Utility Of Auramine-Rhodamine Stain And Gene Xpert Pcr In Diagnosis Of Tuberculosis During Fnac Procedure

By

DR.CHIATRA T

Dissertation submitted to

In partial fulfillment for the degree of

DOCTOR OF MEDICINE
IN
PATHOLOGY

Under the guidance of

DR. KARIGOUDER M M.D.

PROFESSOR

DEPARTMENT OF PATHOLOGY

BLDE (DEEMED TO BE UNIVERSITY)

SHRI B. M. PATILMEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE

VIJAYAPUR – 586103

2019

LIST OF ABBREVATIONS

FNAC	-	Fine Needle Aspiration Cytology
TB	-	Tuberculosis
PTB	-	Pulmonary Tuberculosis
EPTB	-	Extra-Pulmonary Tuberculosis
WHO	-	World Health Organisation
ZN	-	Ziehl-Neelsen
AR	-	Auramine Rhodamine
AFB	-	Acid Fast Bacilli
FM	-	Fluorescent Microscopy
CBNAAT	-	Cartridge based nucleic acid amplification test
PCR	-	Polymerase chain reaction
LN	-	Lymph node
APC	-	Antigen Presenting Cell
IL-12	-	Interleukin-12
IFN-γ	-	Interferon gamma
ELISA	-	Enzyme Linked Immunosorbent Assay
LJ	-	Lowenstein-Jensen
RNTCP	-	Revised National Tuberculosis Control Programme

RT-PCR	-	Reverse Transcriptase PCR
RIF	-	Rifampicin
PPV	–	Positive Predictive Value
NPV	–	Negative Predictive Value
H & E	–	Haematoxylin and Eosin
PAP	–	Papanicolaou

ABSTRACT

Background: Tuberculosis (TB) is a major public health problem in India. The most common presentation of extrapulmonary TB is lymphadenopathy. The clinical parameters for the diagnosis of EPTB are neither specific nor does their absence exclude tuberculosis involvement. Recently WHO recommended Gene Xpert to be used as one of the initial diagnostic test in patients suspected to have extrapulmonary TB. The Gene Xpert test is a cartridge-based nucleic acid amplification assay which diagnoses TB by detecting the presence of causative bacteria, as well as Rifampicin resistant strains of TB.

Objective: Statistical evaluation of Auramine-Rhodamine (AR) stain and Gene Xpert (Nucleic acid amplification test) utility in the detection of Mycobacterial tuberculosis (including Rifampicin resistance), in FNAC procedure for clinically suspected EPTB.

Methods: All clinically suspected cases of extrapulmonary TB, referred for FNAC procedure at Department of Pathology, Shri B.M.Patil Medical College, Hospital and Research Center, BLDE (Deemed to be University), Vijayapura was included in the present study. Routinely stained FNAC smears with Giemsa, PAP and H&E stain were examined. Special methods like Ziehl-Neelsen (ZN) stained and Auramine Rhodamine (AR) stained smears were examined for acid-fast bacilli(AFB). Aspirated material remaining in the needle hub/syringe was rinsed with phosphate buffer and subjected for Gene Xpert test. Procedure for sampling for AR stain and Gene Xpert test have been elaborated.

Results: In the present study of 108 cases, the mean age was 30.6years. Overall ZN stain positivity was in 18.5%, AR stain showed 31.4% positivity and Gene Xpert test showed positivity of 56.4%. On combining AR stain and Gene Xpert assay, additional 31.4% cases of TB were picked up. 25% of additional cases which were AR stain negative, were found positive on Gene Xpert. 9.2% of cases did not show correlation between cytomorphological features and ZN stain, AR stain and Gene Xpert test.

Conclusion: From the present study, we conclude that by using the AR stain and Gene Xpert test, we can confirm more cases of TB along with Multi-Drug Resistant cases on FNAC procedure and help in early initiation of treatment.

Keywords:- Extrapulmonary TB, Auramine-Rhodamine stain, Gene Xpert assay.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Tuberculosis (TB) is a major public health problem in developing countries like India. Globally, TB is one among the top ten leading causes of death accounting for 1.3 million deaths every year. India accounts for two-thirds of global tuberculosis burden.[1]

Tuberculosis is an infectious disease which is caused by Mycobacterium tuberculosis (MTB) bacteria especially, in human beings.[2] It is an airborne disease and is seen commonly affecting the lungs causing Pulmonary Tuberculosis (PTB). It also affects other sites of the body other than the lungs which are called Extra Pulmonary Tuberculosis (EPTB). Most commonly affected organ being the lymph nodes.[3]

Although, based on history, clinical examination and radiological evidence, diagnosis of tuberculosis is difficult. These clinical parameters neither gives definite diagnosis nor their absence rules out the infection. The Diagnosis of the disease mainly rests on the demonstration of the characteristic granuloma with or without caseous necrosis and demonstration of causative organism Mycobacterium tuberculosis.[4] In such scenario Fine needle aspiration cytology (FNAC) plays an important role in establishing accurate diagnosis.[2] FNAC also provides a good non-invasive alternative to the excisional biopsy.[5]

Conventional Ziehl-Neelsen (ZN) stain used for detecting acid-fast bacilli (AFB) plays an important role in the diagnosis and monitoring of the treatment of tuberculosis. But it carries a low sensitivity ranging from 20-43%.[6,7] Culture test for MTB is a gold standard method but is time-consuming and requires specialized safety procedures in laboratories. Serological tests posses decreased sensitivity and specificity.[8]

Fluorescent microscopy (FM) also has a major role in detecting the tubercle bacilli. Since FM using Auramine-Rhodamine (AR) stain is less time consuming and cost-effective, this method is always considered superior to the ZN stain.[2]

One of the latest methods, Gene Xpert MTB/RIF assay (Cartridge based nucleic acid amplification assay-CBNAAT) has been recommended by WHO because of its rapidity, sensitivity,

specificity and cost-effectiveness. It not only detects the presence of tubercle bacilli but also simultaneously identifies the presence of Rifampicin resistant strain.[9] The Gene Xpert technique can be performed on the sputum specimen, gastric aspirates, urine samples, lymph nodes or abscess aspirates and other materials except blood.[70]

Hence, in the present study, we aim at assessing the accuracy and rapidity of AR stain and Gene Xpert test in diagnosing extrapulmonary tuberculosis during the FNAC procedure.

OBJECTIVE OF THE STUDY

Statistical evaluation of Auramine-Rhodamine stain and Gene Xpert (PCR) utility in the detection of Mycobacterial tuberculosis (including Multi-drug resistant tuberculosis), in FNAC procedure in suspected tuberculosis cases.

REVIEW OF LITERATURE

In ancient times, tuberculosis was otherwise called as King's evil, Phthisis, Pott's disease, Rajyakshma, Tapedic, etc. Bones of pre-historic man dating back to 8000 BC, it has been described as Yakshma. Sushrutha described the disease and said that it was difficult to treat. Dating back around 460-377BC of Greek literature, Hippocrates explained scrofula on the skin of phthisic pigs.[10]

Tuberculosis was also called as 'Captain of the ship of death' in previous eras. Fracastonius stated that the disease can be transmitted through the air to humans for which he later named it 'Contagium vivum'. Franciscus Sylvius (1614-1672) first coined the term 'Tubercle', where he noticed tubercles in the lungs of individuals with 'Phthisis'. Benjamin Martin (1720) suggested that it can be an infectious disease. Laurent Bayle (1774-1816) first introduced the term 'Tuberculosis'. Later Robert Koch on 24th March 1882, identified the specific causative agent, Mycobacterium tuberculosis causing tuberculosis. After which, 24th March was celebrated as World Tuberculosis Day every year by WHO (World Health Organization). [10]

Tuberculosis of lymph nodes was also called with the historical names 'Scrofula' or 'The king's of evil'. August Hirsch stated, "It denotes an inflammation kind of tumor more particularly in the neck".[11,12]

For many years, it was believed that the royal house of France and England had a supernatural gift of curing scrofula by touching the affected people. At the end of 19th century, it was identified that tuberculosis infection was the cause of scrofula.[12,13]

Initially, Mycobacterium bovis was thought to be causative agent, but thereafter many studies and researches stated that M. tuberculosis or atypical mycobacterium especially Mycobacterium avium complex as a causative agent.[14]

Tuberculosis presents as both pulmonary as well as extra-pulmonary form. Extra-pulmonary tuberculosis (EPTB) accounts for 10-42% of all cases of tuberculosis and in India, it accounts for 10-15%.[15]

Mycobacterial lymphadenitis is the commonly encountered extra-pulmonary presentation of tuberculosis in both immunosuppressed and immunocompetent individuals.[16]

Lymph nodes (LN) are the most common organ to get affected by tuberculosis accounting for 58% of all new extra-pulmonary tubercular cases, among which cervical lymph node is the most commonly affected site accounting for 75%.[3] EPTB is not only seen affecting the lymph nodes but also affects other systems of our body such as gastrointestinal tract (GIT), skeletal system, genitourinary system and central nervous system.[17]

Amongst the neck nodes, supraclavicular chains of LN's and posterior cervical LN's are most commonly affected by the disease, followed by pre-auricular and submandibular LN's. But less commonly affected LN's are axillary and inguinal group of LN's accounting for less than 10% of cases.[18] EPTB affecting pleura, LNs, skin, bone and genitourinary system is seen in 15% of cases.[19]

Structure of tubercle bacilli

In view of the betterment of diagnostic modalities, it is must to know about the structure and pathogenesis of tubercle bacilli. These bacilli are thin, slender, aerobic rods which grow in branching or straight chains. They measure up to 0.2-0.6 μm in size. These bacilli have a unique, waxy cell wall containing mycolic acid which is responsible for their acid-fast nature. Bacilli stains weakly with gram-positive stain.[20,21]

Pathogenesis of tuberculosis[22]

Pathogenesis of tuberculosis is divided into various steps from the entry of pathogen to the causing fulminant disease.

