'Captain of ship of death'. Frascatorious (1483-1553) postulated that it may be transmitted by human airborne living particles and later he named it as 'contagiumvivum'.The term "tubercle" was coined byFranciseusSylvius (1614-1672) where he noticed tubercles in the lungs of people with 'phthisis'. Benjamin Martin (1720) suggested that it may be an infectious disease. The term 'Tuberculosis' was introduced by Laurent Bayle (1774-1816). Robert Koch in 24th March 1882 identified the specific agent Mycobacterium tuberculosis causing TB which was celebrated as world TB day every year by WHO.[5]

'Scrofula' or the king's of evil' are the historical names of TB lymphadenopathy. The term scrofula meaning glandular swelling (Latin) and full necked sow (French). August Hirsch stated "It denotes an inflammatory kind of tumor more particularly in the neck".[6,7,]

It was widely believed for many centuries that the royal house of England and France had a supernatural gift to cure scrofula by touching the sufferers. Ceremony consisted of king touching the afflicted subject while court chaplain recited prayers and presented on the touch piece. It was at the end of 19th century that the tuberculosis infection was identified as the cause of scrofula.[7,8]

Lymphadenopathy is the most common presentation of extra pulmonary TB in both immunosuppressed and immunocompetent population.[9,10]

In beginning Mycobacterium bovis (M. bovis) was a common infective agent, currently most cases are caused by M. Tuberculosis or Atypical mycobacteria especially inMycobacteriumavium complex (MAC).[11]

The incidence of infection in immunocompetent population is due to spread of the organism from the primary focus to regional lymph nodes. Most commonly primary focus is from lung and it spreads by hematogenous or lymphatic route. When the source of infection is milk contaminated with M. bovis, the primary focus is the tonsils or pharynx.[11]

Clinically tuberculous lymphadenopathy is most often found in head and neck region. The posterior cervical and supraclavicular chains are the sites most commonly involved and it is less frequent in submandibular and preauricular nodes. Rare in axillary or inguinal nodes constituting less than 10% of cases.[12]

Mediastinal, peribronchial, and paratrachealis most often seen as part of primary TB in children or disseminated disease in immunocompromised patients. Involvement of thoracic and abdominal lymph nodes are frequently seen in HIV infected patients.[13]

The typical scenario for superficial nodal lymphadenopathy is gradual, painless enlargement over several months to weeks. Initially the overlying skin is not inflamed, but with the passage of time the epidermis become shiny, pink to red, fairly tender and warm to touch. Mating of nodes may occur due to periadenitis. Eventually if left untreated, the skin may be breached and fistula forms which leads to serous to purulent discharge .[14]

The physical appearance of superficial TB lymphadenitis isclassified into 5 stages:[15]

Stage 1- Enlarged firm, mobile and discrete nodes.

Stage 2-Large, rubbery nodes fixed to surrounding tissue due to periadenitis.

Stage 3-Central softening due to abscess formation.

Stage 4- Collar stud abscess formation.

Stage 5- Sinus tract formation.

The process progresses indolently and is usually not accompanied by systemic symptoms. Miliary TB should be suspected when the lymphadenopathy is generalized, localized outside the cervical chain accompanied by systemic symptoms such as fever, night sweats and weight loss.[16]

STRUCTURE OF TUBERCLE BACILLI

It is important to know the structure of tubercle bacilli and immune-pathogenesis to understand the various diagnostic modalities.These are slender, aerobic rods that grow in straight or branching chains and measure 0.2 to 0.6 μm in size. They stain weakly positive with gram stain and they have a waxy cell wall composed of mycolicacid, which they make them acid fast.[17,18]

Cellular Constituents-

The cell envelope has special properties. The mycobacterium has unique cell wall responsible for its virulence, diagnostic staining and also can induce delayed hypersensitivity.[19]

The envelope consists of two distinct parts:

1. Cell wall
2. Plasma membrane

CELL WALL

The cell wall consists of upper and lower compartments. Before the lower segment there is a membrane which is also known as cell wall core.

It is unsolvable matrix of mycobacterial cell wall after removal of all soluble proteins, lipids and carbohydrates. It contains three covalently attached macromolecules: Peptidoglycans,Arabinogalactan and mycolic acid-MAGP (MycolylArabinogalactan Peptidoglycan) complex. The upper segment consists of free lipids, proteins,Phosphotidylinositolmannosides (PIMs), phtiocerol containing lipids, lipomannan(LM) and lipoarabinomannan (LAM).[20]

All these lipids, proteins, and lipoglycans are the signaling and effector molecules in the disease process. Whereas the insoluble core is essential for the feasibility of the cell in the situation of new drug development.[20]

A. Biologic function of free lipids:

Mycobacteria are rich in lipids and are largely bound to proteins and polysaccharides. Knowledge of their roles in signaling events, pathogenesis and the immune response is now emerging.[20]

Muramyl dipeptide (from peptidoglycan) complex with mycolic acids can cause granuloma formation whereas phospholipids induce caseous necrosis.[21]

CORD FACTOR/TDM (TRECHALASE 6 6’ DIMYCOLATE)

A “cord factor” has been extracted from virulent bacilli with petroleum ether. Cord formation is correlated with virulence. Virulent strains of tubercle bacilli form microscopic “serpentine cords” in which AFB are arranged in parallel chains. It induces cytokine mediated events, such as systemic toxicity, antitumor activity and release of chemotactic factors. It can cause chronic granuloma and can serve as an immunologic adjuvant.[22]

SULFOLIPIDS

The most virulent strains were productive in elaborating acidic lipids, whereas asweakenedone were notably deficient in these compounds. Theyact as an antagonist to the fusion of secondary lysosome with phagosome, thus promoting intracellular survival of the pathogen.[23]

LIPOARBINOMANNAN

It is lipopolysaccharidelipoglycansfound in mycobacteria, it has properties analogues to gram negative O-antigenic PAS (Lipopolysaccharide), such as T-lymphocyte activation and inhibition of antigen responsiveness of cells. LAM also inhibit interferon -gamma mediated activation of macrophages. Thus it hadassociated with interaction of pathogen and host cell in the down regulation of T cell response of various types. It is also major B cell immunogen.[24]

PTHIOCEROL DIMYCECROSATE (DPM/PDIM)

Major lipid of tubercle bacillus referred to as ball wax or wax in bacillus. It is known to have wide spectrum of virulence.

Analysis of lipids by gas chromatography reveals pattern that help in organization of different species.[24]

B. PROTEINS

Each type of mycobacteria encloses several proteins and are associated with cell wall and are powerful immunogens. Proteins are destined to a wax fraction and on injection prompt tuberculin sensitivity. They can also provoke the development of variety of antibodies.[25]

C.POLYSACCHARIDES

Mycobacteria comprises a variety of polysaccharides. Their role in the pathogenesis of disease is indeterminate. They can induce the immediate type of hypersensitivity and can serve as antigens in reactions with era of infected persons.[25]

PATHOGENESIS OF TUBERCULOSIS.

Route and site of infection:

Mycobacterium tuberculosis is an obligate aerobic, intracellular pathogen, which has a tendency towards lung tissue. In most cases the tubercle bacilli go into the body via respiratory route. The bacilli spread from the initial site of infection (lung) through lymphatics or by hematogenous route to other parts of body. The apex & regional lymph node being favored sites. Extra pulmonary TB of the pleura, lymphatics,skin bone, genito-urinary system occurs in 15% of cases. [26]

EVENTS FOLLOWING ENTRY OF BACILLI[26]

- Surface binding of M.tuberculosis to macrophages.
- Phagosome-lysosome fusion.

- Mycobacterial growth inhibition/Killing.
- Recruitment of accessory immune cells for local inflammatory response.
- Presentation of antigens to T cells for development of acquired immunity.

BINDING OF M. TUBERCULOSIS TO MONOCYTES/ MACROPHAGES

Complement receptors (CR1, CR2, CR3 & CR4), Mannose receptors(MR), and other cell surface receptor molecules plays avital role in binding of organisms for phagocytosis.M.Tuberculosisenter macrophages by endocytosis which is mediated by several macrophage receptors. MR attaches to thelipoarabinomannan, a glycolipid in the bacterial cell and complement receptors binds opsonized bacteria.[26]

PHAGOLYSOSOME FUSION

Phagocytosed microorganisms are degraded by intra-lysosomal acidic hydrolases upon phagolysosome fusion. This highly regulated event constitutes a significant antimicrobial mechanism of phagocytes. Inside the macrophage, M. Tuberculosisduplicates within the phagosome by preventing fusion of phagosome and lysosome.[27]

This is an active process as live bacteria blocks the formation of phagolysosome complex, which involves inhibtion of Ca^{2+}signals by blocking the recruitment &association of the proteins which helps in fusion ofphagosomeand lysosome complex. Mycobacterial sulphatides, derivates of multiacylated trehlose-2 sulphate, also have the ability to inhibit phagolysosome fusion.[27]

Thus, the earliest stage of primary TB (<3 weeks) in the non-sensitized individuals is characterized by proliferation of bacteria in pulmonary alveolar macrophages and air spaces, which results in bacteremia and seeding at multiple sites. Although there is

proliferation of bacteria in blood, most of the patients at this stage presents with mild flu like illness or asymptomatic**.**[28]

Macrophages by generating various antimycobacterial effector molecules like reactive oxygen intermediates (ROI) and reactive nitrogen intermediates (RNI) handle the engulfed mycobacteria.[29]

Evasion of host immune response by tuberculosis:

The immune evasion strategies are:

1) Modulation of phagosome by modulation of antigen presentation to avoid elimination by T-cells.

2) Proteins secreted by M. TB such as superoxide dismutase & catalase are antagonistic to ROI.

3) Mycobacterial components such as sulphatides, LAM and phenolic glycolipid I are potent oxygen radical scavengers.

4) M. Tuberculosis infected macrophages appears to be diminished in their ability to present antigens to CD4 +T cells, which leads to persistent infection by production of inhibitory cytokines like TGF-Beta, IL-10 or IL-6.[30]

HIV-TB CO INFECTION

Studies have shown higher incidence of TB among HIV infected individuals.HIV positive patients are at increased risk of developing recently acquired infection as well as reactivation or latent infection. 60-70% of HIV positive individuals will develop TB in their life time. Most of the HIV positive persons presents with extra pulmonary or disseminated disease over Non HIV patients.[31]

TB and HIV infections are both intracellular and known to have profound influence on progression to each other. HIV infection leads to reduction in CD4+T cells, which plays a main role in immunity to TB .[31]

Since CD4+ receptors of the T cells are bound by HIV through the gp-120 antigen, interaction of these cells with APC (antigen presenting cells) in the context of class II MHC (major compatibility complex) molecules is impaired, which results in hypo-responsiveness to soluble tubercle antigen.[31]

FINE NEEDLE ASPIRATION CYTOLOGY (FNAC)

The primary purpose of FNAC of an enlarged lymph node is to decide whether to do/not to do biopsy of lymph node for histological examination. The cytological examination can decide whether lymphadenopathy is due to reactive hyperplasia, infections, metastatic malignancy or lymphomas.[32]

At first lymph node aspirations for diagnostic purpose were performed at the beginning of the century. In 1927, Forkner published an elaborate study in lymph node aspiration cytology. In between 1930 and 1940 the works of Martim and willis, pavlovski, stahel, Rohr and Weil appeared.[33]

Knowledge of the structural, histological features of normal lymph node is essential in evaluation of FNAC smears from enlarged nodes, whether the pathology is reactive, infective or due to lympho-proliferative disorder. A brief outline of structure of a normal lymph node is therefore included following description of the normal cell population.[34]

The lymph node parenchyma is surrounded and divided by fibrous capsule with attached septa. Anatomically it is divided into outer cortex, an inner medulla and an intervening paracortex and sinus system.[35]

The cortex contains primary and secondary follicles. Primary follicles consist of aggregates of small resting B cells, are found in unstimulated node. Secondary follicles develop after antigen stimulation and consists of narrow mantle zone of small B lymphocytes surrounding a germinal Centre, several types of cells are found in germinal centre, the vast majority being B cells in the form of Centroblast and Centrocytes. Macrophages containing phagocytosed debris are also present.[35]

Mature immunoglobulins secreting B cells, familiar as plasma cells, are the principal cell type found in the medulla. The paracortex contains many small lymphoid cells which are of T-phenotype. In addition, activated T-cells and Immunoblasts are also present.[35]

Normal Cytology

The lymphocytes constitute 87-99%, the plasma cells 0-5% and remainder cells form 1-3% of the total population in the aspirate from a normal lymph node.[36]

1. Mature lymphocytes-

These are small, round lymphocytes measures 7-10 µ, having dense nuclear chromatin with thin rim of pale cytoplasm often visible at one edge of the cell.[36]

2. Follicular center cells

A)Small cleaved lymphocytes- These are 10-12 µ in diameter. Their nuclei show deep indentation of nuclear membrane, and hence the term cleaved and have moderate cytoplasm.[36]

B)Large cleaved lymphocytes

These are round cells measuring 20-40µ in diameter and nuclei appears cleaved as a result of deep indentation of the nuclear membrane and nucleoli are not prominent and the cells have abundant basophilic cytoplasm.[36]

C) Small non-cleaved lymphocytes

These are larger measuring 12-20µ in diameter. They are round and have scant amount of cytoplasm unlike mature lymphocytes and they contain prominent nucleoli.[36]

D) Large non-cleaved lymphocytes

These are 20-40µ in diameter. They have large round vesicular nucleus with two or three prominent nucleoli and moderate amount of cytoplasm .[36]

3.Immunoblast

These are large cells measuring 20-40µ in diameter, three times larger than small lymphocytes. They have round irregular nuclei, with fine granular chromatin with prominent nucleoli that is centrally/ eccentrically placed with abundant amount of cytoplasm .[36]

4.Plasma cells

They have eccentrically placed nucleus with dense coarse chromatin that may be arranged in typical cart-wheel like pattern with deeply basophilic cytoplasm and with para-nuclear clear zone.[36]

5.Macrophages

These cells show wide variation in size, in the resting phase the cell measures 14 to 34µ in diameter. There cytoplasm contains many phagocytosed fragments of degenerated cells (tingible bodies). The nucleus is round to oval and contain evenly distributed reticulated chromatin.

Other cells like interdigitating reticulum cells, endothelial cells can also be seen in lymph node aspirate. Eosinophils, plasma cells and neutrophils may also be seen depending on pathological process.[36]

6. Lymphoid globules (Lymphoglandular bodies)

They are the rounded cytoplasmic fragments measuring 8µ in diameter and scattered in the background. The fragments stain an even pale blue identical to the cytoplasm of intact cells with Giemsa stain. They are characteristic finding in smears of lymphoid tissue. They differ from necrotic debris by their regular round shape and uniform staining.[37]

The cause of lymphadenopathy in all the cases may be due to non-neoplastic/benign, primary/metastatic disease. FNAC will be beneficial in the diagnosis of such lesions. In positive cases of malignancy FNAC is the first indication of choice and it is useful to search for hidden neoplasms. As management decision depends on type of tumor or non-tumorous process, FNAC plays an important role indiagnosis of cause of lymphadenopathy. Many studies showed that overall sensitivity of 92.7% and specificity of 98.5%. FNAC is recommended as a gold screening procedure in all cases of lymphadenopathy.[38]

In a retrospective analysis of 1448 lymphadenopathy cases for FNAC study emphasized its increasing popularity as the first line diagnostic procedure in evaluation of lymphadenopathy as shown by four-fold increase in number of FNAC performed in recent years.[39]

FNAC can be employed at the bedside as well as in the outpatient department without giving any prior anesthesia or any fear to the patient. Even this technique is

superior to lymph node biopsy in the sense that samples can be taken at different sites at different times without much inconvenience to the patients.[40]

FNAC, not only provides a provisional diagnosis and interpretation of the adequacy of the sample, but they are also utilized for ancillary studies, such asimmuno-histochemicalstudies on cell blocks or Cytospin preparation to augment the diagnostic accuracy of FNAC findings.[41]

FNAC emerged as a safe and reliable diagnostic procedure in pediatric group reducing the need for excision biopsy. It has decreased the chances of surgical trauma to the childrenpermittingassortment of the best lymph nodebiopsy whenever needed. No significant complications were encountered in any of cases.[42]

Epidemiology of TB-

According to WHO 2 billion people that is one third of the world population is suffering from TB. In world 8000 people are dying every day that is 2.3 million people deaths are occurring every year due to TB.[43]

Most cases are estimated to be in Asia and Africa (58% and 27% respectively), with the highest incidence in India (range 2.0-2.4 million) and China (0.9 -1.1 million), together accounting for 38% of the total number of cases.[43]

They had recently launched a new global TB strategy for the “post-2015 era” aimed at “ending the global TB epidemic” by 2035**.**[44]

It includes Vision, Goal, Milestones for 2025 and Targets for 2035.[44](Table 1).

TABLE 1: New global TB strategy for the "post-2015 era" by WHO[44].

VISION	A world free of tuberculosis –zero deaths, disease and suffering due to tuberculosis
GOAL	End the global tuberculosis epidemic
MILESTONES FOR 2025	–75% reduction in tuberculosis deaths (compared with 2015); –50% reduction in tuberculosis incidence rate (compared with 2015) (less than 55 tuberculosis cases per 100 000 population) –No affected families facing catastrophic costs due to tuberculosis

FNAC OF TB LYMPHADENOPATHY

The cytological features of tuberculosis on FNAC might be reflection of pathogenesis of basic lesion of the disease process with the granuloma.[45]

The most common cause of granulomatous lymphadenitis in developed countries is sarcoidosis but in many tropical countries and patients with immunodeficiency, most common cause is tuberculosis.[46]

Other conditions like leprosy, cat scratch disease, Paracoccidomycosis, histoplasmosis, Leishmaniasis, LGV (Lympho Granuloma Venerum), brucellosis, tularemia and rarely foreign bodies such as talc or silca can also give rise to the granulomatous lymphadenitis.[46]

Cytomorphological features of the tuberculous lymphadenitis shows four major patterns[47]:

1. Epithelioid granulomas with Caseous necrosis
2. Epithelioid granulomas without Caseous necrosis
3. Necrosis alone without epithelioid granulomas.
4. Acute suppurative inflammation

Epithelioid granulomas are small clusters of epithelioidhistiocytes which have elongated nuclei described as slipper shaped, which are arranged in syncytial fashion with abundant ill-defined cytoplasm mixed with reactive lymphocytes, fibroblasts and with or without Langhan's giant cells.[48]

Langhan's giant cells are large multinucleated cell with their nuclei polarized at one part of cell border or in horse shoe shaped pattern.[49]

On cytology Caseous necrosis appears as granular pale stained amorphous material in the background and lack recognizable cell remnants. For definitive diagnosis, AFB should be identified using ZN stain or other special stains for AFB.[50]

The Cytodiagnosticcriteria of tuberculosis on cytological smears is based on presence of Epithelioid cells, multi nucleated giant cells and necrotic background.All these cytomorphological patterns are confirmed by ZN stain. If the ZN stain was negative they are confirmed by AF method or by Culture.[51] (Table-2)

Table :2

Cytodiagnostic criteria for Tuberculous lymphadenitis.[51]

Cytologic feature	ZN stain	Diagnostic label.
A. Epithelioid cells, Multinucleated giant cells and necrosis.	A)AFB positive B) AFB negative Confirmed by either Auto-fluorescence/ culture study for accurate results.	A)Tuberculous lymphadenitis. B)Granulomatous lymphadenitis. Likely to be of tuberculous etiology, however stain for AFB is negative
B. Necrotic material without epithelioid cells	A) AFB positive B) AFB negative Confirmed by either Auto-fluorescence/ culture study for accurate results.	A) Tuberculous lymphadenitis. Repeat FNAC advised for cytodiagnosis, ZN staining and /or for culture of AFB.

The diagnosis of tuberculosis depends on demonstrating mycobacteria by using special stains like ZN, AR, AF after performing FNAC. If the ZN stain is negative for AFB on the smear, the cytological features of caseous necrosis or granulomatous inflammation on FNAC are sufficient for initiation of treatment in endemic cases. It helps to avoid unnecessary complications due to surgical interventions and avoids the delay of treatment. Hence FNAC is generally considered

to be acheap, reliable and sole indicative tool in diagnosis of tuberculous lymphadenitis.[52]

The characteristic necrotic background comprising of eosinophilic granular material containing nuclear debris was described as “Tubercular diathesis”. Many cases lacking the typical findings and showing scattered epithelioid cells with or without granuloma or only necrotic material with neutrophilic infiltration were diagnosed as tuberculous lymphadenitis, even though AFB were absent in these smears.[53]

In a retrospective study, criteria required to make an FNAC diagnosis of tuberculosis were reassessed with 70 cases. The sensitivity of Fine needle aspiration (FNA) cytology with histological confirmation emphasize that all criteria for the diagnosis of TB in FNA sample must be utilized and that particular caution should be exercised in making a diagnosis of acute necrotizing granulomatous inflammation including metastatic lesions.[54]

The FNAC smears of tuberculosis were hypo-cellular or in some cases, normo-cellular but never hyper-cellular. FNAC on correlation with histopathology showed that its diagnostic efficacy, particularly in tubercular lymphadenitis has been reported to be as high as histopathological studies.[55]

Although culture is considered confirmatory for diagnosis, a negative culture does not eliminate the diagnosis of TB, as enlarged lymph node do not necessarily contain live bacilli. In conditions where the resources are limited, FNA alone is taken as diagnostic of TB.[56]

Tuberculousof intercostal lymph node generally present as cold abscesses. Such abscesses are commonly presumed to be soft tissue TB.Therefore, if necrosis or a granulomas are encountered in soft tissue swellings, particularly in soft tissue of chest,

or in the axillary region, the possibility of intercostal tuberculous lymphadenitis should be suspected.[57]

The diagnosis of tuberculosis is early and simple, when the disease is florid or disseminated.At times TB of extra pulmonary organ or tissue pose a diagnostic problem. In such cases FNAC offers a simple, safe and rapid and accurate diagnosis in such cases.[58]

Isolation of AFB by staining:

The detection of AFB by ZN stain technique is the most commonly used technique in developing countries due to the cost involvement, equipment required and time involved with other modalities.[59]

The cell wall of mycobacteria has unique ability of binding the fuchsin dye so that it cannot bedestained by acid alcohol. This property of AFB is valuable help in the early detection of infection .[60]

Aspiration of pus (purulent material) from a lymph node aids in differentiation between acute suppurative and tuberculous lymphadenitis. The cytological picture between these two conditions is identical -Numerous neutrophils lying in an abundant, necrotic background. Differentiation between these two conditions depends upon presence or absence of AFB. One clinical observation is of great help in such cases such as tuberculous lesions present as cold abscesses, while acute suppurative lymph nodes present as hot abscesses.[59]

For bacilli to be detected on the smear, their number should be minimum 5,000 to 10,000 per ml of material. If the number is less than this, they may not be detected on the smear.[61]

In 1990 Das DK*et al*[51] correlated cytomorphological features of TB lymphadenitis with AFB positivity and bacillary counts in 174 cases. They found that in presence of epithelioid,Langhan giant cells the AFB positivity was lower while the representation was just reverse in presence of necrosis and infiltration of neutrophils.[59]

The activated macrophages aggregate around the center of the lesion to form granulomas and to effectively phagocyte the mycobacteria without producing any destruction to tissue leading to Caseous necrosis. If the macrophage activating mechanism is poor, it leads to tissue destruction. The lesion starts enlarging further the Caseous material liquifies. This liquefied Caseous material contain large number of bacilli.[59]

When the smears were devoid of granuloma and show features of only acute inflammation, tuberculous etiology can be established only by doing special stains such as ZN.[62]

Cytodiagnosisaccompanied with AFB smear and culture examination helps in accurate diagnosis. A negative mycobacterium does not exclude the possibility of TB when smears are positive as it can result from inadequacy of the material submitted for culture or anti-tuberculous therapy**.**[63]

A presumptive diagnosis of mycobacteria can be attained by a cautious examination of Romanowsky stained smears helps in identification unstained images of AFB. The presence of mycobacteria as negative images has been detected in Romanowsky stained smears of peripheral blood, bone marrow buffy coat and lymph node aspirates. These images have also been described in Geimsa and Gram stained sputum samples.[64]

Closer observation of these unstained images appears as bundled, curved or criss cross within the cytoplasm of histiocytes.[64]

In contrary to other mycobacteria MAI has anexceptional staining character in addition to ZN stain, they are positive in PAS stain. This staining characteristic is of diagnostic importance because only few bacteria like nocardia and corynebacterium show both acid fast and PAS positivity.[64]

The detection of AFB in stained smear is the easiest and most rapid procedure include ZN (hot stain), Kinyoun (cold stain) and fluorochrome using auramineO, with or without a second fluorochrome, Rhodamine.[60]

With carbolfuchsin, the AFB stains bright red against either blue or green background depending on the counter stain used.[62]

The property of AFB is due to thick, waxy capsule that surrounds the mycobacterial cell. For aqueous carbolfuchsin to penetrate through the wax, the capsule should be softened. This is done by ZN procedure. The dye will penetrate in to the waxy capsule by heating and binds to the cell wall. The bacterial cells cooldown after the heat is removed, the capsule again hardens and protects the bounded dye and cell wall from the acid alcohol decolorizer.[60]

It should be emphasized thatAuramineRhodamine stain was a fluorescent antigen- antibody technique, rather it was a direct physio-chemical binding of the stain to mycolic acid rich cell wall.[60]

METHOD OF REPORTING AFB[52]

The grading scale (Table 2), originally recommended for grading sputum smear for AFB was used to grade the smears of lymph node aspirates stained by ZN method

Table 3 Grading of AFB

No. of AFB seen	Grade	Minimum number of fields to be examined
>10 per oil immersion field	3+	20
1-10 per oil immersion field	2+	50
10-99 in 100 oil immersion field	1+	100
1-9 in 100 oil immersion field	Scanty	200

Diagnostic utilization of UV-induced fluorescence for detection of infective pathogens was first described by Graham and later Mann in 1983.[65,66]

Ghaliet al[67] (1984) first demonstrated AF of pneumocysticcarinii in PAP stained smears.

In 1995, Küpper *etal*[68] reported significant difference between the sensitivities of PAP fluorescence (FMP), A-R fluorescence and Z-N staining respectively for their ability to detect atypical Mycobacterium kansasi in cytological samples in bronchial secretions.

Wright *et al*[2] conducted a prospective study from 1991 to 2001 of 1,044 superficial lymph node aspirates on HIV positive and negative cases and assessed morphology of lymph node on routine PAP stained smears. The same smears were seen under fluorescent microscopy for AFB and compared with culture technique. On comparison with culture, they found that fluorescent had shown many false positive results.

In 2012 Joshi *et al*[69], conducted a study on 212 patients who were suspected of tuberculosis on clinical examination. They eliminated 20 cases due to blood mixed material and other 20 due to reactive lymphadenitis. Out of 80 patients ZN stain was positive only in 30 cases and AF was positive in 46 cases. Culture was positive on 40 cases. By taking Culture as a gold standard method they concluded that AF was more superior than ZN stain. They had observed three different cytomorphological patterns in the TB lymph node - Granulomatous pattern, Caseating necrotizing lymphadenitis and acute suppurative.

Brijesh*et al*[70], conducted a study on 145 clinically suspected cases of TB presenting with lymphadenopathy for a period of 1 year (Aug 2010 to July 2011).They made four smears from each aspirate Giemsa, ZN stain, AR stain and one was wet fixed for PAP stain and needle washes for incubation in Lowenstein-Jensen medium for culture study. Out of 145 cases 90 cases were positive. Among 90 cases only there observed that 24 cases were positive byZN, 31 cases were positive by AR ,38 were positive on AF and culture was positive only in 25 cases. Using Culture as a reference method theyconcluded that AF is more sensitive in compare to AR and ZN stain as it is an inexpensive technique. They concluded that limited use of AF was better because of increase of false positive rates and subjective biased errors. AF should always be used as an adjacent to other techniques.

Krishna M *et al*[71] done similar study on clinically suspected cases of TB lymph nodes in 2015 on 88 cases. They stained all the lymph nodes aspirates with PAP, ZN and for AR. Out of 88 cases they concluded that AF was more effective than AR and ZN staining in demonstration of AFB on FNAC of lymph node.

MATERIALS AND METHODS:

SOURCE OF DATA

Patients of both OPD and IPD, referred to the department of Pathology in BLDE University Shri B.M. Patil Medical college, hospital and research centre, Vijaypur in who fine needle aspiration (FNAC) of lymph node is suspected case of tuberculosis is requested.

Study period: 1st November, 2015 to 30 June, 2017.

INCLUSION CRITERIA:

All the patients who are clinically diagnosed having tuberculosis with lymphadenopathy referred for FNAC to the department of Pathology.

EXCLUSION CRITERIA:

Patients who are having non palpable lymph nodes, skin diseases are excluded.

METHODS OF COLLECTION OF DATA.

A prospective study of 153 samples of suspected cases of tuberculosis satisfying the inclusion and exclusion criteria, referred to the Pathology department of BLDE University's Shri B.M Patil medical college, Hospital and Research centreVijayapur, Karnataka was taken for the study.

A detailed history of patients was elicited and complete general physical examination and systemic review of the patients was undertaken. Informed consent was taken from the patients for the FNAC procedure.

Smears were made from aspirate and stained with H&E, Giemsa,PAP& ZN stains. PAP stained smears were examined for AFB for their AF under fluorescent microscope using the blue excitation filter.

ZN stained smears were examined for AFB under oil immersion of compound microscope. AR stain was taken as a gold standard method and AR stained smears were scanned under fluorescence microscope.

MATERIAL

- Aspirated material from the lymph node.
- Equipment.
- 5ml/10ml disposable syringe
- 22-26-gauge needle
- Cameco syringe holder
- Clean non grease glass slides
- Cotton swab and methylated spirit
- 95% ethyl alcohol in coupling jars for fixation
- PAP stain— (Hematoxylin, OG6, EA 36, and graded alcohols).
- ZN stain- (Carbolfuschin, Acid alcohol, methylene blue,).
- Auramine stain - (Auramine O, Acid alcohol and Potassium Permanganate).