1) Invasion of tubercle bacilli into the body

Mycobacterium tuberculosis is an obligate aerobic, intracellular organism that has an affinity for the lung tissue. Bacilli enter the human body through respiratory route. Once it reaches the lungs which is the primary site of infection, it spreads to the different organs through lymphatics and hematogenous route. The apex of the lungs and regional LN's are the most favorable sites. Since the organism affects different organs, disease manifests with different clinical signs with respect to the organ affected.

2) Journey of bacilli in causing tuberculosis

i) Tubercle bacilli and macrophage interaction

With the help of complement receptors (CR1, CR3 and CR4), CD14, mannose receptors(MR) and other cell surface receptors the process of phagocytosis begins in which the organism is engulfed into macrophage by endocytosis in the alveoli of the lung. Mannose receptors binds to glycolipid of bacterial cell and complement receptors binds to the opsonized bacteria.[19]

ii) Survival of bacilli within the macrophage

Tubercle bacilli prevent the formation of phagolysosome by inhibiting the gathering of the proteins and blocking the calcium signals. Once the fusion is inhibited, bacteria will start replicating unchecked within the vesicle.[23]

In non-sensitised individuals, during the early stage of the disease, bacteria replicates and proliferates within the pulmonary alveolar macrophages causing bacteremia and seeding it into multiple sites of the body. This results in

the extrapulmonary spread of tuberculosis. [23]

iii) Immune response to the tubercle bacteria[23]

Once the mycobacterial antigen enters regional LN's, they are presented to the T-cells through antigen-presenting cells (APC's). This interaction leads to the differentiation of T-cell to form helper-T-cell in the presence of IL-12 produced by APC's.

These helper-T-cells produce interferon-gamma (IFN-γ) which are critical mediators for macrophages containing M. tuberculosis bacteria. Thereby IFN-γ carry on three functions-

a) It stimulates phagolysosome maturation and allows the bacteria to face bactericidal actions of lysosomes.
b) It also stimulates the formation of nitric oxide, which combines with other oxidants to form nitrogen intermediates which in-turn again has a killing effect on the bacterium.
c) IFN-γ produces antimicrobial peptides which again has a lethal effect on the bacteria.

iv) Granuloma formation

The hallmark feature of tuberculosis infection is the formation of granuloma or tuberculoma. IFN-γ transforms activated macrophages to epithelioid histiocytes which aggregate to form granulomas. Once these epithelioid cells start disintegrating from the center, it gives the appearance of central granular caseous necrosis. The periphery of the necrotic zone is surrounded by activated epithelioid cells and lymphocytes. Few of these epithelioid cells combine and form multinucleated giant cells (Langhan's giant cells).[23,31]

v) Cavitary tuberculosis

When the formation of caseous necrosis predominates, it gets transformed into a pus-filled cavity. This abscess cavity without the features of inflammation is called as cold abscess. The tubercle bacilli multiply to the highest number within this cavity. If the cavity expands and erodes blood vessels, bacilli spread to the whole body. Whereas if the cavity gets ruptured into the airway, it becomes the mode of transmission.[31]

vi) Secondary TB infection

Secondary TB infection or reactivation of TB is seen when the dormant tubercle bacilli reactivate after the primary infection. It can present as a pulmonary or EPTB infection. Several risk factors are responsible for development of secondary TB namely immunosuppressed state due to HIV infection, renal failure, sepsis, diabetes mellitus, malnutrition and long term use of corticosteroids. The lifetime risk of developing secondary TB is 10% in immunocompromised individuals.[24]

Clinical manifestations of tuberculosis

Tuberculosis presents with varied clinical signs and symptoms depending upon the site and duration of infection.

Primary infection is asymptomatic and it acts as an immune stimulant. Active infection develops in 5-10% of individuals with primary infection. This is seen once the host defense mechanism is impaired.[25]

Primary infection is always seen in the lungs. Clinically these patients present with low-grade fever lasting for 3 weeks with other non- specific symptoms like productive cough, pharyngitis and fatigue. [26]

Diagnosis of EPTB is difficult. Clinical presentation depends on the organ involved. Lymph nodes are the most commonly affected organ in EPTB and manifest as painless enlargement. Though they are painless initially, they later become painful. With the progression of the disease, LN's becomes matted. In the absence of signs of inflammation, it proceeds to form cold abscess. If left untreated, it starts to form discharging sinus to the skin along with ulceration and scarring. [27] Since spine is the common site for skeletal TB, it presents with back pain. [28] The disease can involve any part along the length of GIT.[29]

Systemic symptoms like fever, weight loss and night sweats with generalized lymphadenopathy which is localized outside the cervical chain, in such scenarios miliary tuberculosis should be suspected.[30]

HIV-TB Co-infection

The immune system involved in M.tuberculosis is cell-mediated immunity and T-lymphocytes are the controller of infection. In HIV patients, there is a progressive and severe impairment of cell-mediated immunity which plays as a potent risk factor for acquiring tuberculosis. [31,32] There is a 60-70% chance of acquiring TB among HIV positive individuals in their lifetime. TB presents as extra-pulmonary or disseminated form among HIV positive individuals. [33] The risk of acquiring TB is more during the early phase of HIV indicating an immune defect independent of CD4+ cell count. [31]

Fine Needle Aspiration Cytology (FNAC)

It was in the 1950's and 1960's, FNAC as a technique, was started by a group of pathologists in Europe. FNAC is a method that is applied to superficial lesions that are easily accessible, palpable lesions of skin, soft tissue, thyroid, breast, salivary glands and superficial LN's. [34]

In the beginning, FNAC was used to confirm a metastatic or clinically suspicious, locally recurring known cancer without any surgical intervention. After conducting many studies and experiments, it was concluded that FNAC is not only limited in diagnosing neoplastic conditions but also has got value in diagnosing infectious, inflammatory and other non-neoplastic conditions. FNA material was not only used for cytological preparations but also for biochemical and microbiological assays. [34]

Since LN's are the commonly affected organ, FNAC of lymph node helps in deciding whether a biopsy is indicated or not, for histopathological examination. Also FNAC helps in determining the cause for lymphadenopathy, whether it is due to infections, reactive conditions, primary or metastatic malignancies. [35] Many studies have shown that FNAC with a sensitivity of 92% and specificity of 98%, is recommended as a golden screening technique in all the cases of lymphadenopathy. [36]

Role of FNAC in the diagnosis of Tuberculosis[37]

FNAC is a non-invasive, simple and cost-effective technique in diagnosing tuberculosis when compared to core-needle biopsy or excisional biopsy. It not only helps in the initial diagnosis of tuberculosis but also in the follow up of patients after completion of anti-tubercular treatment.

Many studies have been conducted on using FNAC as a technique in diagnosing pulmonary and extra-pulmonary tuberculosis in various other sites like LN, breast, salivary gland and thyroid gland. FNAC under imaging guidance, it was possible to even detect deep-seated TB infection in liver, spleen, pancreas, bone, spine and intestine.

FNAC procedure not only avoids physical but also psychological trauma which was seen in patients during the surgical open biopsy. FNAC also provides the advantage of carrying out the procedure on the out-patient department basis.

Epidemiology of TB[1]

World Health Organization (WHO) says, about 9-11 million of the world population is affected by TB and about 1.3 million die every year due to TB. Most of these cases are seen in South-East Asia accounting for 44%. Amongst South East Asia region, India stands first accounting for 27% having the highest TB burden in the world.

Within India, 1.8-3.7 million of the population was affected by TB with increased incidence in adult age group. In view of reducing the global TB burden, WHO has launched new global TB strategy for the "post-2015 era" aiming at "ending the global TB epidemic" by 2035. [38]

The strategy includes Vision, Goal, Milestone for 2025 and Targets for 2035. [38] (Table 1)

Table: 1- New global TB strategy for the "post-2015 era" by WHO states that -"

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 100000 population) -No affected families facing catastrophic costs due to tuberculosis"

Laboratory diagnosis of tuberculosis

The definitive diagnosis of EPTB is still a challenge in a developing country. Diagnosis depends on cytomorphological demonstration of granulomas with/without caseous necrosis and demonstration of a causative organism by using special stains like Ziehl-Neelsen (ZN) stain, Fluorescent microscopy on FNA material along with isolation of organism by

culture. Culture is always a gold standard method for isolation of Mycobacterium tuberculosis. Since it takes longer duration and requires specialized laboratory, this method is not routinely preferred for the diagnosis of TB. [39]

With the advancement of technologies, other specialized techniques are available for the diagnosis of TB such as ELISA, Immunochromatography. Because of the lack of specificity, again these techniques were not preferred.[40] Polymerase Chain Reaction (PCR) being an important milestone in the detection of DNA/RNA, using this principle WHO has come up with new diagnostic modality known as Gene Xpert MTB/RIF assay in the diagnosis of TB. Recently WHO has recommended that Gene Xpert to be used as one of the initial diagnostic test in patients having EPTB. [41]

Culture method in diagnosis of TB

Though culture is time-consuming, it remains as a gold standard method to isolate MTB. Species of MTB takes 3-8 weeks to grow on an LJ medium which in turn results in delay in the diagnosis of TB. But this method has an increased sensitivity when compared to microscopy. [42]

Cytomorphological diagnosis of EPTB

LN's are the most commonly affected organ in EPTB. The diagnostic features of tubercular lymphadenitis has four morphological features. [43]

a) Epithelioid granulomas with caseous necrosis
b) Epithelioid granulomas without caseous necrosis
c) Caseous necrosis alone
d) Acute suppurative inflammation

The diagnostic criteria for tuberculosis on cytology include the presence of epithelioid cells, multinucleated giant cells with/without necrotic background. [44] The typical necrotic background comprised of pink granular material containing nuclear debris is called as 'Tubercular diathesis'. Cases not fulfilling the above criteria but shows only scattered epithelioid cells or only necrosis along with neutrophilic infiltration with/without granulomas were also diagnosed as tubercular lymphadenitis. [45]

Tuberculosis affecting intercoastal LN's always presents as a cold abscess. Such cases are considered as soft tissue tuberculosis. On cytology, if it demonstrates granulomas or caseous necrosis especially in the chest region and axillary region, these cases should be suspected for TB. [46]

Thus, FNAC provides a simple, reliable, safe, rapid and cost-effective tool in diagnosis of EPTB where it poses a diagnostic problem. [47, 48]

Isolation of tubercular bacilli by ZN stain

ZN staining method is the most commonly used technique in diagnosing pulmonary as well as extra-pulmonary tuberculosis. Because of its cost-effectiveness, less time consuming and reduced need for equipments especially in developing countries, it is the most commonly used method. Demonstrating ability of acid-fast bacilli (AFB) in FNA material is directly proportional to the concentration of bacilli present in the sample. [49]

The acid-fast property of the bacilli allows them to get stained with carbol fuschin, which is a component of ZN stain. A gentle amount of heat was used to make dye penetrate the bacilli. Later on, with the decolorizing solution (acid alcohol), smear is decolorized. Since decolorizer cannot penetrate bacterial cell wall, except acid-fast bacilli, rest other bacilli are decolorized leaving the Mycobacterium bacilli stained red. To enhance the Mycobacterium bacilli visualization, smears are counterstained with methylene blue.[50]

A minimum of 5000-10000/ml of bacillary load is required in the FNA sample, to visualize under the microscope. If the load is less than 5000, there will be a chance of not detecting the bacilli on smears resulting in false-negative cases. [51]

Method of reporting AFB[52]

The grading system (Table 2), recommended by RNTCP for the grading sputum smear for AFB by ZN stain.