SAMPLE SIZE:

With the prevalence rate of tuberculosis is 2.22% and considering 95% of confidence level and +/- 3 margin of error. [6]

So calculated sample size was 153.

Using statistical formula

Where, $n = z^2 \frac{p(1-p)}{d^2}$

n= Sample size.

Z = 1.96 at 95% confidence limit

P = prevalence of tuberculosis=2.22%

d = Allowable error +/-3,

Hence, minimum of 153 samples was included in the study.

STATISTICAL ANALYSIS:

The following statistical analysis was done:

- Sensitivity
- Specificity
- Positive predictive value
- Negative predictive value

RESULTS

153 Patients were included in the study. The smears of the lymph node aspirates were stained with PAP, Giemsa, H&E and special stains like AR and ZN stain are examined. PAP stained smears were examined for AFB for their AF. ZN stained smears are also examined for AFB under oil immersion of compound microscope. AR stain was taken as a gold standard method to prove the accuracy of AF over ZN staining in detection of AFB.

Table 4.1: Distribution of cases according to site of lymph node.

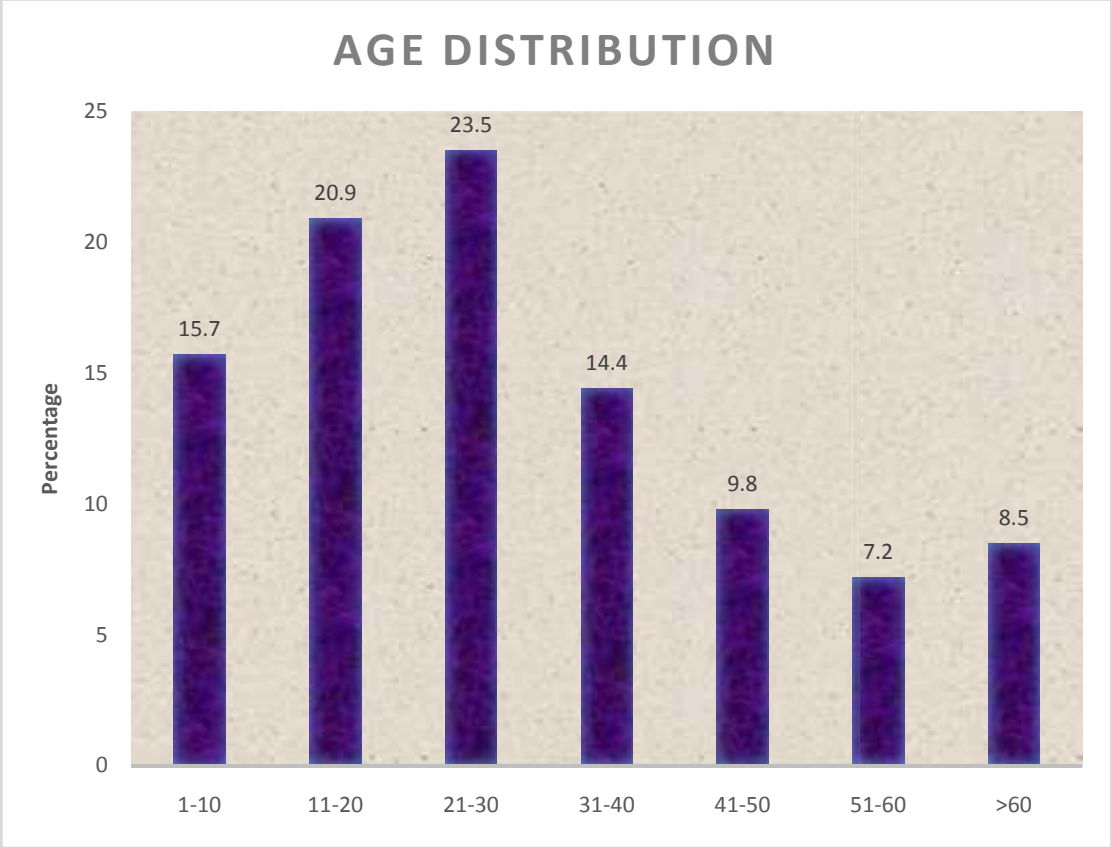
Lymph node	**N**	**%**
Cervical	71	46.4
Submandibular	20	13.1
Axillary	15	9.8
Supraclavicular	15	9.8
Submental	9	5.9
Preauricular	7	4.6
Posterior triangle	3	2
Cervical & SM	2	1.3
Inguinal	2	1.3
Para midline	2	1.3
Parotid	2	1.3
B/l inguinal	1	0.7
Gluteal	1	0.7
Groin	1	0.7
Infra auricular	1	0.7
Jugulodigastric	1	0.7
Total	153	100

In this study the most common site of involvement was cervical lymph node accounting for 71cases (46.4%). (**Table 4.1)**

Table 4.2: Distribution of Cases According to Age

Age (Yrs.)	N	%
1-10	24	15.7
11-20	32	20.9
21-30	36	23.5
31-40	22	14.4
41-50	15	9.8
51-60	11	7.2
>60	13	8.5
Total	153	100

Figure 1: Bar Diagram Showing Distribution of Cases According to Age

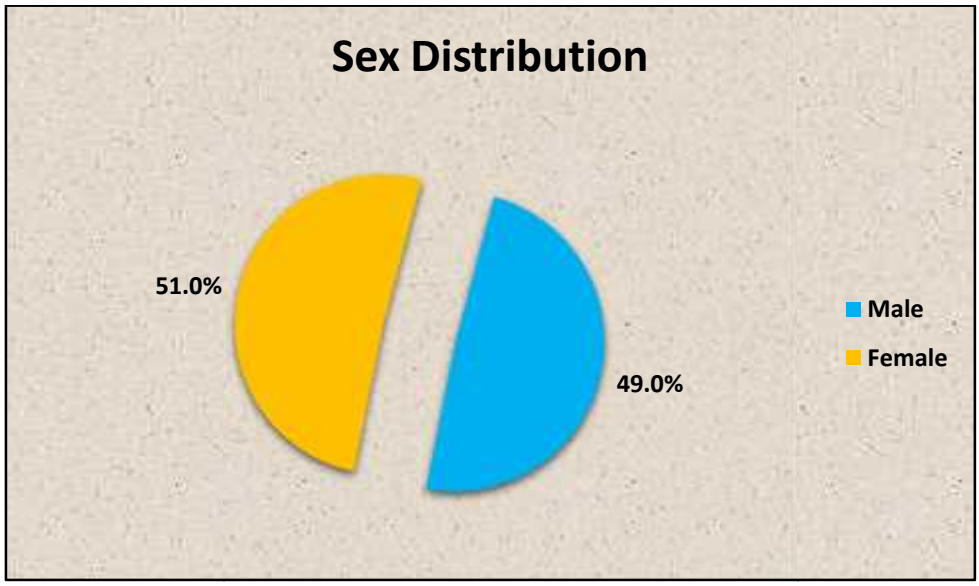


In the present study, the age ranged from 1 to 75 years. The mean age of presentation in our study was 23±5 years. **(Table 4.2 and Figure 1)**

Table 5: Distribution of Cases According to Sex

Sex	N	%
Male	75	49
Female	78	51
Total	153	100

Figure 2:Pie Chart Showing Distribution of Cases According to Sex

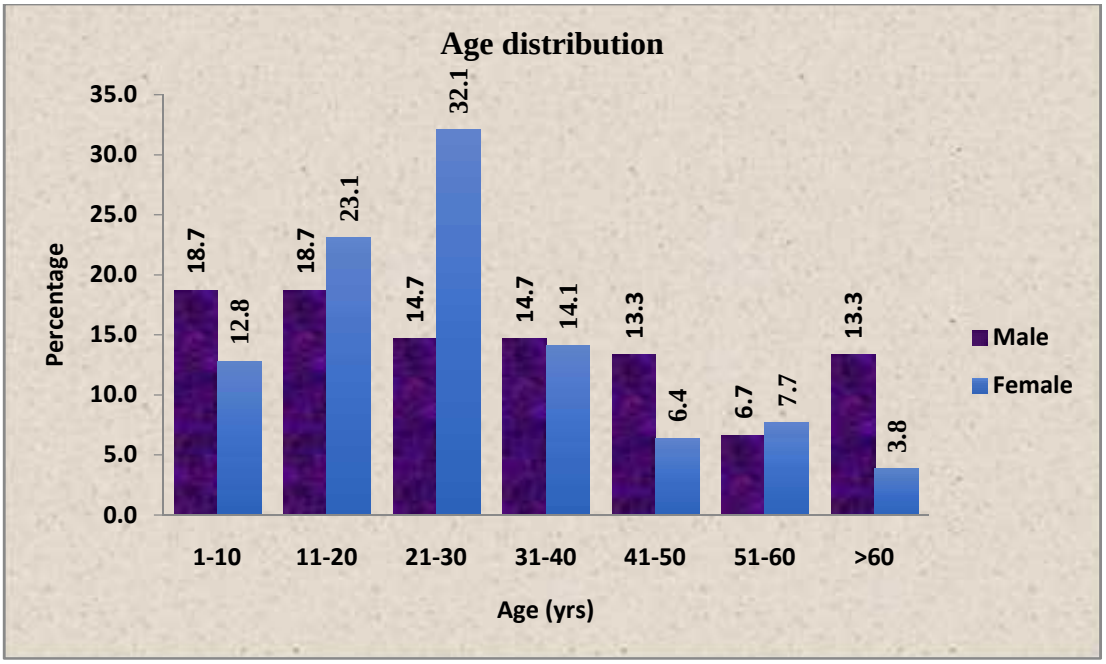


In the present study female 78(51%) preponderance was noted.

Table 6:Showing Associationof Age with Sex

Age (Yrs.)	Male		Female		p value
	N	%	N	%	
1-10	14	18.7	10	12.8	
11-20	14	18.7	18	23.1	
21-30	11	14.7	25	32.1	
31-40	11	14.7	11	14.1	
41-50	10	13.3	5	6.4	<0.06
51-60	5	6.7	6	7.7	
>60	10	13.3	3	3.8	
Total	75	100.0	78	100.0	

Figure 3: Bar Diagram Showing Association of Age with Sex

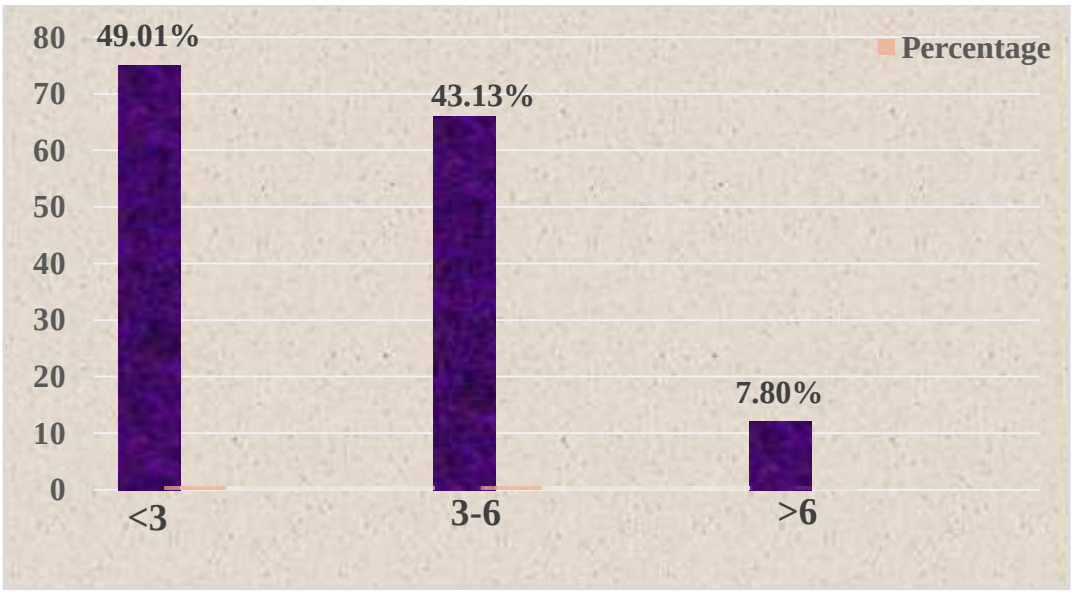


The most common age group affected in males was 1 to 20 years accounting for 37.4% and where as in females the most common age group affected was 21 to 30 years accounting for 32.1% which is not statistically significant (P<0.06).

Table 7: Duration of Lymphadenopathy

Duration(months)	No of cases	Percentage
<3	75	49.01%
3-6	66	43.13%
>6	12	7.80%
Total cases	153	100%

Figure 4: Bar diagram showing distribution of cases according to duration of lymphadenopathy

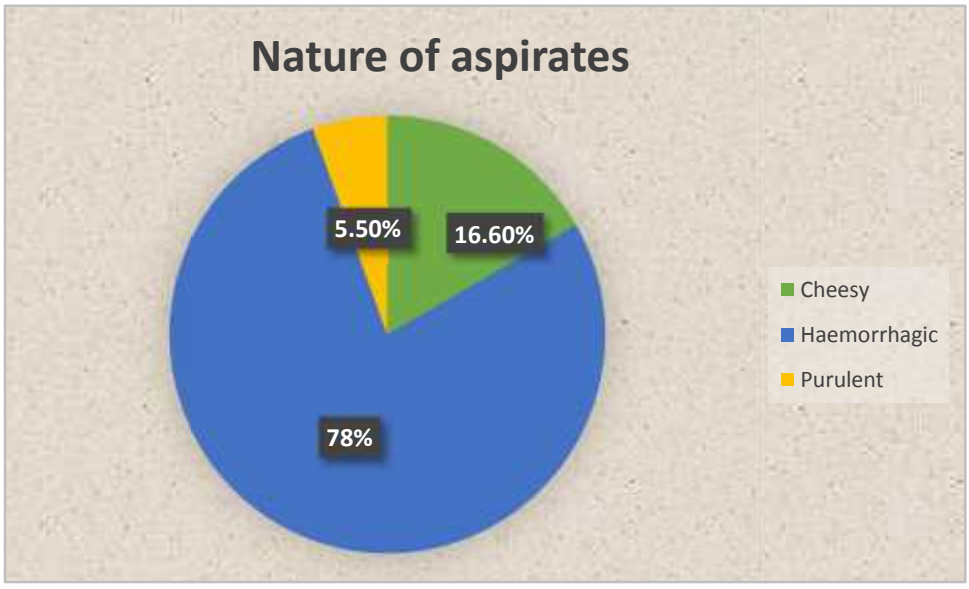


Most patients had lymphadenopathy of less than 3 months duration 49.01% (75/153), followed by 3-6 months 43.13% (66/153) and more than 6 month's duration was seen in 7.80% (12/153) patients.