Table 2- Grading of Acid Fast Bacilli by ZN stain

No of AFB seen	Result	Grade	Minimum no of fields examined
>10 per oil immersion field	Positive	3+	20
1-10 per oil immersion field	Positive	2+	50
10-99 per 100 oil immersion field	Positive	1+	100
1-9 per 100 oil immersion field	Scanty	Scanty	200
Absence of AFB in 100 oil immersion fields	Negative	0	100

When the FNA smears doesn't show granulomas but show acute inflammatory features, establishment of TB diagnosis becomes difficult. In such cases, special stains like ZN helps to reach the diagnosis. [53]

A definitive diagnosis of TB should be established by combining cytomorphological features, AFB and culture. Anyone of them showing negative for Mycobacterium doesn't rule out the possibility of TB. [54] Because the ZN stain has low sensitivity, it is of less diagnostic value especially in FNAC smears.[8]

Fluorescent microscopy in diagnosis of TB

The fluorescent staining method always holds a superior position when compared to ZN stain. [55, 56] Fluorescent staining technique uses the same principle as ZN stain. But in this stain, instead of carbol fuschin, fluorescent dye i.e, Auramine is used as a primary stain to stain the tubercle bacilli. As a counterstain potassium permanganate is been employed for the betterment of visualization of bacilli which highlights the organism. [57]

On Fluorescent microscopy, AFB appears as golden yellow, rod-shaped, slender bacilli. [58] AR stain is less time consuming and allows in low power examination, thus reduces the time taken for screening the whole slide for the bacilli.[2]

Reporting of Auramine Rhodamine stain[59]

The grading system (Table:2), recommended by WHO for grading AFB by Fluorescent microscopy.

Table 3- Grading of Acid Fast Bacilli by Fluorescent microscopy

Result (WHO scale) (1000x field = HPF)	**Fluorescence** (400x magnification: 1 length = 40 fields = 200 HPF)	**Minimum no of fields examined**
Negative	Zero AFB / 1 length	40
Scanty	1–19 AFB / 1 length	40
1+	20–199 AFB / 1 length	40
2+	5–50 AFB / 1 field on average	20
3+	>50 AFB / 1 field on average	8

The fluorescent staining method is more sensitive when compared to the conventional ZN method. It is of high diagnostic value because it picks up the bacilli in patients having low bacillary index which are thought to be missed on conventional ZN stain.[58,60]

Hence, the diagnosis of Mycobacterium tuberculosis becomes easy when fluorescent staining method is combined with clinical parameters and cytomorphological features in LN aspirates. [58]

Gene Xpert

Due to several limitations of ZN stain and culture, many studies were conducted in view of identifying a method that has got higher sensitivity and specificity in the diagnosis of TB. Finally in December 2010, WHO has recommended Gene Xpert as one of the 'first line' diagnostic test in the patients suspected to have EPTB.[41]

The Gene Xpert test was developed by Cepheid Inc. for the identification of anthrax bacilli. This assay works on molecular beacon technology for the detection of DNA sequence which is amplified in a hemi-nested RT-PCR. It is a cartridge-based real-time nucleic acid amplification system which incorporates microfluidics technology for the purification, concentration, detection and identification of the target nucleic acid sequence from the clinical samples.[61]

It uses single-use cartridge made up of plastic with multiple chambers which are preloaded with lyophilized reagent and liquid buffer. These reagents are required for further processing of the sample, extraction of DNA and hemi-nested RT-PCR. Machines are available with one, four, sixteen and forty-eight modules. [61]

Working of Gene Xpert MTB/RIF assay [61]

Respiratory or non-respiratory samples are first treated with sodium hydroxide and sample reagent (SR) containing isopropanol and incubated at room temperature for 15 minutes. This step reduces the viability of the tubercle bacilli in-turn reducing the biohazard risk. After this step, the processed sample is transferred to the cartridge and loaded to the Gene Xpert machine. Later steps are fully automated.

A syringe drive, rotary drive and a filter is being incorporated by cartridge, on which tubercle bacilli is deposited, after getting liberating from the processed sample. Ultrasonic lysis of the bacilli is carried out and genetic material is obtained. Thus the test starts amplifying the target gene rpoB gene by using hemi-nested RT-PCR reaction. This assay has lyophilized Bacillus globigii spores as an internal control.

TB bacilli are identified by the five overlapping molecular probes (A-E) which are complementary to the target gene. Along with Tuberculosis bacilli, the assay also helps in detection of rifampicin (RIF) resistant strains.

Gene Xpert assay in the diagnosis of EPTB

Gene Xpert test is used for the diagnosis of EPTB in adults and children. [62,63] Diagnosis of EPTB is a diagnostic challenge for various reasons like insufficient sample and in a sample having less bacillary load. In such conditions, Gene Xpert plays an important role in diagnosis of EPTB. [64]

Samples that can be used for Gene Xpert test comprised of respiratory samples like sputum, bronchoalveolar lavage, gastric lavage and extrapulmonary samples such as FNA, biopsy material, pus, other body fluids like CSF, ascitic fluid, pericardial and pleural fluid. Samples that are not recommended are stool, blood and urine. [65]

Gene Xpert to detect M tuberculosis, the specimen of any type should have a threshold level of 130-150cfu/ml of bacillary load. This PCR assay amplifies DNA of dead as well as live tubercle bacilli. [66]

WHO has put up criteria for using Gene Xpert in the diagnosis of EPTB along with rifampicin resistance in adults and children. It states that -“

a) Xpert MTB/RIF should be used in preference to conventional microscopy and culture as the initial diagnostic test for CSF specimens from patients suspected of having TB meningitis

b) Xpert MTB/RIF may be used as a replacement test for usual practice (including conventional microscopy, culture or histopathology) for testing specific non-respiratory specimens (lymph nodes and other tissues) from patients suspected of having EPTB" [67]

Gene Xpert test also poses some limitations such as machine requires a constant power supply, control of temperature and calibration of the machine annually. [68]

In a study conducted by Brijesh *et al*[2], 145 cases which were suspicious for tuberculosis were included. They studied cytomorphological features, special methods for the detection TB like ZN stain, AR stain, culture and Autofluorescence (AF) for all the 145 cases. 57.8% of cases showed granulomatous cytomorphological features. ZN stain was positive in 26.7% , AR stain was positive in 34.4% and AF was positive for 42.2% of cases. Using culture as a reference method, they concluded that AF and AR stain has more sensitivity when compared to ZN stain.

Vamseedhar *et al*[58] studied a total of 102 fine-needle aspirates of LN's which were suspicious for EPTB. Out of 102 cases, they found that ZN stain was positive in 44.1% and AR stain was positive in 81.3% of cases. Thus they concluded that AR stain is more sensitive when compared to ZN stain.

In 2016, Vikas *et al*[69] conducted a study on 115 cases suspected to have EPTB. This study showed 36.5% cases positive for ZN stain and 51.3% cases positive for AR stain. They concluded that, fluorescent stain (AR stain) method has helped to detect more number of TB cases when compared to ZN stain.

In a study conducted by Sunil *et al*[70], 289 cases suspicious for EPTB were studied for cytomorphological features, fluorescent microscopy (AR stain) and CBNAAT (Gene Xpert). 51% cases showed cytomorphological features of TB, 39.7% cases positive for fluorescent

microscopy and 49.1% cases positive for TB on CBNAAT. They showed that, CBNAAT detected TB in 6.5% more cases which was negative on cytomorphology and fluorescent microscopy.

In a study conducted by Majeb *et al*[73], out of 1146 FNAC cases, 40 cases were selected for evaluation of TB which were suspicious for EPTB. 62.5% cases were positive for AFB stain (ZN stain). Gene Xpert test detected TB in 37.5% cases which were negative on ZN stain. They concluded that Gene Xpert showed higher sensitivity and specificity when compared to ZN stain.

Asma *et al*[74] studied 174 samples of lymph node for the presence of TB. They found that ZN was positive in 23.6% and Gene Xpert was positive in 77% cases. By taking the culture method as a reference method, they concluded that Gene Xpert had higher sensitivity (87.5%) and specificity (73.3%). Because of rapidity and simplicity, Gene Xpert was considered as most useful method in detection of lymph node TB in this study.

In a study conducted by Fantahun *et al*[75], fine needle aspirates of 231 suspected cases for lymph node TB were studied. By taking culture as a reference method, fluorescent microscopy showed 35.5% and 99.5% sensitivity and specificity respectively. Gene Xpert assay showed 93.5% and 69.1% sensitivity and specificity respectively. They concluded that, Gene Xpert was more sensitive method in diagnosing lymph node TB from FNA material.

MATERIALS AND METHODS

SOURCE OF DATA

All cases of clinically suspected tuberculosis referred for fine-needle aspiration cytology to the Department of Pathology, Shri B.M Patil Medical College, Hospital and Research Center, BLDE (Deemed to be University), Vijayapura were taken.

Study period: 1st December 2017 – 30th June 2019.

INCLUSION CRITERIA

All the cases with suspicion of tuberculosis like lymphadenopathy, abscess, soft tissue swelling, breast lump/abscess, parotid swelling etc.

EXCLUSION CRITERIA

Neoplastic swellings/mass including benign and malignant or metastatic lesions diagnosed by FNAC procedure.

METHODS OF COLLECTION OF DATA

A prospective study of all cases of lymphadenopathy, cold abscess suspicious for tuberculosis coming under cytology section, referred to the Department of Pathology, Shri B M Patil Medical College, Hospital and Research Centre, Vijayapur was taken for the study.

A detailed clinical history and laboratory investigations were collected on OPD/IPD basis. Informed and written consent was obtained. For all the cases FNAC procedure was performed by using 21-25 gauge needle. Routinely stained FNAC smears with Giemsa stain, PAP stain and H & E stain showing tubercular granulomas and/or caseous necrosis were included in the study. Two unstained air-dried smears were subjected to ZN stain and AR stain each.

Aspirated material remaining in the needle/syringe hub was rinsed with 2ml of phosphate buffer solution and mixed well until a homogenous suspension is obtained. 0.7ml of this solution is

then transferred to the sterile conical, screw-capped tube using pipette. Double the volume of Xpert MTB/RIF sample reagent is added to 0.7ml of homogenized solution. Then it is vigorously shaken and incubated at room temperature for 15min. This solution is subjected for Gene Xpert test.[15]

MATERIALS REQUIRED

- Fine needle aspirated material
- 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
- ZN stain- (Carbol fuschin, Acid alcohol, Methylene blue)
- AR stain- (Auramine O, Alcohol, Pottasium permanganate, Hydrochloric acid)
- Gene Xpert machine- (Phosphate buffer, Xpert MTB/RIF sample reagent, Cartridge)

SAMPLE SIZE

With an expected prevalence of lymph node tuberculosis 77% at 95% confidence level and 0.10 desired precision, calculated sample size was 68.

The sample size was calculated using the formula,

$$n = \frac{Z^2 x\, P\,(1 - P)}{d^2}$$

Where,

n= Sample size.

Z = value of 2 statistic at 5% level of significance

P = expected prevalence rate

d = margin of error

STATISTICAL ANALYSIS

Data was analysed using

- Mean +/- Standard deviation
- Percentages
- Sensitivity and Specificity analysis.
- Negative Predictive value and Positive Predictive value

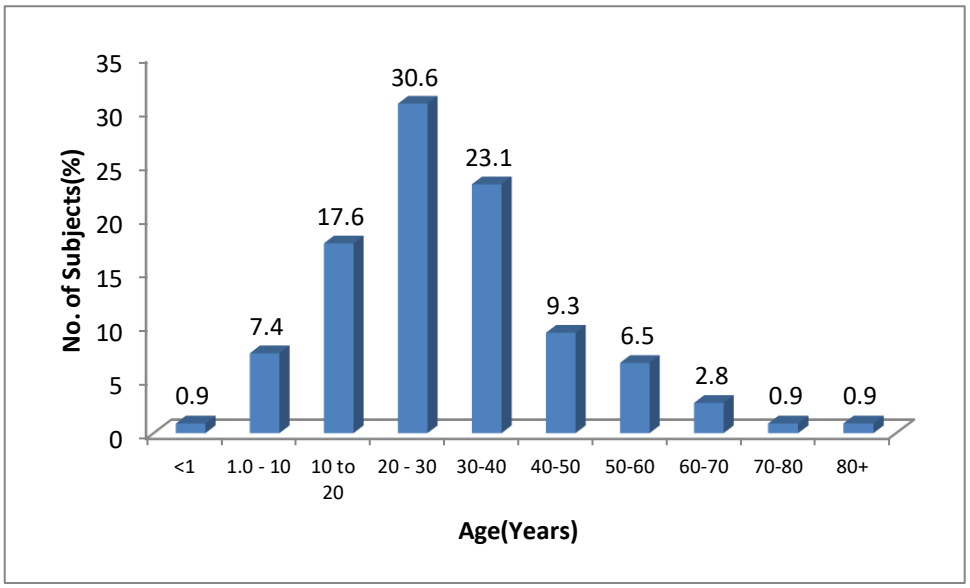
RESULTS

A total of 4641 FNAC's were done in the Cytology section of Department of Pathology during the period from 1st December 2017 to 31st July 2019. Among 4641 cases, 108 cases were suspicious for tuberculosis. These 108 cases were included in the present study. The smears prepared from aspirates were stained with Pap and Giemsa. Also special stains like ZN and AR for the detection of AFB were done. The remaining fine needle aspirate material from the needle hub is collected and sent for Gene Xpert assay. All the parameters were statistically analyzed.

Table-4: Distribution of cases according to Age

Age(Years)	No of patients	Percentage(%)
<1	1	0.9%
1 - 11	8	7.4%
11- 20	19	17.6%
20 - 30	33	30.6%
30-40	25	23.1%
40-50	10	9.3%
50-60	7	6.5%
60-70	3	2.8%
70-80	1	0.9%
80+	1	0.9%
Total	108	100%

Figure-1: Bar diagram showing Distribution of cases according to Age

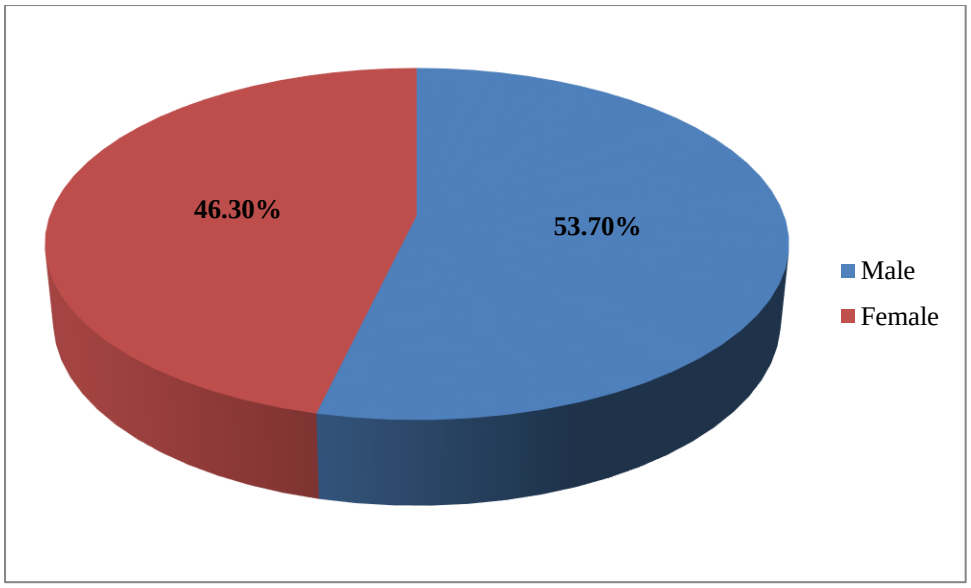


In this study, age ranged between 5 months to 82 years, with the mean age 30.6 years of presentation. (Table 4 and Figure 1)

Table-5: Distribution of cases according to Gender

Gender	**No of patients**	**Percentage(%)**
Male	58	53.7%
Female	50	46.3%
Total	**108**	**100**%

Figure-2: Pie Chart showing Distribution of cases according to gender



In this study, a slight male 58(53.70%) preponderance was seen. (Table 5 and Figure 2)

Table-6 : Distribution of cases according to site of lesion

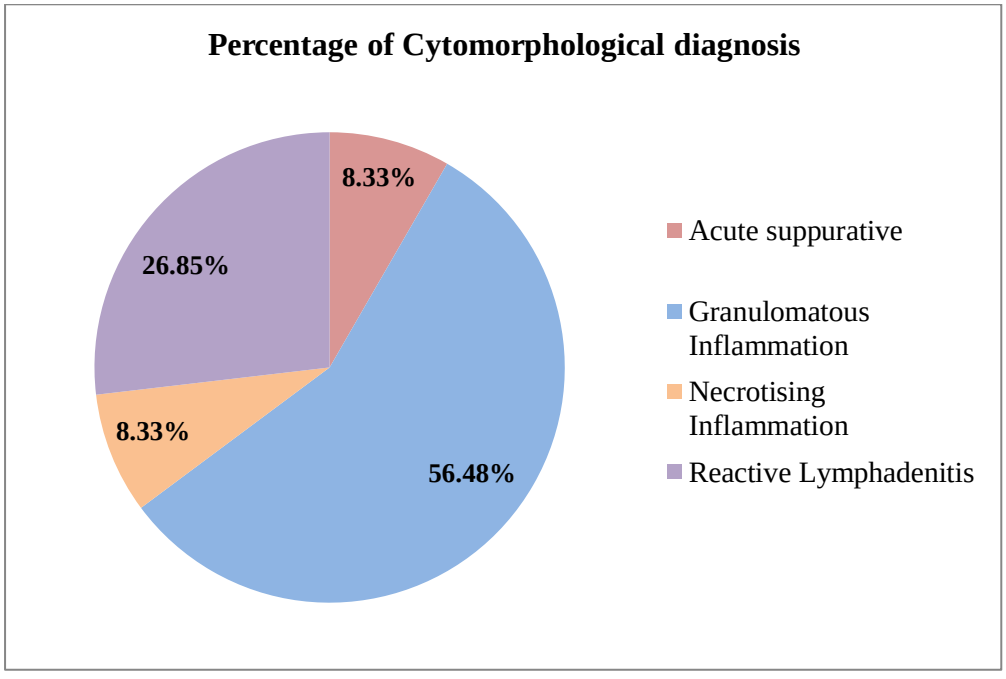
Site of lesion	No. of patients	Percentage(%)
Cervical	76	70.3%
Breast	7	6.5%
Supraclavicular	7	6.5%
Axillary	6	5.6%
Inguinal	5	4.7%
Post-auricular	3	2.8%
Submandibular	2	1.8%
Ant chest wall	1	0.9%
Epididymis	1	0.9%
Total	**108**	**100**%