Table 8: Distributionof Cases According to Nature of Aspirates in TB Positive Cases

Nature	No of cases	Percentage
Cheesy	6	16.60%
Hemorrhagic	28	77.77%
Purulent	2	5.50%
Total cases	36	100%

Figure 5: Pie Diagram Showing Distribution of Cases According toNature of Aspirates in TB Positive Cases.

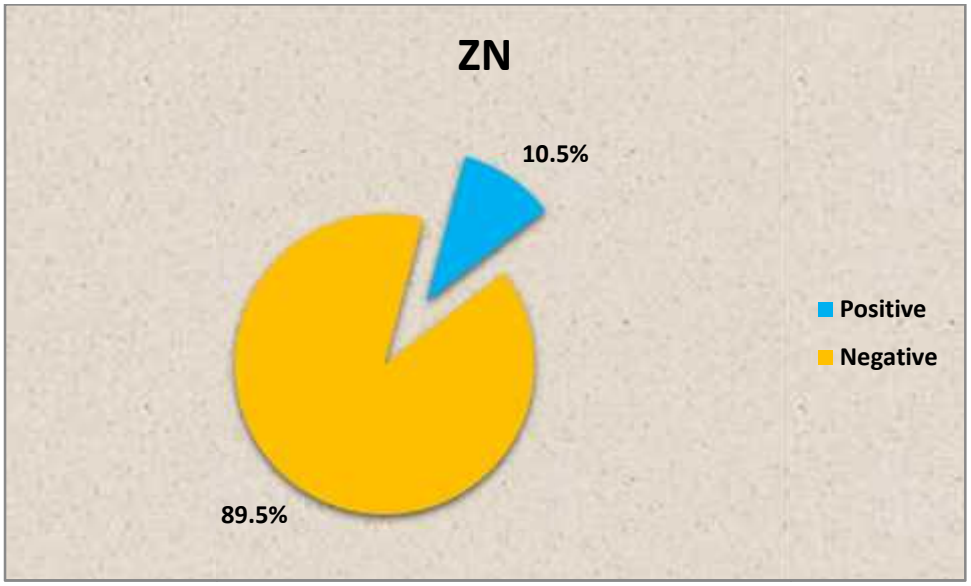


The most common aspirate seen in TB positive cases was hemorrhagic accounting in 78% of cases followed by cheese like material accounting in 16.60% of cases.

Table 9: Distributionof Cases According toZN Positivity

ZN	N	%
Positive	16	10.5
Negative	137	89.5
Total	153	100

Figure 6: Pie Diagram Showing Distribution of Cases According to ZN Positivity

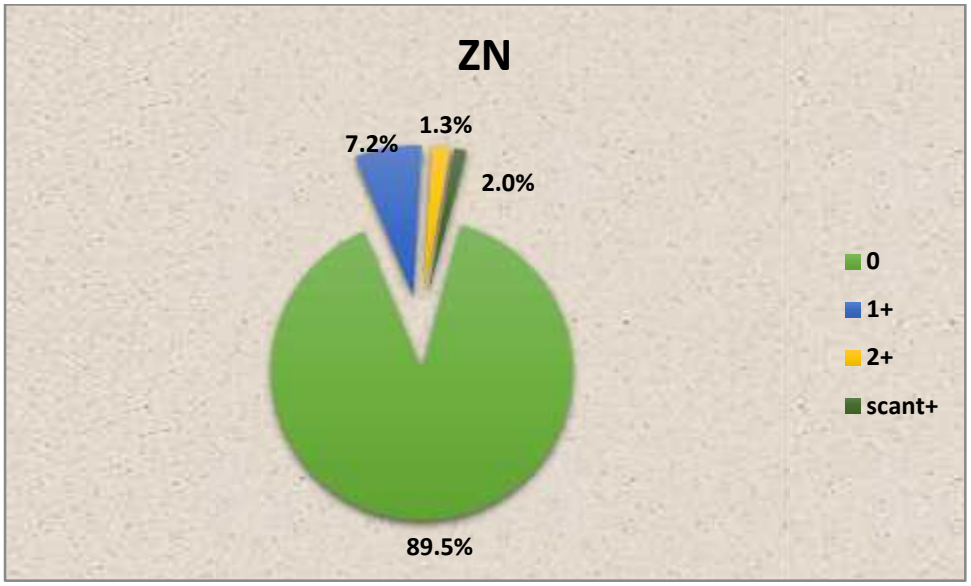


Out of 153 cases only 16 cases were showing positivity on ZN stain (10.5%) and rest 137 cases were negative for ZN stain (89.5%).

Table 10: Distribution of Cases According to Grades of ZN Positivity

ZN	N	%
0	137	89.5
1+	3	2
2+	2	1.3
scant+	11	7.2
Total	153	100

Figure 7: Pie Diagram Showing Distribution of Cases According to Grades of ZN Positivity

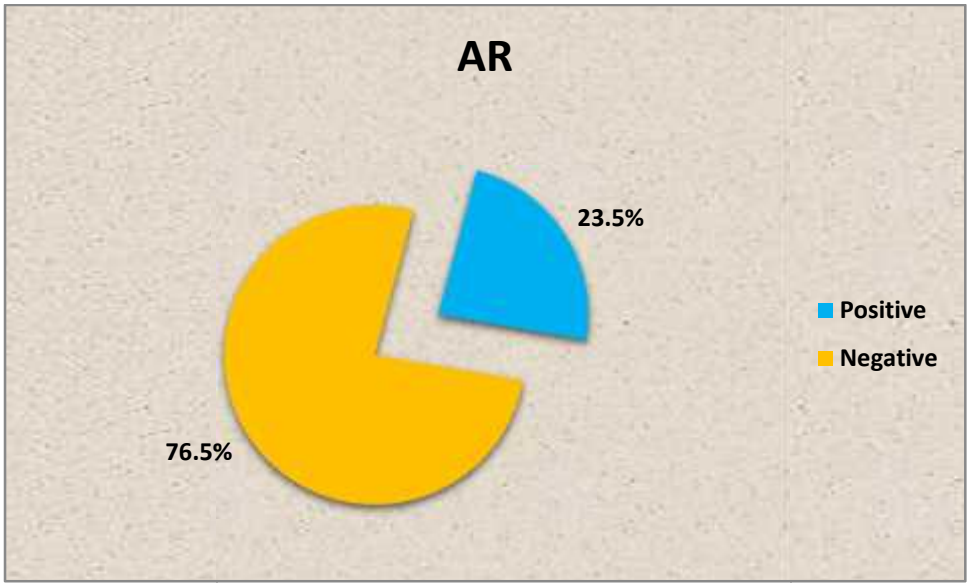


Out of 16 positive cases of Zn, 7.2% (11/153) cases show scant positivity and 2%(2/153) cases show 1+ positivity and 1.3% (2/153) show 2+ positivity.

Table 11: Distribution of cases according to AR Positivity

AR	N	%
Positive	36	23.5
Negative	117	76.5
Total	153	100

Figure 8: Distribution of cases according to AR Positivity

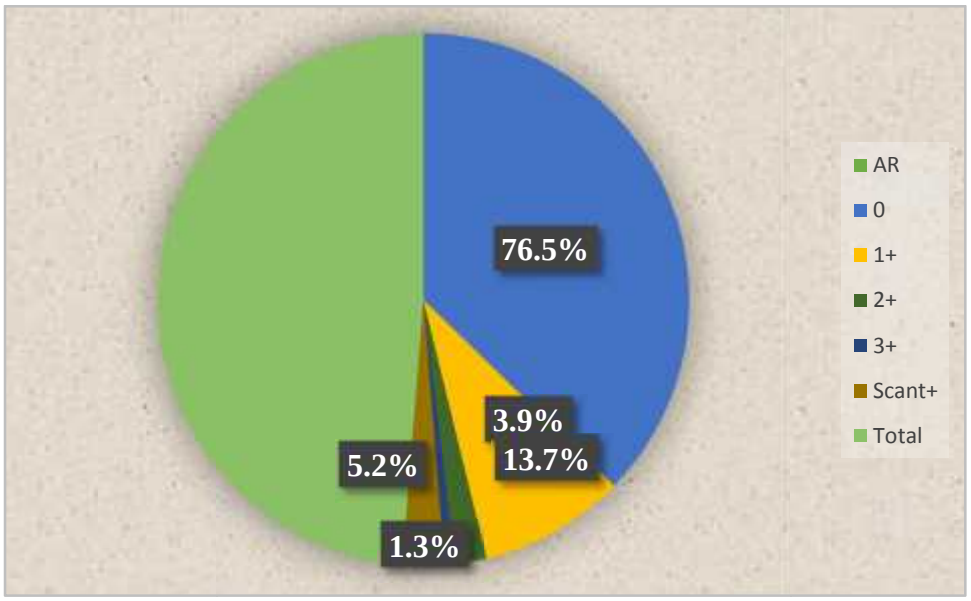


Of 153 cases, 23.5% (36/153) cases were showing positivity on AR and 76.5%(117/153) were negative on AR stain.

Table 12: Distribution of cases according to Grades of AR Positivity

AR	N	%
0	117	76.5
1+	20	13.7
2+	6	3.9
3+	2	1.3
Scant+	8	5.2
Total	153	100

Figure 9: Distribution of cases according to Grades of AR Positivity

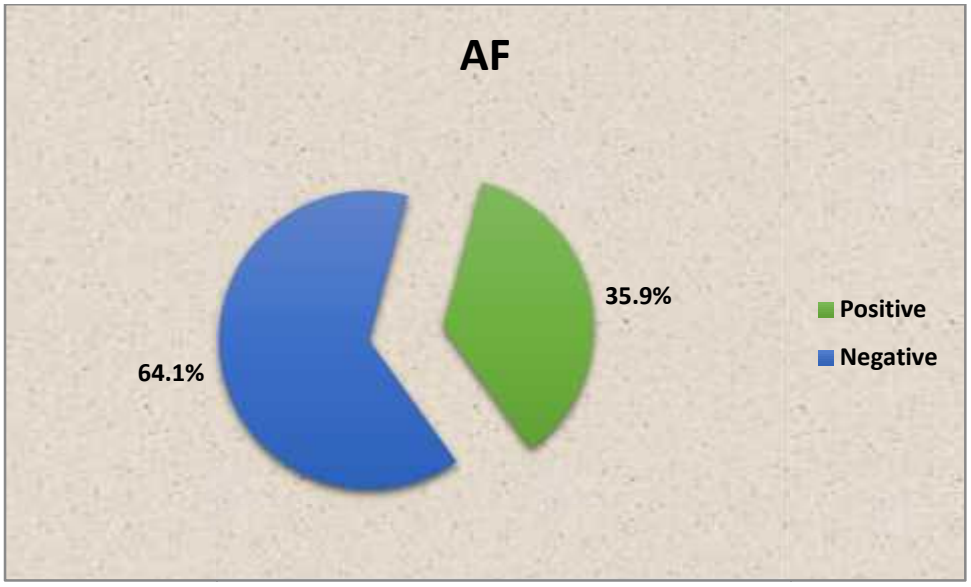


Out of 153 cases, 76.5%(117/153) cases were showing no bacilli so they are graded as negative that is grade 0, 13.7% (20/153) cases were showing grade 1, 3.9% (6/153) were showing grade 2 and 1.3% (2/153) were showing grade 3 and 5.2%(8/153) were showing scant positivity. This grading was done depending on number of bacilli in each High Power field.

Table 13: Distribution of cases according to AF Positivity

AF	N	%
Positive	55	35.9
Negative	98	64.1
Total	153	100

Figure 10: Distribution of cases according to AF Positivity

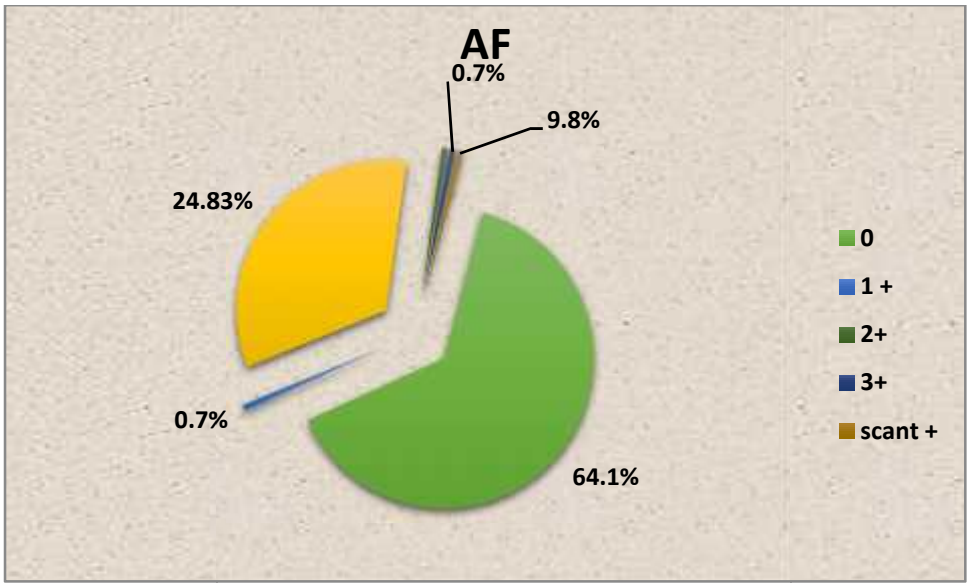


Of total 153 cases, 35.9% (55/153) were showing positive on AF, 64.1% (98/153) cases were negative on AF.

Table 14: Distribution of cases according to Grades of AF Positivity

AF	N	%
0	98	64.1
1+	38	24.83
2+	1	0.7
3+	1	0.7
scant +	15	9.8
Total	153	100

Figure 11: Distribution of cases according to Grades of AF Positivity

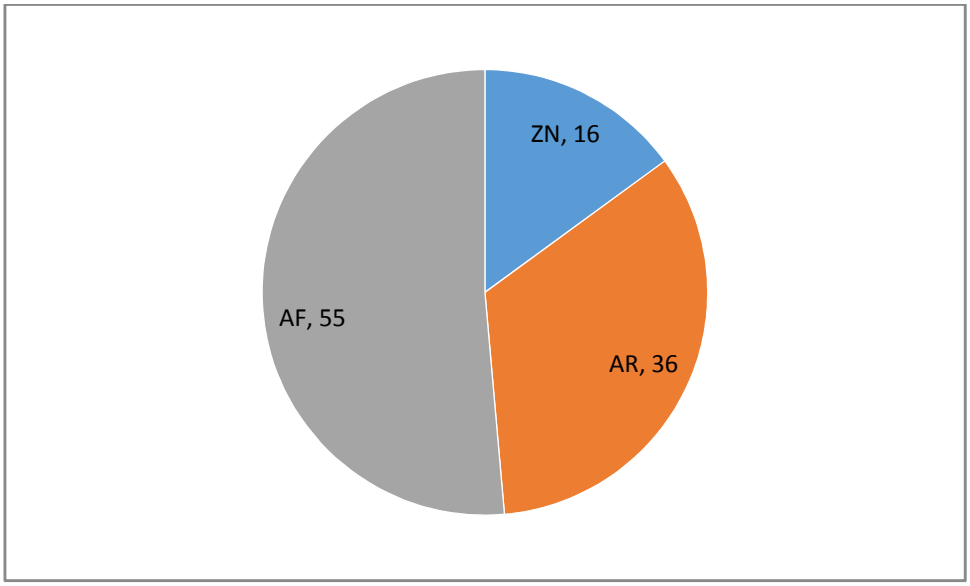


Out of 153 cases, 64.1%(98/153) were negative, 24.83%(38/153) were showing grade 1, 0.7%(1/153) were showing grade 2, 0.7%(1/153) were grade 3, 9.8%(15/153) were showing scant Positive.