In the present study, the most common site involved is the cervical region with 76 cases (70.3%) among 108 cases. (Table-6)

Table-7: Distribution of Cases according to Cytomorphological diagnosis

Cytomorphological diagnosis	No. of patients	Percentage(%)
Acute suppurative inflammation	9	8.33%
Granulomatous Inflammation	61	56.48%
Necrotizing Inflammation	9	8.33%
Reactive Lymphadenitis	29	26.85%
Total	**108**	**100**%

Figure-3: Pie Chart showing Distribution of cases according Cytomorphological diagnosis

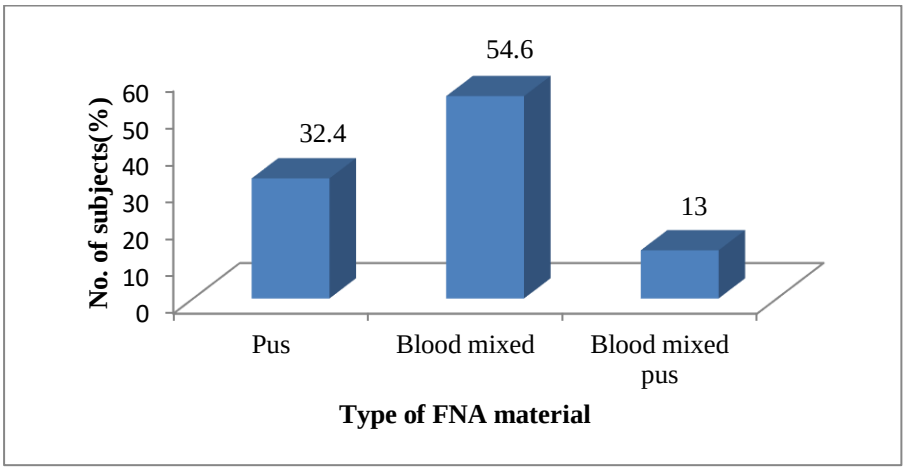


The most common cytomorphological pattern seen in the suspicious cases for TB in the present study is Granulomatous inflammation (56.48%) followed by reactive lymphadenitis (26.85%).(Table 7 and Figure 3)

Table-8: Distribution of cases according to Nature of aspirate

Type of FNA material	No of patients	Percentage(%)
Pus	35	32.4%
Blood mixed	59	54.6%
Blood mixed pus	14	13%
Total	108	100%

Figure-4: Bar diagram showing Distribution of cases according to Nature of aspirate



In the present study, predominantly aspirated material was Blood mixed accounting for 54.6% (Including positive and negative cases of TB) followed by Pus (32.4%) and Blood mixed pus (13%). (Table 8 and Figure 4)

Table-9 : Distribution of cases according to Nature of FNA material among ZN stain, AR stain and Gene Xpert positive cases.

Type of aspirate	ZN stain Cases(%)	AR stain Cases(%)	Gene Xpert Cases(%)
Pus	13(65%)	19(55.8%)	26(42.6%)
Blood mixed	4(20%)	9(26.4%)	23(37.7%)
Blood+Pus	3(15%)	6(17.6%)	12(19.6%)
Total	20(100%)	34(100%)	61(100%)

Figure-5: Bar diagram showing Distribution of cases according to Nature of FNA material among ZN stain, AR stain and Gene Xpert positive cases.

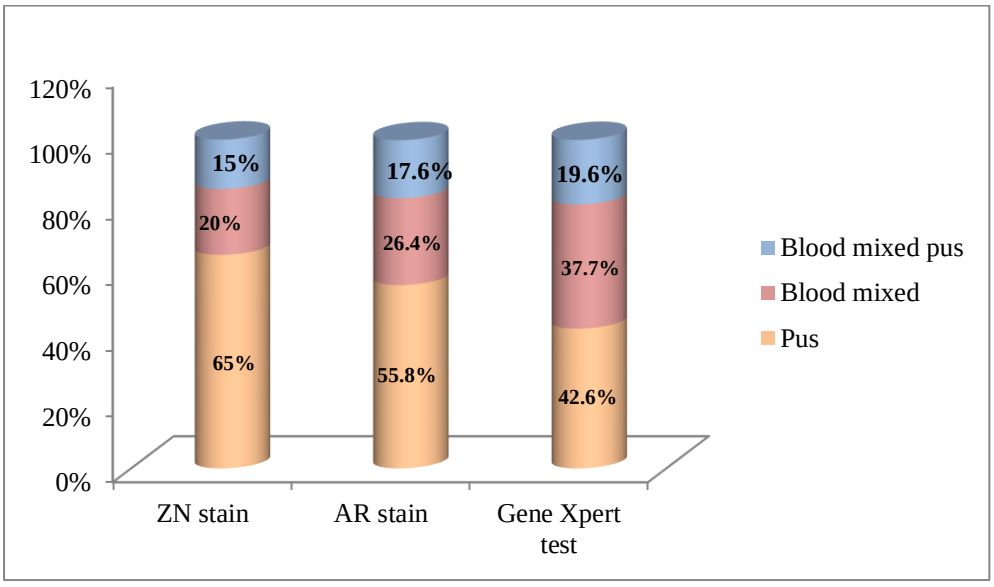


Table-10: Distribution of cases according to the presence of Granulomas

Granulomas	No of patients	Percentage(%)
Seen	61	56.5%
Not seen	47	43.5%
Total	108	100%

Figure-6: Pie Chart showing Distribution of cases according to the presence of Granulomas

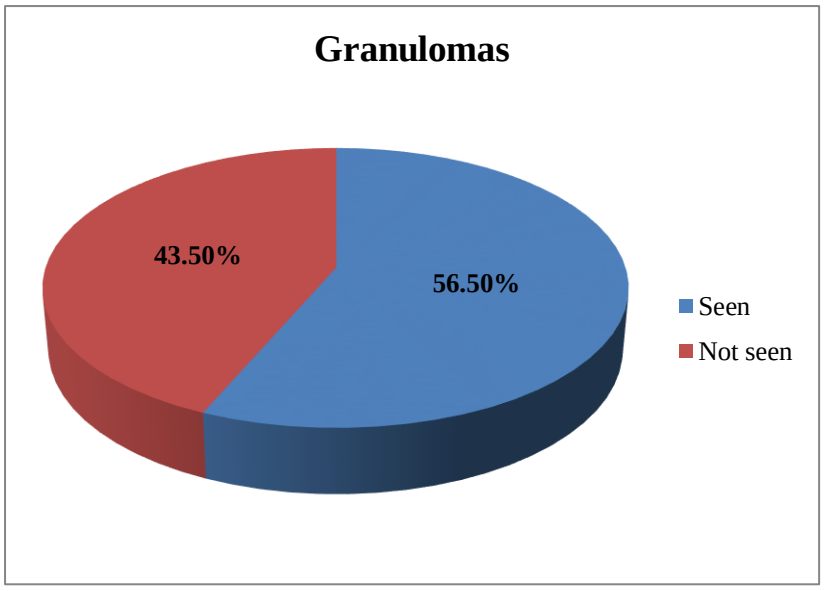
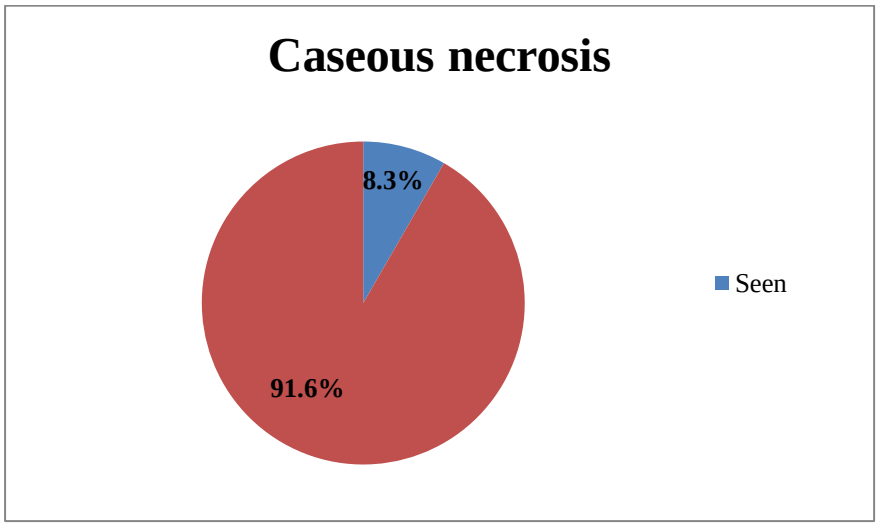


Table-11: Distribution of cases according to the presence of Caseous Necrosis only

Necrosis	No of patients	Percentage(%)
Seen	9	8.3%
Not seen	99	91.6%
Total	108	100%

Figure-7: Pie Chart showing Distribution of cases according to the presence of Caseous Necrosis only

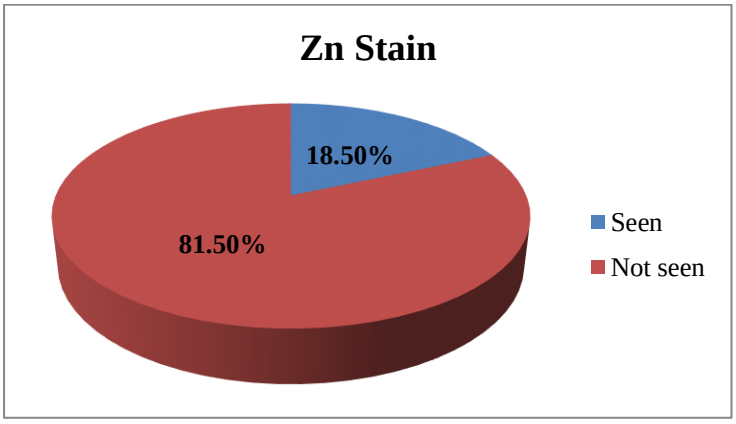


Out of 108 cases, Granulomas were seen in 61 (56.5%) cases (Table 10 and Figure 6) and Caseous necrosis was seen in 09 (8.3%) cases (Table 11 and Figure 7).

Table-12: Distribution of cases according to ZN stain positivity

ZN stain	No of patients	Percentage(%)
Positive	20	18.5%
Negative	88	81.5%
Total	108	100%

Figure-8: Pie Chart showing Distribution of cases according to ZN stain positivity

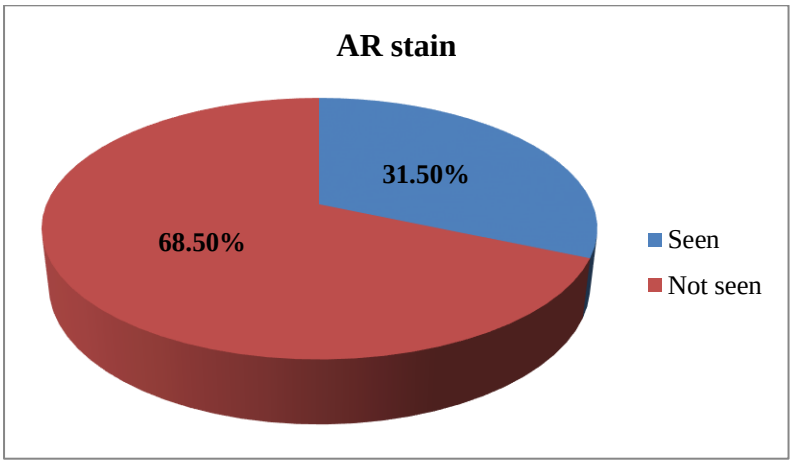


Out of 108 cases, 20(18.5%) cases showed positivity for ZN stain, whereas rest 88(81.5%) cases were negative. (Table 12 and Figure 8)

Table-13: Distribution of cases according to AR stain positivity

AR stain	No of patients	Percentage(%)
Positive	34	31.5%
Negative	74	68.5%
Total	108	100%

Figure-9: Pie Chart showing Distribution of cases according to AR stain positivity

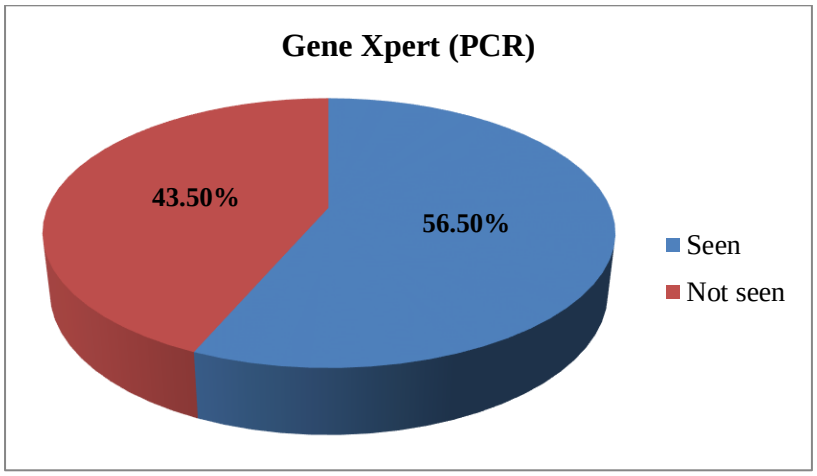


Among 108 cases, 31.5%(34/108) cases showed positive for AR stain. Rest 68.5(74/108) were negative for AR stain. (Table 13 and Figure 9)

Table-14: Distribution of cases according to Gene Xpert (PCR) assay positivity

Gene Expert (PCR)	No of patients	Percentage(100%)
Positive	61	56.5%
Negative	47	43.5%
Total	108	100%

Figure-10: Pie Chart showing Distribution of cases according to Gene Xpert(PCR) assay positivity



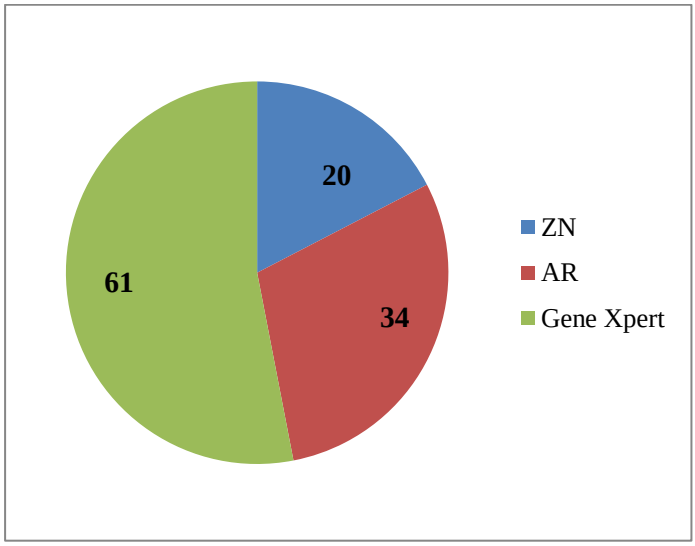
With the help of Gene Xpert (PCR) assay, MTB was detected in 56.5%(61/108) of cases. Whereas, in 43.5%(47/108) cases MTB was not detected. (Table 14 and Figure 10)

Out of 108 cases subjected for Gene Xpert assay, none of them showed Rifampicin resistance TB.

Table-15: Distribution of cases according to ZN, AR and Gene Xpert positivity

	ZN	**AR**	**Gene Xpert**
Cases	20	34	61
Percentage (%)	18.5%	31.5%	56.5%

Figure-11: Pie chart showing the distribution of cases according to ZN, AR and Gene Xpert positivity



In the present study, out of 108 cases, 20 cases were positive for ZN stain, 34 cases were positive on AR stain and in 61 cases MTB was detected by Gene Xpert assay. (Table 15 and Figure 11)

Table-16: Table showing comparison chart of ZN stain, AR stain taking Gene Xpert assay as a gold standard

Results	**ZN stain**	**AR**
TP (True Positive)	20	34
FP (False Positive)	00	00
FN (False Negative)	41	27
TN (True Negative)	47	47

Table-17: Table showing statistically analyzed values of ZN stain, AR stain taking Gene Xpert assay as gold standard

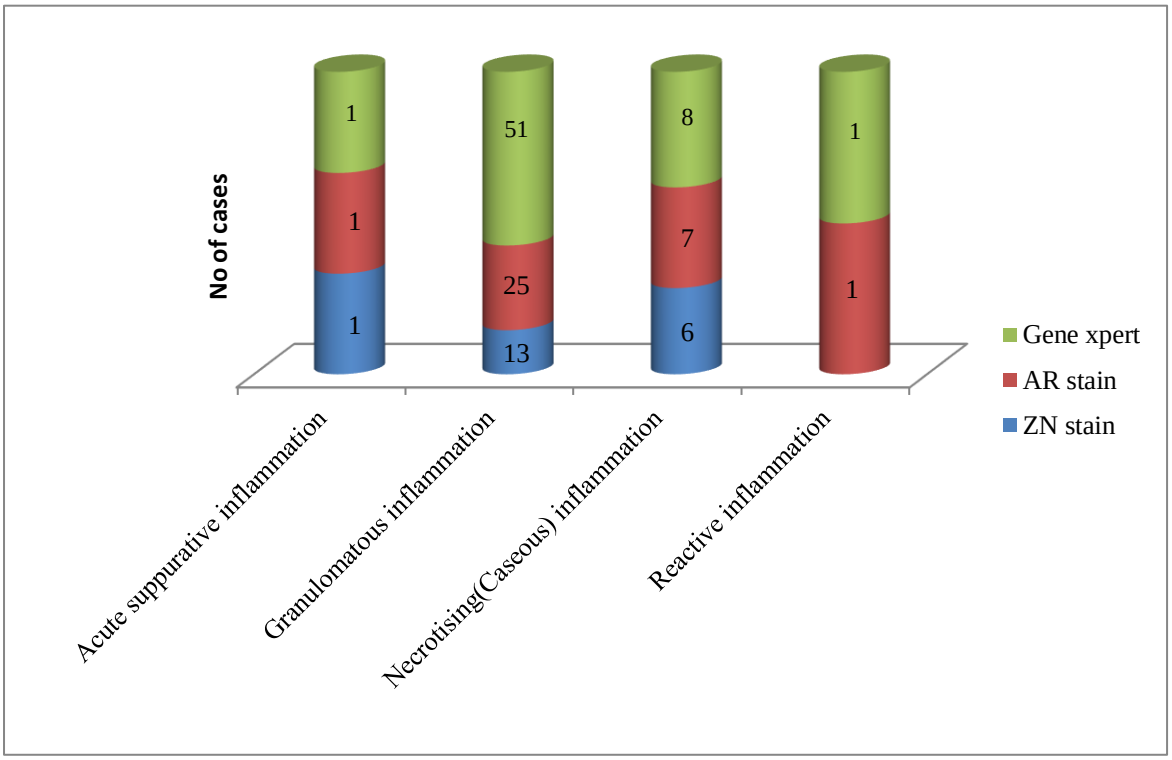
Values	ZN stain	AR
Sensitivity	32.78%	55.73%
Specificity	100%	100%
Positive predictive value	100%	100%
Negative Predictive value	53.41%	63.51%
Accuracy	62.04%	75%

Taking Gene Xpert assay as a Gold standard test, ZN stain showed sensitivity of 32.78%, specificity of 100%, PPV 100%, NPV 53.41% with an accuracy of 62.04%. AR test showed sensitivity of 55.73%, specificity of 100%, PPV 100%, NPV 63.51% with accuracy of 75%. These values found to be statistically significant. (Table 17)

Table-18: Table showing overview of the result of the present study

		Ziehl-Neelsen (ZN) stain Cases(%)		**Auramine Rhodamine (AR) stain** Cases(%)		**Gene Xpert (PCR) assay** Cases(%)	
Cytomorphological Diagnosis	Total no cases	Positive	Negative	Positive	Negative	Positive	Negative
Acute suppurative inflammation	09	01(11.1%)	08(88.8%)	01(11.1%)	08(88.8%)	01(11.1%)	08(88.8%)
Granulomatous inflammation	61	13(21.3%)	48(78.6%)	25(40.9%)	36(59%)	51(83.6%)	10(16.3%)
Necrotising(Caseous) inflammation	09	06(66.6%)	03(33.3%)	07(77.7%)	02(22.2%)	08(88.8%)	01(11.1%)
Reactive inflammation	29	00(0%)	29(100%)	01(3.4%)	28(96.5%)	01(3.4%)	28(96.5%)
Total no of cases	108	20	88	34	74	61	47
% of positive results		**18.5%**	81.4%	**31.4%**	68.51%	**56.4%**	43.5%

Figure-12: Bar diagram showing predominant cytomorphological pattern among ZN stain, AR stain and Gene Xpert assay Positive cases.