Table 15: Distribution of cases according to ZN, AR and AF positivity

ZN	AR	AF
16	36	55

Figure 12:Distribution of cases according to ZN, AR and AF positivity.

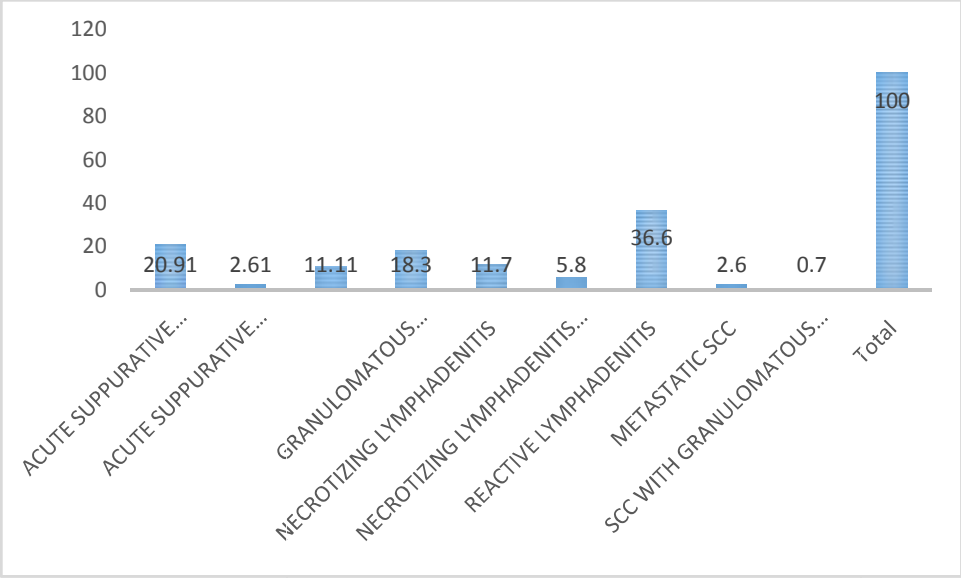


In the present study, 16 cases were positive on ZN, 36 on AR and 55 were positive on AF**.**

Table 16: Distribution of cases according to dominant Cytomorphological Patterns in various lymph node aspirates.

Impression of TB	N	%
ACUTE SUPPURATIVE INFLAMMATION	32	20.91
ACUTE SUPPURATIVE INFLAMMATION WITH AFB POSITIVE	4	2.61
GRANULOMATOUS LYMPHADENITIS	17	11.11
GRANULOMATOUS LYMPHADENITIS WITH AFB POSITIVE	28	18.30
NECROTIZING LYMPHADENITIS	18	11.7
NECROTIZING LYMPHADENITIS With AFB POSITIVE	6	5.8
REACTIVE LYMPHADENITIS	56	36.60
METASTATIC SCC	4	2.6
SCC WITH GRANULOMATOUS REACTION	1	0.7
TOTAL	153	100

Figure 13: Bar Diagram showing Distribution of cases according to dominantCytomorphological Patterns in various lymph node aspirates.

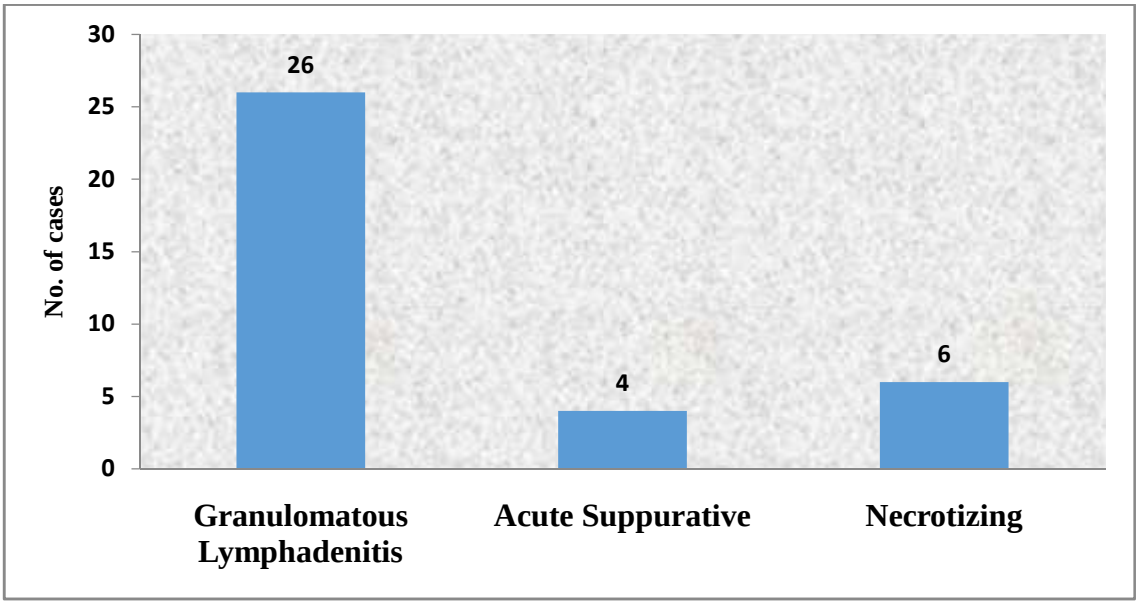


The most common cytomorphological pattern encountered in Suspicious cases of TB was reactive lymphadenitis accounting in 36% of cases followed by Granulomatous lymphadenitis accounting in 29% of cases followed by Acute suppurative inflammation in 22% of cases.

Table17.Dominant cytomorphological patterns in TB Lymphadenitis

Cytomorphological patterns	No of cases
Granulomatous Lymphadenitis	26
Acute Suppurative	04
Necrotizing	06
Total	36

Fig 14: Dominantcytomorphological patterns in TB Lymphadenitis



The most common cytomorphological pattern encountered in the tuberculosis positive cases was Granulomatous lymphadenitis in 26 cases constituting the major group followed by Necrotizing and Acute Suppurative Lymphadenitis.

Table 18: Comparative chart of results of the detection of AFB by ZN and AF with reference to AR stain

Results	ZN	AF
TP (true positive)	15	22
FP (false positive)	1	33
FN (false negative)	22	14
TN (true negative)	116	84

Taking AF as a reference method the 15 cases by ZN, 22 cases by AF stain were cytomorphological suggestive of TB.

Table 19: Taking AR as a reference method, analysis of statistical values of ZN and AF.

	ZN	AF
Sensitivity	41.67%	61.11%
Specificity	99.15%	71.79%
PPV	93.75%	40.00%
NPV	84.67%	85.71%
Accuracy	85.62%	69.28%

Taking AR stain as gold standard the AF showed a sensitivity of 61.11%, specificity of 71.79%, Positive predictive value (PPV) of 40.00% and negative predictive value (NPV) of 85.71%. On other hand ZN stain revealed a sensitivity of 41.67%, specificity of 99.15%, PPV of 93.75%, NPV of 84.67%. These differences are found to be statistically significant.

PHOTOMICROGRAPHS

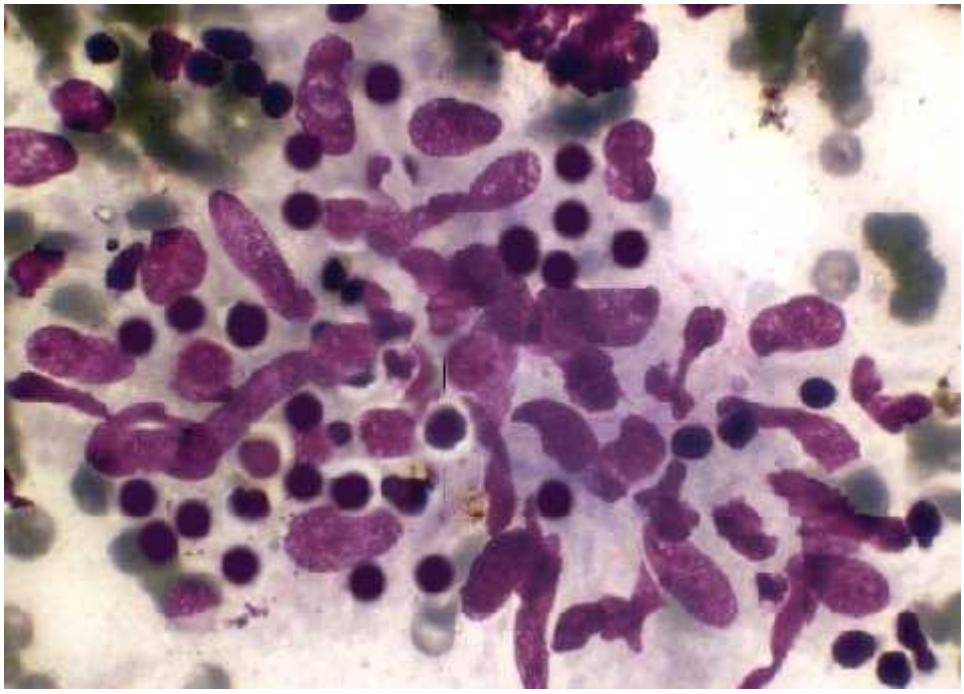


Figure- 15
Microphotograph Showing features of Granulomatous Lymphadenitis (PAP,400X)

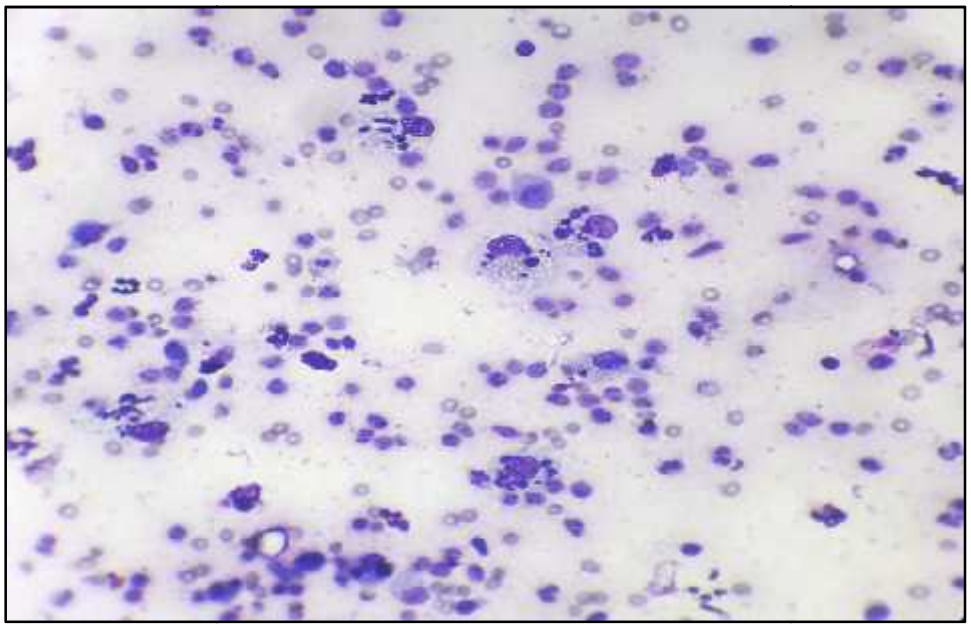


Figure –16

Microphotograph Showing features of Acutesuppurative Lymphadenitis (PAP, 100X)

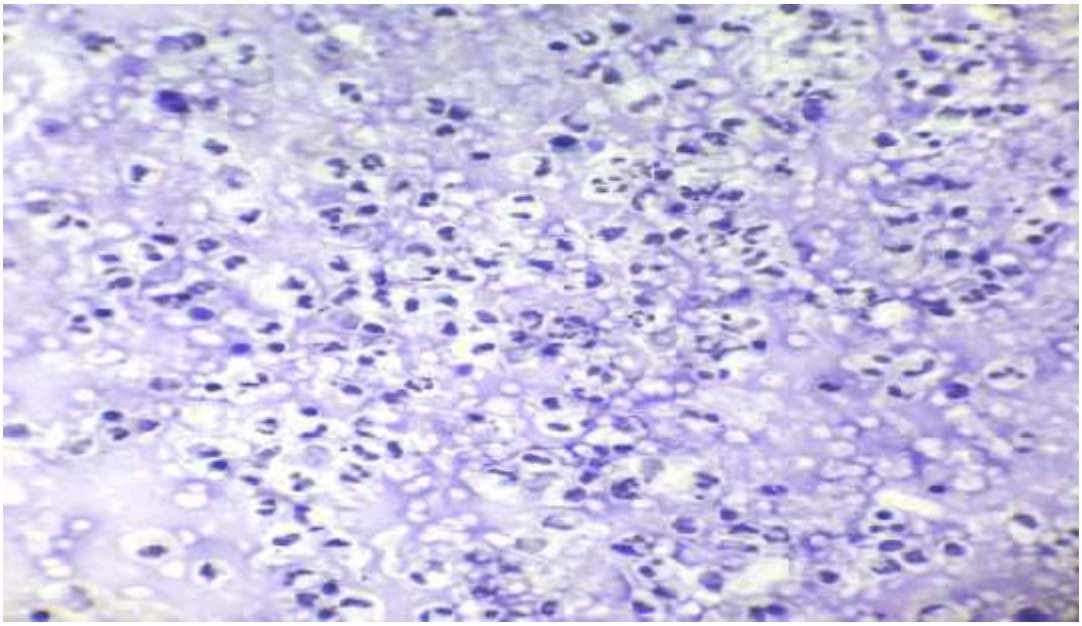


Figure-17

Microphotograph Showing features of Necrotizing Lymphadenitis (PAP 100X)