In the present study, out of 9 cases with cytomorphological diagnosis as acute suppurative inflammation, one case showed positive in ZN stain, AR stain and Gene Xpert assay. Among 61 cases with Granulomatous inflammation as cytomorphological diagnosis, 13 were positive in ZN stain, 25 were positive in AR stain and 51 were positive in Gene Xpert test. Out of 9 cases showing necrotizing (caseous) inflammation, 6 were positive for ZN stain, 7 were positive in AR stain and 8 were positive in Gene Xpert assay. Among 29 cases with cytomorphological diagnosis as Reactive inflammation, none were positive in ZN stain and one case showed positive in AR stain and Gene Xpert assay. (Table 18 and Figure 12)

PHOTOMICROGRAPHS

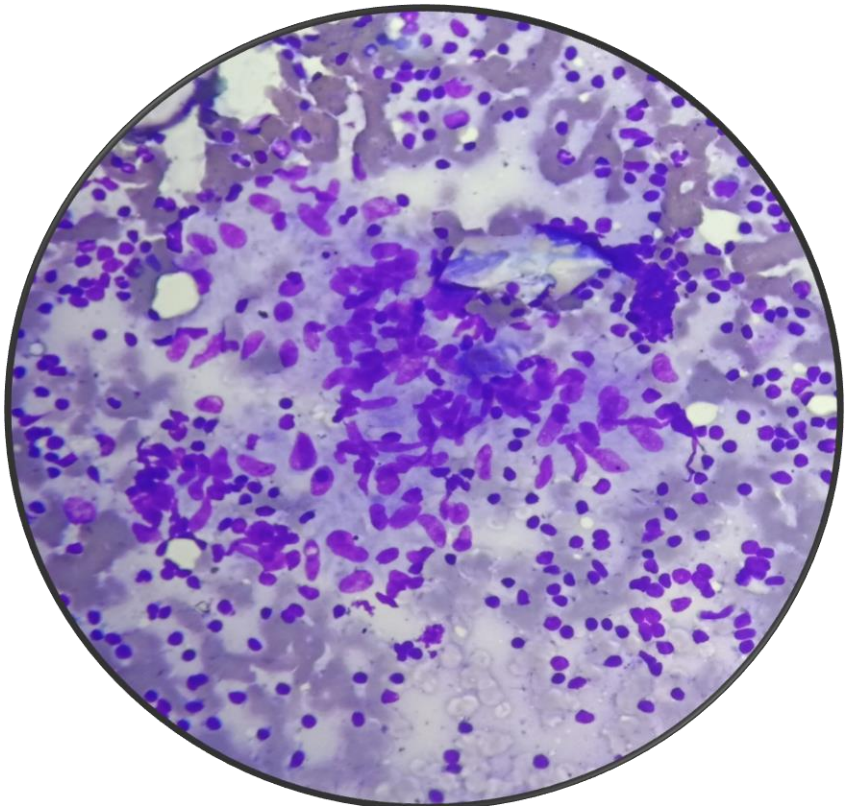


Figure:13(Giemsa,X400)- Cytological smear showing well formed Granulomas comprised of epithelioid cells and lymphocytes.

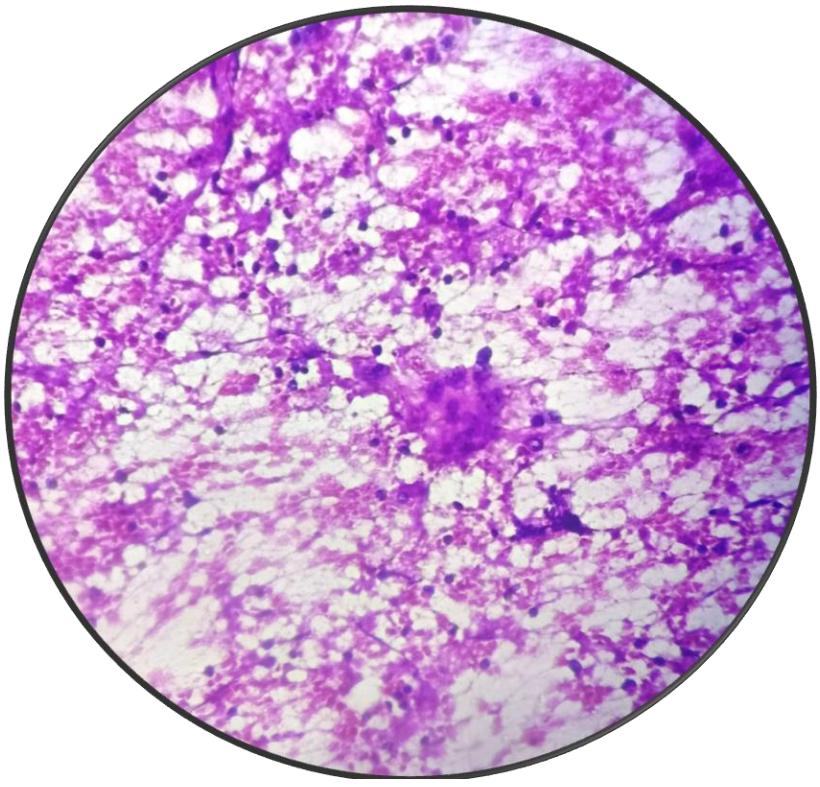


Figure:14 (PAP,X400)- Cytological smear showing Langhan's type of giant cells

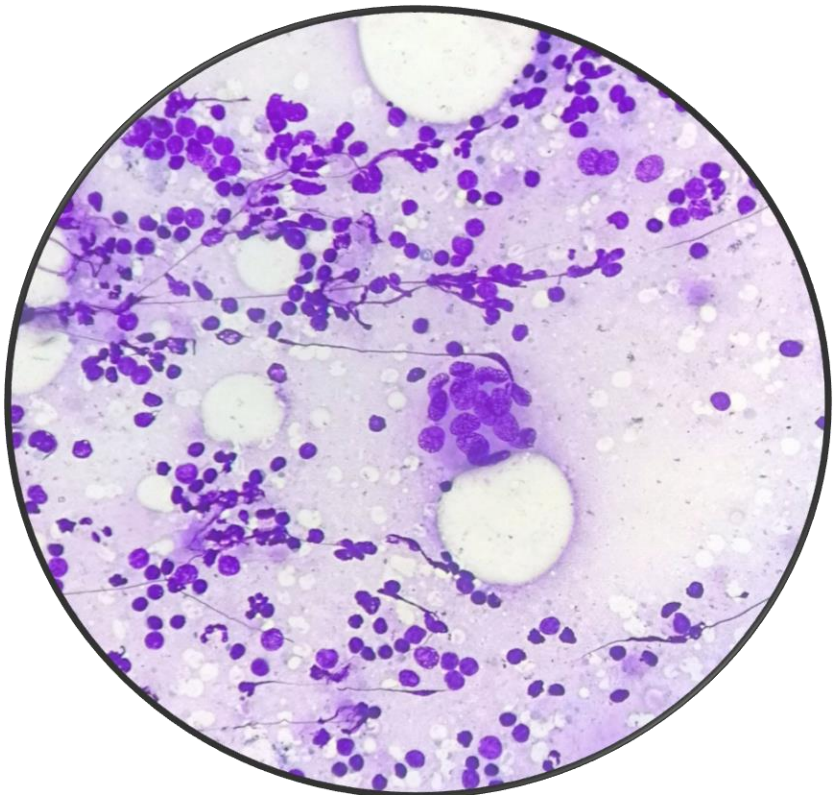


Figure:15 (Giemsa,X400)- Cytological smear showing cluster of epithelioid cells.