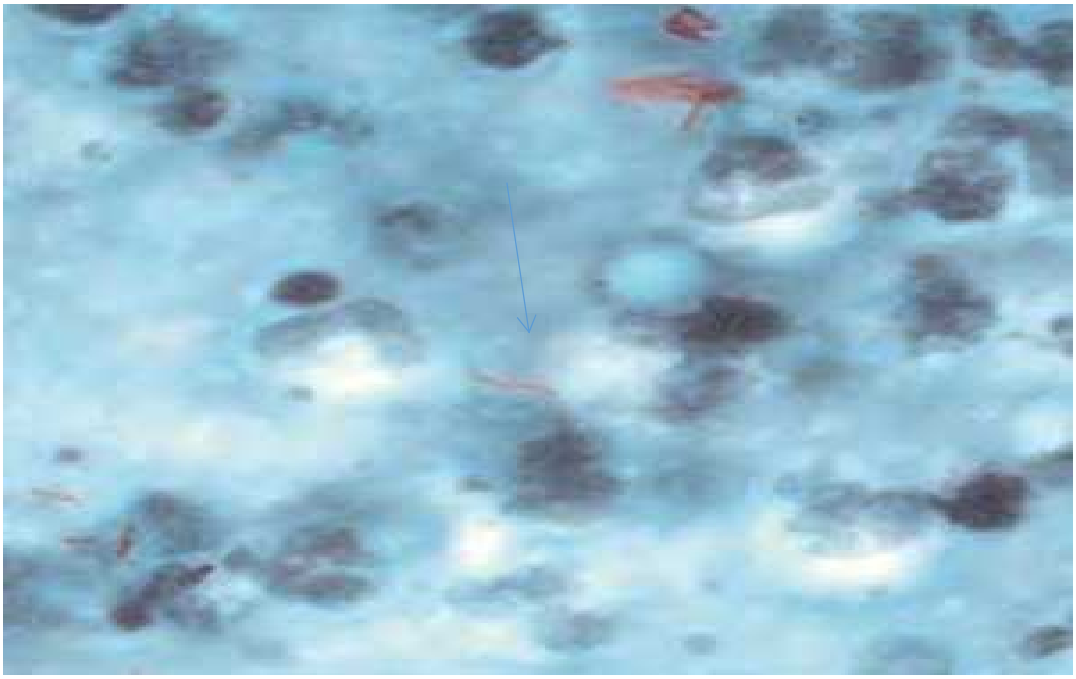


Figure-18

Microphotograph showing AFB by Conventional method (ZN Stain, 1000x)

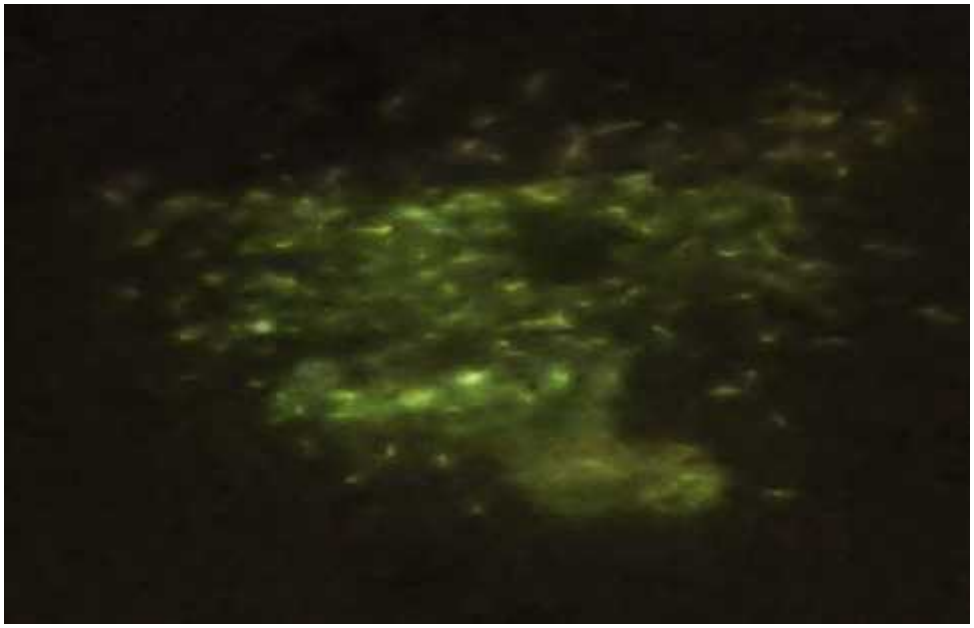


Figure-19

Microphotograph Showing AFB Under Fluorescence Microscope by AF method (400X)

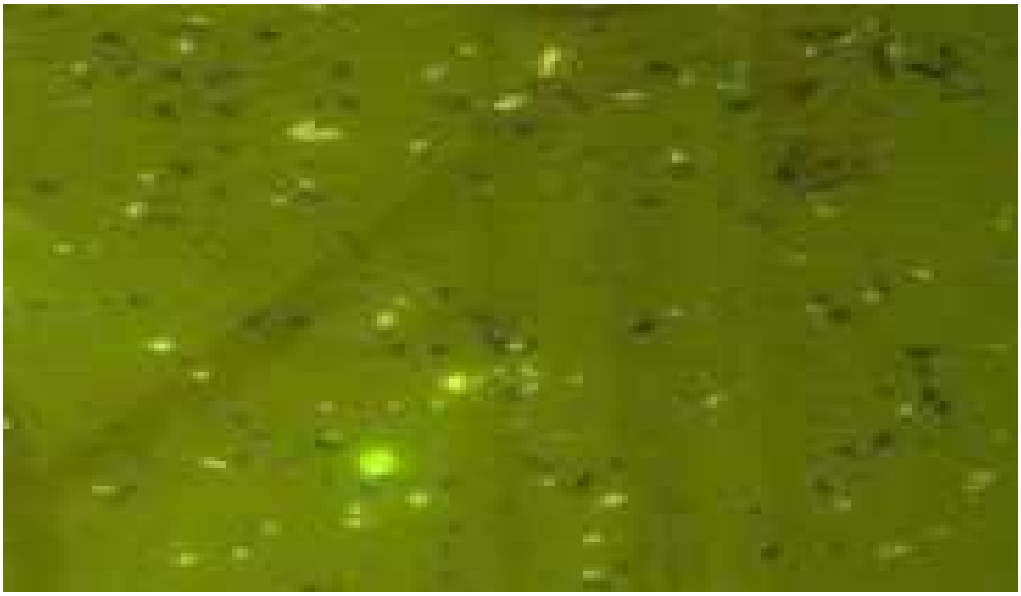


Figure-20

Microphotograph showing AFB under Fluorescence Microscope by AR method (400X)

DISCUSSION

Tuberculosis is an ancient infection that has immense impact on the health and socio economic development since time immemorial and still continues to remain a major health problem especially in developing countries like India.

It is a disease that can affect multiple organs of the body. Tuberculous lymphadenitis is the most common type of manifestation of extra pulmonary TB. Diagnosis of TB is easy when the disease is floridhowever; if there is a localized involvement of extra pulmonary organs sometimesit poses a difficulty in diagnosis.

Diagnostic modalities must be customized according to population and epidemiology of TB in that region. These include improved microscopy, usage of culture, new modalities like AF in diagnosis of extra pulmonaryTB, chemical and physical detection of mycobacterial antigens in paucibacillary condition, antigen capture, antibody detection, cellular immune recognition, nucleic acid amplification and phage assay.

In developing countries, the diagnosis of TB is made on symptoms based algorithms. The diagnosis of TB by cytomorphology is not a new entity. Initially the diagnosis was made based on certain patterns like Granulomatous, Caseousnecrosis,Langham's type of giant cells. But many viral infections, bacterial infections can also simulate the same cytomorphological patterns. So laboratory test plays an important role to establish the cause of such lymphadenopathy because the prognosis and treatment will differ.

Since many years, the ZN stain is used to detect AFB in lymphadenitis as it is cheap and easily available. But the disadvantage of this method is that it has low sensitivity. Other molecular techniques such polymerase chain reaction (PCR), are not

recommended in developing countries as they are expensive. Fluorescent microscopy using dyes like AR can be done but the major disadvantage is the dyes are carcinogenic. So in search of new modalities "AF"for detection of AFB is explored. This technique is simple, fast and cost effective especially in developing countries like India.

Tuberculouslymphadenopathy can occur in any age group.In the present study the youngest patient was 12 months old and oldest was 75 years old. The mean age of distribution was 23±5. In the study conducted by Ahmad *et al*, the youngest patient was four-year-old and the oldest was 63 years old and the mean age of distribution was 21±10yrs. Majority of the patients were in 3rd decade of life.Similar age distribution was seen in studies conducted by Krishna *et al*[71],Brijesh*et al*[70], Joshi *et al*[69],Purohit *et al*[73] and Dandapat*et al*[74]on Tuberculous lymphadenitis.

In the present study the cervical region was most commonly affected region, involved in 71% of the cases. Thiswas in concordance with Bezabih*etal*[75]who observed cervical involvement in 74.2% of cases.Similar results were found in the studies conducted by Joshi *et al*[69], Brijesh*et al*[70]and Krishna *et al*[71].

The most common pattern encountered in the present study was Granulomatous lymphadenitis which was in concordance with other studies. However, the most common pattern encountered in Wright *et al*[2],Brijesh*et al*[70]study was Caseating necrotizing which was found to be statistically significant. The possible explanation for this might be due to the failure of macrophage activating mechanism which leads to tissue destruction, later leads to caseous necrosis. (Table 21)

Table 20: Comparative chart of most common cytomorphological pattern in present study with other studies

PUBLICATION	CASES	DOMINANTCYTOMORPHOLOGICAL PATTERNS
Wright *et al*[2] (2004)	330	Necrotizing lymphadenitis
Annam *et al*[7] (2009)	60	Granulomatous lymphadenitis
Joshi *et al*[69] (2012)	80	Granulomatous lymphadenitis
Brijesh*et al*[70](2013)	90	Caseating Necrotizing
Krishna *etal*[71](2016)	88	Granulomatous lymphadenitis
Present study	36	Granulomatous lymphadenitis

Brijesh*et al*[70]studied 90 cases of TB lymphadenitis and they observed that the most common needle aspirate was blood mixed material. Out of 90 cases 43 cases showed blood mixed material, and 26 cases showed cheesy white material. Remaining 21 cases showed pus like material. In the present study most common Fine needle aspirate was blood mixed in cases of TB lymphadenitis. Out of 36 cases 28 cases showed blood mixed material and rest all cases showed cheese like and purulent aspirates.

Table21: Comparison chart of detection of AFB positive cases in different studies by ZN, AF & AR methods.

PUBLICATION	CASES	ZN STAIN	AR STAIN	AF STAIN	CULTURE
Joshi *et al*[69](2012)	80	30(37.5%)	Not compared	46(57.5%)	40(50%)
Brijesh*et al*[70] (2013)	90	24(26.7%)	31(34.4%)	38(42.2%)	27(27.8%)
Krishna *et al*[71](2016)	88	33(37.5%)	72(81.82%)	76(86.36%)	Not compared
Present study	153	16(10.5%)	36(23.5%)	55(35.9%)	Not compared

In the present study we took around 06 cases and sent for culture. The culture was negative in all the 06 cases. It might be due to ineffective sampling contamination, treatment prior to culture and bacterial load. Similar results were found in studies conducted byJoshi *et al*[69]&Krishna *et al*[71].

Brijesh*et al*[70]studied around 90 cases and they concluded that AF was more sensitive in identifying the AFB than ZN stain. In their study 8 cases showed AF positive but negative on AR or on culture. Laterthey observed that the cause of more positive cases on AF than AR was due to organisms like Bacillus subtilis, Staphylococcusaureus, Nocardia, budding yeast also exhibit spontaneous emission spectra or may be due to air dying artifacts on PAP stained smears or it might be due to subjective biased errors.

Krishna *et al*[71] studied around 88 cases of extra pulmonaryTB.They found that AF was more superior than ZN stain. They also took AR as a gold standard reference as in present study and made a comparison between both ZN stain and AF

stain and concluded that ZN stain had less sensitivity than AR. They concluded that if the cytomorphology features of lymph node was pursuant with mycobacterial infection and they are identified by ZN/AR/AF then the possibility of false positive results is low.

Taking AR stain as a reference method, analysis of statistical values of ZN and AF was made in the present study and it was noted that the ZN stain had high specificity 99.15%, and high PPV 93.75% than AF. But the AF had more sensitive 61.11% and high NPV of 85.71% than ZN stain. (Table 22)

Table 22: Comparison of results of Sensitivity,Specificity, Positive Predictive Value (PPV) & Negative predictive Value(NPV) of AFB in present study with previous studies

ZN & AF STAINS	Result of the present study ZN & AF	Wright *et al*[2](2004) ZN & AF	Joshi *et al*[69](2012) ZN & AF	Brijesh*et al*[70](2013) ZN & AF
Sensitivity	41.67%&61.11%	62.0%&65.9%	62.50%&95%	80.0%&96.0%
Specificity	99.15%&71.79%	97.0%&73.0%	87.50%&81.81%	93.85%&78.46%
PPV	93.75%&40.00%	66.05%&76.5%	70.0%&82.6%	83.33%&63.16%
NPV	84.67%&85.71%	97.0%&61.7%	83.33%&94.7%	92.42%&98.08%

Many studies which were conducted for detection of AFB from various specimens, like sputum, CSF, FNA, pus, and other body fluids were examined by ZN and AR stains, for AFB showed that AR was more sensitive as compared to ZN stain.

In a study, conducted by *Wright etal*[2] they found that the sensitivity of AF (65.9%)and ZN (62.0%) stains were nearly equal. Contrary to their study, we found

AF (61.11%)was more sensitive than ZN (41.67%). This could be due to differences in techniques including careful scanning, possibly, a higher bacterial load in our patient's.

In conclusion, the present study was undertaken to increase the utility of fluorescence microscopy by using normal PAP stained smears via AF method. In the present study AF had high sensitivity rates of 61.11% than ZN stain 41.67%. It had been proven in other studies. It was a novel method of detection of AFB, cheaper, easily available and less time consuming than other methods. AR also had high sensitivity rates as AF but the AR stainhas carcinogenic properties.

CONCLUSION

The morphologic spectrum in TB lymphadenitis varies depending on the stage of the disease and the immunity of the host. The presence of caseation and granulomas comprised of epithelioid cells, lymphocytes, Langhan's typegiant cells on cytology smears will help in the diagnosis of TB in the developing countries such as India, where it continues to be the most common cause of lymphadenopathy compared to other causes of granuloma formation.

The problems in diagnosis of TB lymphadenitis occurs when the aspirate shows polymorphous population of lymphocytes with occasional epithelioid cells but absence of typical Langhan's giant cell or necrosis, making it to resort to excision biopsy for a definitive diagnosis. In such conditions the routinely performed ZN staining had low sensitivity as it rarely detects AFB in aspirates. The bacillary index should be 10^4 bacilli/ml to get positivity on ZN stain. However, in the present study we used AF method for detection of AFB which had high sensitivity rates than ZN staining method.

It is also observed thatwhenever there is scant AFB positivity, searching for them is a tedious process on conventional ZN stain as compared to AF method. The adaptable property of AFB for AF makes them easily detectablein Pap stained smears. On AF, AFB bacilli are easily detectable under low power view; large area of smear can be screened within a short period of time, which reduces the turnaround time. As Pap stainisfrequentlyutilized for routinecytologicsmears, no any special stain is required, detection of AFB in Pap stained smearsis a rapid,relatively simple & cost effective method.

SUMMARY

The present study was undertaken to correlate autofluorescence method on Papanicolaou stain with conventional ZN stain method in detection of AFB in lymph node aspirates.

1) This study was carried out on 153 patients with clinically suspected cases tuberculous lymphadenopathy referred to the department of pathology for FNAC.
2) Out of 153 patients, 5 aspirates were diagnosed as malignancy and 148 clinically suspected Tuberculous lymphadenitis cases evaluated and the results were analyzed.
3) Predominant age group involved in the present study was 21-30 years (23.5%) followed by 11-20 years (20.9%).
4) Female preponderance was seen in the study (Female 51% & Male 49%).
5) Most of patients presented with lymphadenopathy of less than 3 months' duration seen in 60.55% of cases.
6) Lymphadenopathy predominantly involved cervical region seen in 71% cases followed by Sub-mandibular 20% cases.
7) Nature of the aspirate was hemorrhagic in 77.77% of cases.
8) Out of 148 cases, 36 cases were diagnosed as TB lymphadenitis based on AFB positivity on AR stain.
9) Overall AFB positivity was seen in 16 cases (8.7%) by conventional ZN method.
10) Out of 16 cases 11 were scant positive, 2 were 1+ and 3 were 2+ by conventional ZN method.

11) Out of 36 positive cases 28 cases were 1+ positive, 6 cases showed 2+ positivity and rest 2 cases show 3+ positivity on fluorescence microscopy.

12) The sensitivity of AF method was 62.0% whereas ZN was only 41.67%.

13) Thus the AF method for detection of AFB in lymph node aspirate was more sensitive than conventional ZN method. Moreover, the method was safe, inexpensive and easy to perform and requires no additional staining.

LIMITATIONS OF THE STUDY

- As the period of study was short, the procurement of the number of TB positive cases wasless.
- As it is a new technique for detection of AFB, limited use of AF technique is required because of it increased false positive rates and subjective biased errors. Hence, it is recommended to be used with other ancillary techniques.

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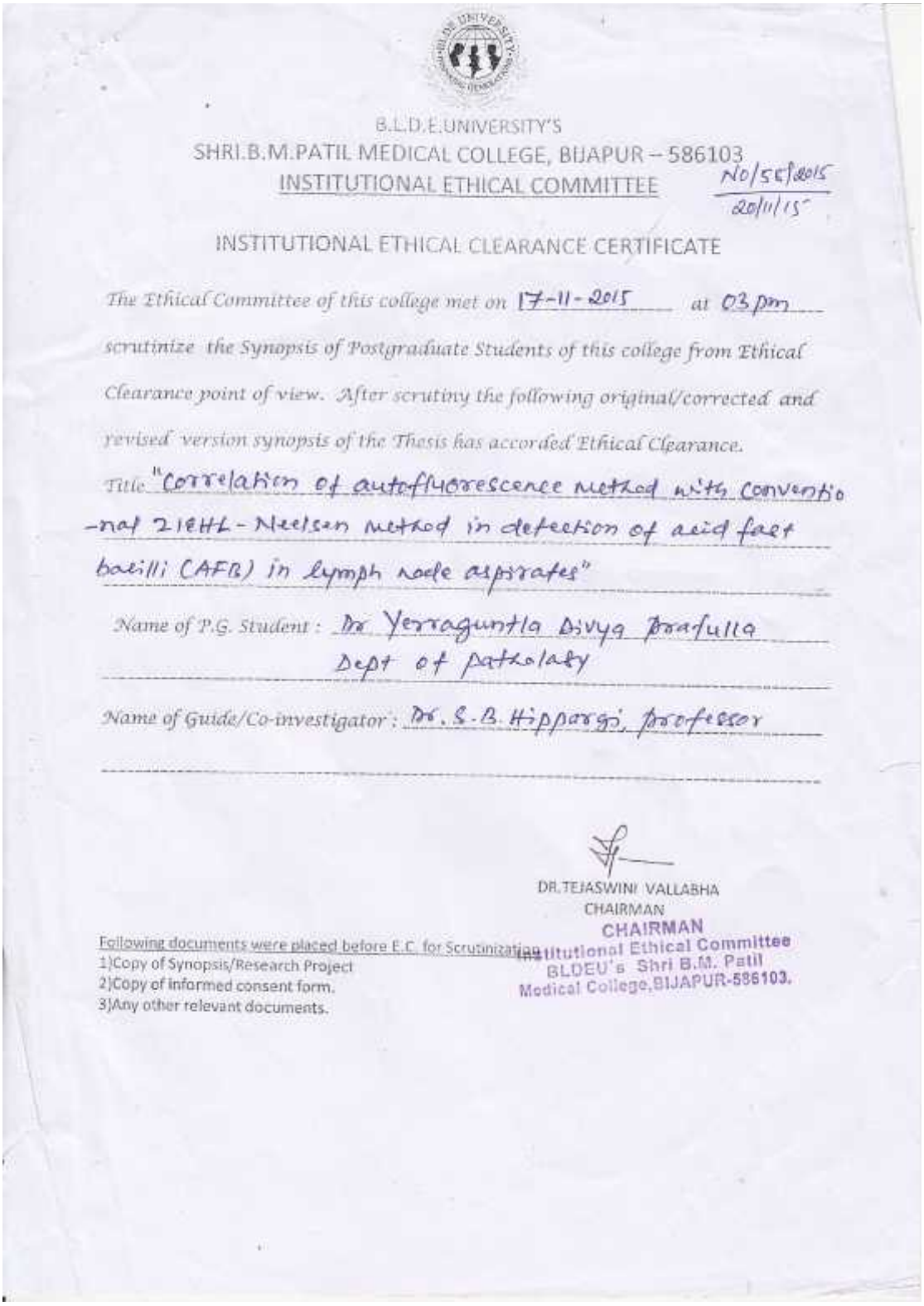
ANNEXURE-I



B.L.D.E.UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR – 586103
INSTITUTIONAL ETHICAL COMMITTEE

No/55/2015
20/11/15

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 17-11-2015 at 03 pm scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has accorded Ethical Clearance.

Title "Correlation of autofluorescence method with conventio-nal ZIEHL-Neelsen method in detection of acid fast bacilli (AFB) in lymph node aspirates"

Name of P.G. Student : Dr Yerraguntla Divya Prafulla
Dept of pathology

Name of Guide/Co-investigator : Dr. S.B. Hipparagi, professor

DR.TEJASWINI VALLABHA
CHAIRMAN

CHAIRMAN
Institutional Ethical Committee
BLDEU's Shri B.M. Patil
Medical College,BIJAPUR-586103.

Following documents were placed before E.C. for Scrutinization
1)Copy of Synopsis/Research Project
2)Copy of informed consent form.
3)Any other relevant documents.

ANNEXURE-II

B.L.D. E UNIVERSITY SHRI B.M. PATILMEDICAL COLLEGEHOSPITAL AND RESEARCH CENTRE, VIJAYPUR-586103

RESEARCH INFORMEDCONSENT FORM

TITLE OF THE PROJECT:CORRELATION OF AUTOFLUORESCENCE METHOD WITH CONVENTIONAL ZIEHL-NEELSEN METHOD IN DETECTION OF ACID FAST BACILLIIN LYMPH NODE ASPIRATES**.**

PRINCIPAL INVESTIGATOR: DR. YERRAGUNTLA DIVYA PRAFULLA

P.G DEPARTMENT OF PATHOLOGY

P. G GUIDE **:** DR. S.B.HIPPARGI$_{MD}$

PROFESSOR, DEPT OF PATHOLOGY.

PURPOSE OF RESEARCH:

I have been informed that the present study is correlation of autofluorescence method with conventional ZN method in detection of acid-fast bacilli(AFB) in lymph node aspirates.

PROCEDURE:

I understand that I undergo detailed history and after which necessary investigations will be done.

RISK AND DISCOMFORTS:

I understand that, there is no risk involved in procedure performed.

BENEFITS:

I understand that my participation in the study will help to know the role of autofluorescence microscopy in Papanicolaou stained lymph node aspirate in diagnosis of tuberculosis.

CONFIDENTIALITY:

I understand that the medical information produced by the study will become a part of hospital record and will be subjected to confidentiality and privacy regulations of the hospital. If data is used for publications the identity of patient will not be revealed.

REQUEST FOR MORE INFORMATION:

I understand that the medical information produced by the study will become a part of hospital record and will be subjected to confidentially and privacy regulations of hospital. If the data is used for publications the identity of patient will not be revealed.

REQUEST FOR MORE INFORMATION:

I understand that I may ask more information about study at any time.

REFUSAL FOR WITHDRAWL OF PARTICIPATION:

I understand that my participation is voluntary and that I may refuse to participate or may withdraw from the study at any time.

INJURY STATEMENT:

I understand that in the unlikely event of injury to me during the study I will get medical treatment but no further compensations.

I have read the fully understood this consent form. Therefore, I agree to participate in the present study.

I have explained the patient the purpose of study, the procedure required and possible risk and benefit of my ability in the vernacular language.

Investigator/P. G

Date:

ANNEXURE III

PROFORMA FOR STUDY:

Demographic details:

Name:

Age:

Sex: M/F

Occupation:

Residence:

OPD/IP NO:

Lab. No/Sample No.:

Chief complaints:

History of present illness:

Past history:

Family history:

General physical examination:

Description of lymphadenopathy:

Duration:

Unilateral/bilateral

Site:

Size:

Number of lymphnodes: Other swellings:

Consistency: soft/firm/rubber/hard/stony hard

Matted: Yes/No

Sinus: Yes/No

Cytomorphology:

Pap smears will be screened for cytomorphological evidence of tuberculosis.

Special stain:

Smears stained with Z-N stain will be screened for the presence of AFB.

Positive /Negative, if present, / OIF

Auto fluorescent microscope:

Pap smears will be screened for the presence of auto fluorescent AFB by using fluorescent microscope.

TB bacilli: Present/Absent

If present, /OIF

Final diagnosis:

Comment:

KEY TO MASTER CHART

RL	Reactive lymphadenitis
GL	Granulomatous lymphadenitis
ASI	Acute suppurative inflammation
TL	Tubercular lymphadenitis
NL	Necrotizing lymphadenitis
SCC	Squamous Cell Carcinoma

MASTER CHART

Sl No	FNAC no.	IP NO	Name	Age	Yrs	Sex	Lymphnode	ZN	AR	AF	IMPRESION TB
1	919/15	IP/2015/15423	Revubai Laxman Bandennavar	65	Years	Female	Submental	0	0	0	ASI
2	986/15	IP/2015/16153	Baganna Channappa Hadapad	2	Years	Male	R.Preauricular	Scant +	2+	1+	TL
3	1018/15	OP/2015/188720	Kavita T	23	Years	Female	Lt.Cervical	0	0	Scant+	GL
4	1100/15	OP/2015/203408	Mallamma Halegouda	35	Years	Female	Lt.Cervical	0	0	0	GL
5	1295/15	OP/2015/231269	Laxmi Madagond	9	Years	Female	Pre auricular	Scant+	2+	1+	GL S/O TL
6	2016/15	OP/2015/322083	Pundlika Kolahar	50	Years	Male	Rt.Infra auricular	0	0	Scant+	ASI
7	2059/15	IP/2015/29048	Mallamma Shankareppa Bosagi	24	Years	Female	Sub mandibular	2+	3+	3+	GL S/O TL
8	2083/15	OP/2015/331697	Shivagyappa G Sunagar	18	Years	Male	Rt submandibular	0	0	0	RL
9	2084/15	OP/2015/331613	Shrishail Akalwade	46	Years	Male	Rt. Supraclavicular	0	0	0	RL
10	2119/15	OP/2015/336129	Dinesh Gawari	15	Years	Male	Lt.Cervical	0	0	0	RL
11	2132/15	OP/2015/336870	Yasanna Bagali	25	Years	Male	Rt.Supraclavicular	0	0	0	RL
12	2143/15	OP/2015/338161	Sharanamma Natikar	6	Years	Female	Submental	0	0	scant +	RL
13	2177/15	OP/2015/344123	Kavya Guddi	7	Years	Female	Rt.Cervical	0	0	0	RL
14	2204/15	OP/2015/346992	Vilas Roogi	15	Years	Male	Lt.Cervical	0	0	0	RL
15	2206/15	OP/2015/347011	Shantabai	20	Years	Female	Rt Submandibular	0	0	0	RL
16	2208/15	IP/2015/30682	Ishwarappa Parappa Jakati	51	Years	Male	Submental	Scant +	2+	0	ASI S/O TL
17	2320/15	IP/2015/31857	Kallyansing Ramsing Shekadar	68	Years	Male	Rt.Cervical	0	0	0	RL
18	2326/15	IP/2015/31972	Savita Ramu Mulawad	40	Years	Female	Jugulodigastric	Scant +	1+	0	GL S/O TL
19	2328/15	OP/2015/364948	Sneha Mangalur	9	Years	Female	Lt.Cervical	0	0	0	RL
20	2330/15	OP/2015/364120	Sunanda Tevaratti	28	Years	Female	Rt.Cervical	0	0	0	RL
21	2362/15	OP/2015/369514	Bhagya Balin	20	Years	Female	Lt.Supraclavicular	0	0	0	GL
22	2485/15	IP/2015/34328	Kiran Shrishail Biradar	16	Years	Male	Lt.Cervical	0	0	0	ASI
23	2519/15	OP/2015/392254	Daneshwari	25	Years	Female	Lt.Cervical	Scant+	1+	0	ASI OF TB ETIOLOGY
24	2698/15	OP/2015/411172	Sushila Kanakareddy	55	Years	Female	Rt.Cervical	0	0	0	RL
25	2727/15	IP/2015/36972	Surekha Vijay Kulahalikar	55	Years	Female	Lt.Cervical	0	0	0	ASI
26	2846/15	IP/2015/38517	Mallu Vittal Kirsagar	11	Months	Male	Lt.Submandibular	0	0	0	ASI
27	2847/15	OP/2015/436948	Khemu Jadhav	55	Years	Male	Lt.Cervical	0	0	0	GL
28	2809/15	IP/2015/38217	Rehana Sameer Shekh	31	Years	Female	Rt. Axillary	0	1+	1+	GL S/O TL
29	2921/15	OP/2015/449117	Ramya Kumbar	5	Years	Female	Lt.Cervical	0	0	0	RL
30	57/16	OP/2016/7484	Shivanand Nagargoji	22	Years	Male	Lt.Cervical	0	0	0	RL
31	61/16	IP/2016/709	Mangalabai Babu Lamani	8	Years	Female	Rt. groin	0	Scant+	0	GL S/O TL
32	62/16	OP/2016/9317	Sushalawwa Bhagavant Teli	48	Years	Female	Lt.Cervical	0	Scant+	Scant+	GL S/O TL
33	102/16	OP/2016/13723	Nagamma Narayanakar	35	Years	Female	Lt.Cervical	0	0	0	RL
34	203/16	IP/2016/2684	Renuka Yelagond Pujari	20	Years	Female	Rt.Sub mandibular	0	0	0	ASI
35	208/16	OP/2016/36262	Rekha Hanchanal	40	Years	Female	Lt.Cervical	0	1+	scant +	GL S/O TL
36	209/16	OP/2016/36248	Bouramma Majaggi	38	Years	Female	Lt.preauricular	0	0	0	ASI
37	210/16	OP/2016/36441	Anajana Biradar	20	Years	Female	Rt submandibular	scant+	Scant+	1 +	GL S/O TL
38	275/16	OP/2016/45897	Baburay Mosalgi	45	Years	Male	Rt.Cervical	Scant+	1+	1+	GL S/O TL
39	293/16	OP/2016/51429	Bhimashankar	6	Years	Male	Rt.Submental	2+	3+	2+	GL S/O TL
40	295/16	IP/2016/4168	Santosh Ganapati Tulase	30	Years	Male	Rt.Cervical	0	2+	1+	GL S/O TL
41	351/16	IP/2016/4459	Chanaveer Basanagouda Kirasur	25	Years	Male	Rt.Submandibular	0	0	1+	NL
42	353/16	OP/2016/56567	Asma Hanif	21	Years	Female	Rt.Cervical	0	0	0	NL
43	427/16	OP/2016/69780	Abhishek	6	Years	Male	Lt.Submandibular	0	0	0	GL
44	511/16	OP/2016/81607	Mallappa Bargall	32	Years	Male	Rt.Submandibular	0	0	0	GL
45	514/16	OP/2016/82991	Shreedevi Mali	22	Years	Female	Rt.Cervical	0	0	0	RL
46	536/16	OP/2016/84587	Geeta Biradar	26	Years	Female	Lt.Cervical	0	0	0	RL
47	806/16	IP/2016/11125	Sumitra Jogyappa Bantanur	45	Years	Female	Lt.Supra clavicular	0	0	0	RL
48	819/2016	IP/2016/11349	Chanveer Basanagouda Kirasur	55	Years	Male	Rt.submandibular	0	0	0	ASI
49	820/16	OP/2016/124756	Sharada Haveri	24	Years	Female	Rt.posterior triangle	0	0	0	NL
50	937/16	OP/2016/143307	Renuka Kori	10	Years	Female	Lt.parotid	0	0	Scant+	ASI
51	940/16	OP/2016/142866	Bhirappa	45	Years	Male	Rt.Submandibular	0	0	1+	RL

54	982/16	IP/2016/13667	Akash Shreeshail Bannannevar	8	Years	Male	Sub mandibular	0	0	0	ASI
55	990/16	IP/2016/13439	Bharati Girmallappa Naikodi	17	Years	Female	Lt.Cervical	0	0	0	ASI
56	1120/16	OP/2016/169904	Aiman Nadaf	14	Years	Female	Lt.Cervical	0	0	1+	GL
57	1131/16	OP/2016/171665	Gayatri Ambali	4	Years	Female	Lt.Cervical	0	0	0	RL
58	1133/16	IP/2016/15187	Mallikarjun Nagappa Navi	59	Years	Male	Rt.Cervical	0	0	0	MSCC
59	1144/16	OP/2016/173169	Sugalabai Talawar	60	Years	Female	Rt. submandibular	0	1+	1+	TL
60	1243/16	OP/2016/186554	Sharada J Mathapati	39	Years	Female	Rt.Cervical	0	0	1+	GL
61	1258/16	OP/2016/187801	Faruk Jamadar	22	Years	Male	Rt.cervical	0	Scant+	0	GL S/O TL
62	1294/16	OP/2016/192665	Vasanth Rathod	68	Years	Male	Rt Submandibular	0	0	0	MSCC
63	1312/16	IP/2016/18224	Husanabai Shreemanth Bellenavar	55	Years	Female	Rt.Supraclavicular	0	0	0	GL
64	1373/16	IP/2016/18972	Nikita Kisan Rathod	11	Years	Female	Rt.Cervical	0	1+	1+	GL S/O TL
65	1413/16	OP/2016/208432	Manohar Singh	35	Years	Male	Lt.Cervical	0	0	0	NL
66	1414/16	OP/2016/208456	Kajal Bellennawar	17	Years	Female	Rt. Supraclavicular	0	0	0	GL
67	0	OP/2016/216936	Bhimbai Rathod	40	Years	Female	Lt.Supraclavicular	0	0	0	GL
68	1570/16	OP/2016/229881	Jayashri Nakodi	19	Years	Female	Lt.Cervical	0	Scant+	0	GL S/O TL
69	1574/16	IP/2016/21648	Chadram Chenavirappa Shikare	45	Years	Male	Lt.Cervical	0	0	0	ASI
70	1705/16	OP/2016/251369	Ningappa Hadimani	32	Years	Male	Lt.Axillary	0	0	Scant+	RL
71	1736/16	OP/2016/255725	Sadashiva L Yallammanagudi	60	Years	Male	Sub mandibular	0	0	Scant+	RL
72	1768/16	OP/2016/261227	Mahadevi Bidri	25	Years	Female	Lt.Submandibular	0	0	0	NL
73	1828/16	OP/2016/268883	Kavita	19	Years	Female	rt &lt cervical	0	0	0	RL
74	1854/16	OP/2016/272791	Madiwalayya	70	Years	Male	Right cervical	0	0	0	MSCC
75	1880/16	OP/2016/279156	Ramappa T Maigur	40	Years	Male	B/l inguinal	0	0	0	RL
76	2100/16	OP/2016/313221	Pratham Chavan	5	Months	Male	Rt. axillary	0	1+	1+	ASI S/O TL
77	1955/16	IP/2016/27255	Ranjana Peeraji More	25	Years	Female	Rt Submandibular	0	0	0	RL
78	2101/16	IP/2016/29331	Neelawwa Bandeppa Kakandaki	70	Years	Female	Rt.gluteal	0	0	0	ASI
79	2148/16	OP/2016/319255	Atmanand	18	Years	male	Rt.Preauricular	0	0	1+	RL
80	2149/16	OP/2016/319816	Rajeshwari Rathod	28	Years	Female	Rt. Axillary	0	Scant+	0	GL S/O TL
81	2151/16	OP/2016/319909	Chandrashekhar Pawar	25	Years	Male	Rt.Cervical	scant+	1+	0	GL S/O TL
82	2165/16	OP/2016/30346	Bhima shi	78	Years	male	Lt.Cervical	0	0	0	RL
83	2179/16	OP/2016/322005	Kaveri C Patil	6	Years	Female	Lt.Cervical	0	0	1+	RL
84	2191/16	IP/2016/30346	Bhimashi Gurappa Dashavanth	78	Years	Male	Rt.Cervical	0	0	0	RL
85	2204/16	IP/2016/30823	Neelamma Parasappa Madar	22	Years	Female	Lt.Axillary	0	0	0	ASI
86	2224/16	OP/2016/327032	Chandru Bisnal	24	Years	Male	Rt.Cervical	0	0	0	RL
87	2291/16	OP/2016/336055	Kiran Badiger	20	Months	Male	Rt.Sub mandibular	0	0	0	ASI
88	2294/16	IP/2016/31488	Mahadev Mallappa Masuti	61	Years	Male	Rt. Supraclavicular	0	0	1+	RL
89	2296/16	OP/2016/339267	Prathap	3	Months	Male	Lt.Axillary	Scant+	2+	1+	ASI S/O TL
90	2391/16	OP/2016/351401	Ramesh	28	Years	Male	Lt.Cervical	0	Scant+	1+	NL S/O TL
91	2392/16	OP/2016/351749	Kavita Rathod	34	Years	Female	Rt.Cervical	0	0	Scant+	RL
92	2430/16	OP/2016/359389	Laxmi	30	Years	Female	Rt. Cervical	0	0	Scant+	RL
93	2482/16	OP/2016/366514	Siddramesh Kundargi	32	Years	Male	Rt.Cervical	0	0	0	RL
94	2591/16	IP/2016/36388	Shrinivas Hanumanth Hallad	7	Years	Male	Rt.Cervical	0	0	1+	ASI
95	2529/16	OP/2016/372931	Shakuntala	40	Years	Female	Lt.Cervical	0	0	1+	GL
96	2565/16	OP/2016/377833	Praveen B Salagar	19	Years	Male	Rt.Cervical	0	2+	1+	GL S/O TL
97	2664/16	OP/2016/391292	Kavita	20	Years	Female	Rt.Cervical	0	Scant+	Scant+	ASI S/O TL
98	2677/16	OP/2016/385181	Padmavati M	35	Years	Female	Rt.Paramidline	0	0	0	GL
99	2700/16	IP/2016/36500	Anand Hanamantha Bandiwaddar	32	Years	Male	Rt.Paramidline	0	0	1+	NL
100	2760/16	OP/2016/402166	Anita Basri	23	Years	Female	Rt.Supra clavicular	0	1+	1+	NL S/O TL
101	2821/16	OP/2016/413033	Vivekanand Patil	36	Years	Male	Rt.Cervical	0	0	0	GL
102	2760/16	OP/2016/402166	Anita Basri	23	Years	Female	Rt.Supra clavicular	0	1+	1+	NL S/O TL
103	2927/16	OP/2016/428075	Heena Feerajade	25	Years	Female	Rt.Cervical	0	0	0	GL
104	2955/16	OP/2016/429990	S S Biradar	69	Years	Male	Lt.Cervical	0	0	0	GL
105	3036/16	OP/2016/438489	Shivanand Madiwal	18	Years	Male	Rt.Cervical	0	0	0	GL S/O TL
106	3080/16	OP/2016/442783	Parashuram Banasode	24	Years	Male	Rt.Cervical	0	1+	1+	NL S/O TL

110	165/17	OP/2017/436286	Muttappa Malagatti	12	Years	Male	Rt.Post traingle	0	0	0	RL
111	166/17	OP/2017/15172	Sidarya Loni	8	Years	Male	Submental	0	0	0	RL
112	381/17	IP/2017/3015	Ravi Shivappa Singe	25	Years	Male	Rt. axillary	Scant+	1+	1+	TL
113	398/17	IP/2017/3314	Revanasidda Ogeppa Jiddimani	46	Years	Male	Rt.Cervical	Scant+	1+	0	TL
114	511/16	OP/2016/81607	Mallappa Bargall	32	Years	Male	Rt.Submandibular	0	0	0	GL
115	597/17	OP/2017/54402	Savitri Chatri	30	Years	Female	Rt.Cervical	0	0	0	ASI
116	640/17	OP/2017/57170	Geeta Kale	25	Years	Female	Rt.Cervical	0	0	0	GL S/O TL
117	647/17	OP/2017/57363	Shabera	42	Years	Female	Rt.Supraclavicular	0	0	1+	GL S/O TL
118	662/16	OP/2016/102879	Prakash M	47	Years	Male	Rt.Cervical	0	0	0	GL
119	681/17	IP/2017/5554	Shahida Saddam Shaikh	18	Months	Female	Lt.Cervical	0	0	0	RL
120	767/17	OP/2017/69496	Laxmi Patil	25	Years	Female	Lt.Cervical	0	0	0	RL
121	786/17	OP/2017/70376	Subhas Lalsangi	62	Years	Male	Rt. cervical	0	0	0	MSCC WITH GRL.
122	805/17	OP/2017/73185	Vishwanath Bevinakatti	18	Years	Male	Rt.inguinal	0	0	0	ASI
123	806/17	OP/2017/73106	Sannajambanna	27	Years	Male	Lt.Cervical	0	0	1+	ASI
124	831/17	OP/2017/48272	Sunanda Devareddy	30	Years	Female	Rt.Cervical	0	0	0	GL S/O TL
125	840/17	OP/2017/75520	Pooja Chalawadi	20	Years	Female	Submental	0	0	0	RL
126	856/17	OP/2017/76273	Venkatesh	36	Years	male	Rt.Cervical	0	0	1+	NL
127	925/17	OP/2017/82456	Shantamma	60	Years	Female	Rt.Axillary	0	0	0	NL
128	1067/17	OP/2017/97227	Saleem Nadaf	17	Years	Male	Rt.c&SM	0	1+	1+	GL S/O TL
129	1138/17	OP/2017/102149	Mahamad Ayaz	10	Years	Male	Lt.post auricular	0	0	0	RL
130	1241/17	IP/2017/10260	Bhagirathi Shivappa Awati	60	Years	Female	Lt.post auricular	0	0	1+	GL S/O TL
131	1253/17	OP/2017/112967	Heena Kousar	15	Years	Female	Rt.Cervical	0	1+	0	TL
132	1330/17	OP/2017/119548	Sampat Kumar Biradar	4	Years	Male	Rt.Cervical	0	0	0	RL
133	1361/17	IP/2017/11312	Manjunath Chandrashekar Gadekar	1	Years	Male	Lt.Cervical	0	0	Scant+	ASI
134	1404/17	OP/2017/126005	Bhagyashree	9	Years	Female	Submental	0	0	0	NL S/O TL
135	1419/17	OP/2016/319816	Rajeshwari Rathod	28	Years	Female	Rt. Axillary	0	0	0	ASI S/O TL
136	1566/17	OP/2017/142007	Laxmi Madagond	11	Years	Female	Lt.Preauricular	1+	1+	1+	GL S/O TL
137	1686/17	OP/2017/154387	Gourishankar	10	Years	Male	Rt. Cervical	0	0	0	RL
138	1729/17	OP/2017/157692	Nanasab Dhanawade	70	Years	Male	Rt.Supraclavicular	0	1+	0	ASI S/O TL
139	1881/17	OP/2017/170320	Bhimappa Karigar	39	Years	Male	Lt.Axillary	0	0	0	TL
140	1909/17	OP/2016/284273	Amogi Indi	45	Years	Male	Rt.Cervical	1+	1+	1+	TL
141	2073/17	OP/2017/189744	Rakesh Lingareddi	5	Years	Male	Rt.Inguinal	0	0	Scant+	NL S/O TL
142	2083/17	OP/2017/190381	Shivaprasad	18	Years	Male	Rt.Parotid	0	0	0	NL
143	2085/17	OP/2015/332362	Biranna A Talikeri	45	Years	Male	Rt.Posterior triangle	0	0	0	RL
144	2123/17	OP/2017/191988	Shreekantabai	67	Years	Female	Rt.Axillary	0	0	0	RL
145	2187/17	OP/2017/194762	NEETA CHAVAN	48	Years	FEMale	Rt.Cervical	0	0	0	RL
146	2197/17	OP/2017/199864	Abdulsamad Husainsab Nadaf	10	Years	Male	Lt.Supraclavicular	0	0	1+	ASI
147	2276/17	IP/2017/19778	Laxmi Rajakumar Charatti	18	Months	Female	Rt. Cervical	0	0	Scant+	RL
148	2277/17	IP/2017/20065	Puttakka Umakant Gidaganti	28	Years	Female	Lt.Axillary	0	0	1+	ASI
149	2312/17	IP/2017/20392	Laxman Kallappa Umarani	43	Years	Male	Lt.Supra clavicular	0	0	0	RL
150	2315/17	OP/2017/210024	Varsha	16	Years	Female	Rt. axillary	0	0	0	GL S/O TL
151	2408/17	OP/2017/220784	Bouramma Gangashetti	29	Years	Female	Lt.preauricular	0	0	1+	GL S/O TL
152	2604/17	OP/2017/240391	Savita Ramu Rathod	25	Years	Female	Rt.submandibular	0	0	1+	ASI
153	2679/17	OP/2017/246782	Madhu	30	Years	Female	Rt.Supraclavicular	0	0	0	GL S/O TL

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