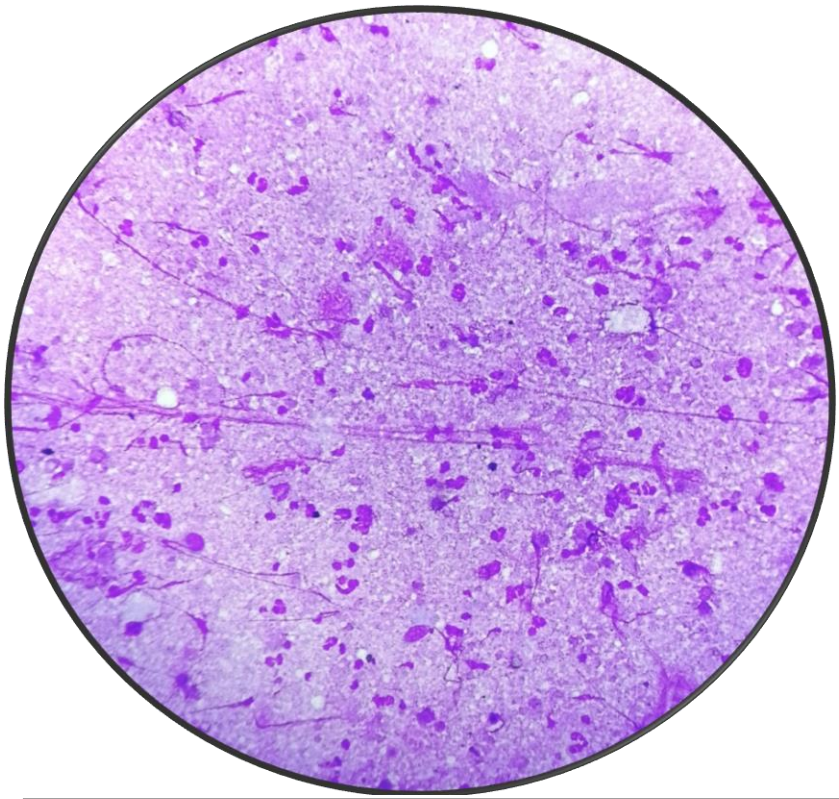


Figure:16 (Giemsa,X400)- Cytological smear showing eosinophilic, granular caseous necrosis.

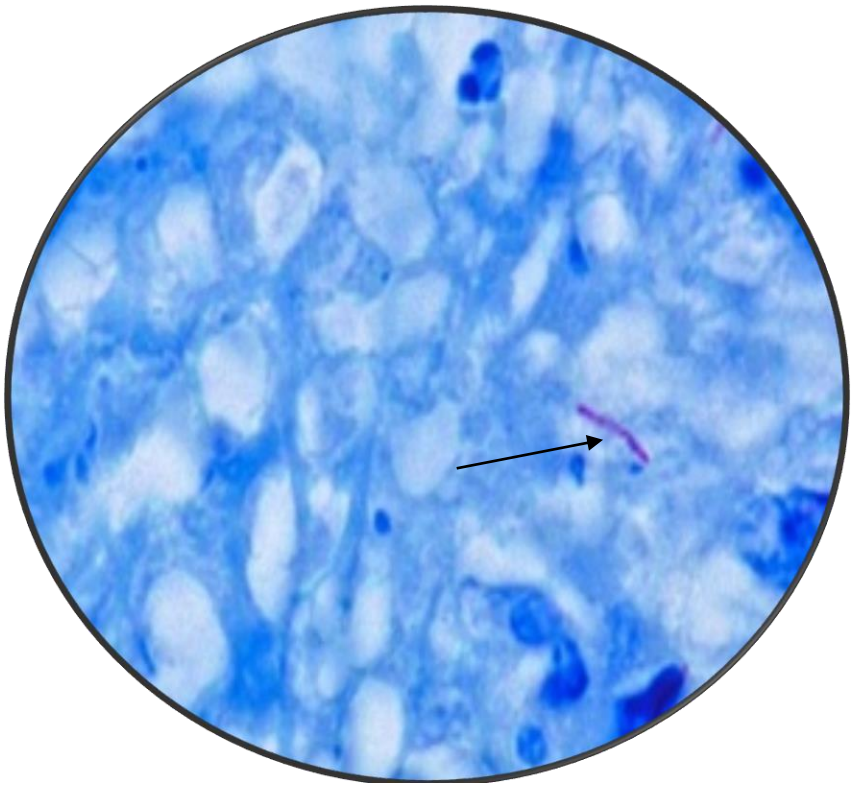


Figure:17 (ZN stain,X1000)- ZN stained cytology smear showing pink, beaded, rod-shaped acid fast bacilli.

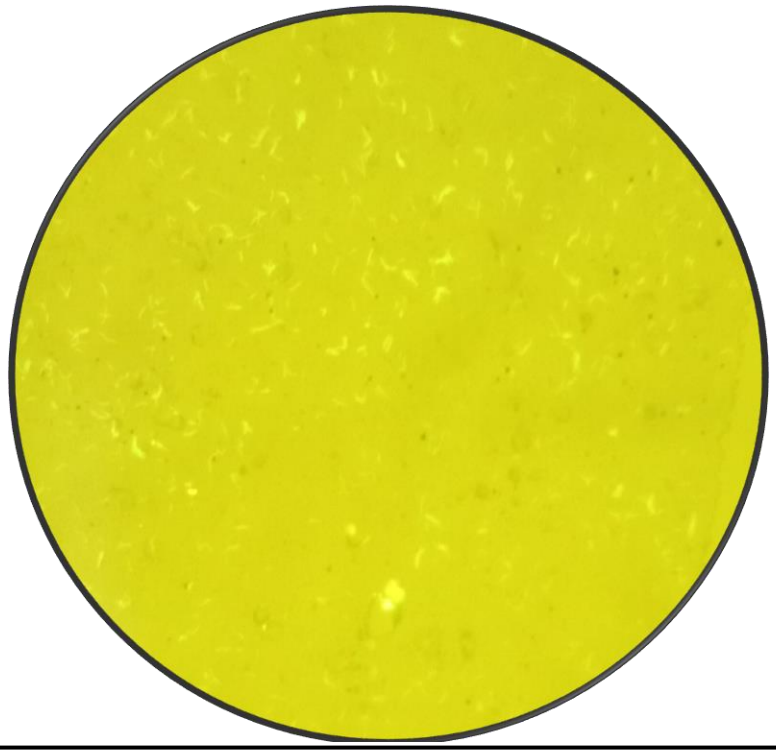


Figure:18 (AR stain,X1000)- AR stained cytology smear showing bright yellow colour, slender, rod-shaped acid fast bacilli.

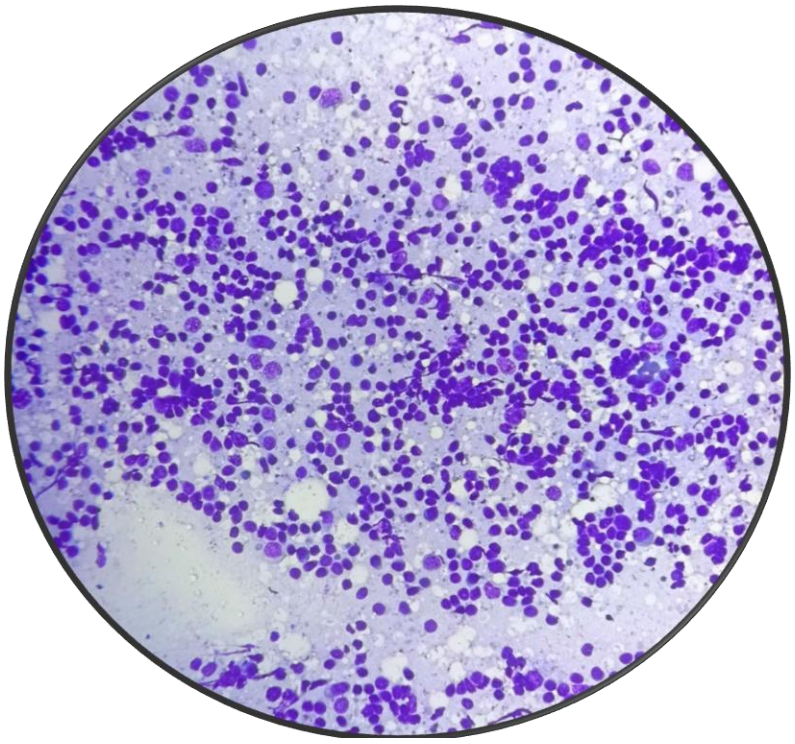


Figure:19 (Giemsa,X400)- Cytological smear showing polymorphous population of lymphoid cells suggestive of reactive lymphadenitis.

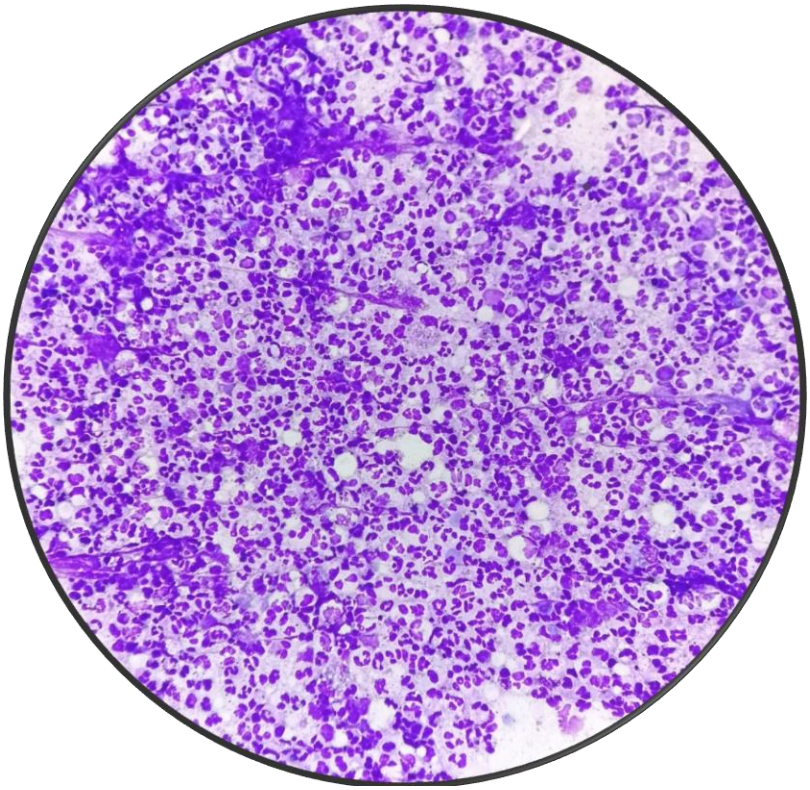


Figure:20 (Giemsa,X400)- Cytological smear showing dense neutrophilic infiltration in the background of suppurative necrosis

Figure:21: Figure showing Fine Needle Aspirated material in a needle hub

Figure:22- Figure showing Gene Xpert MTB/RIF machine along with Cartridge.

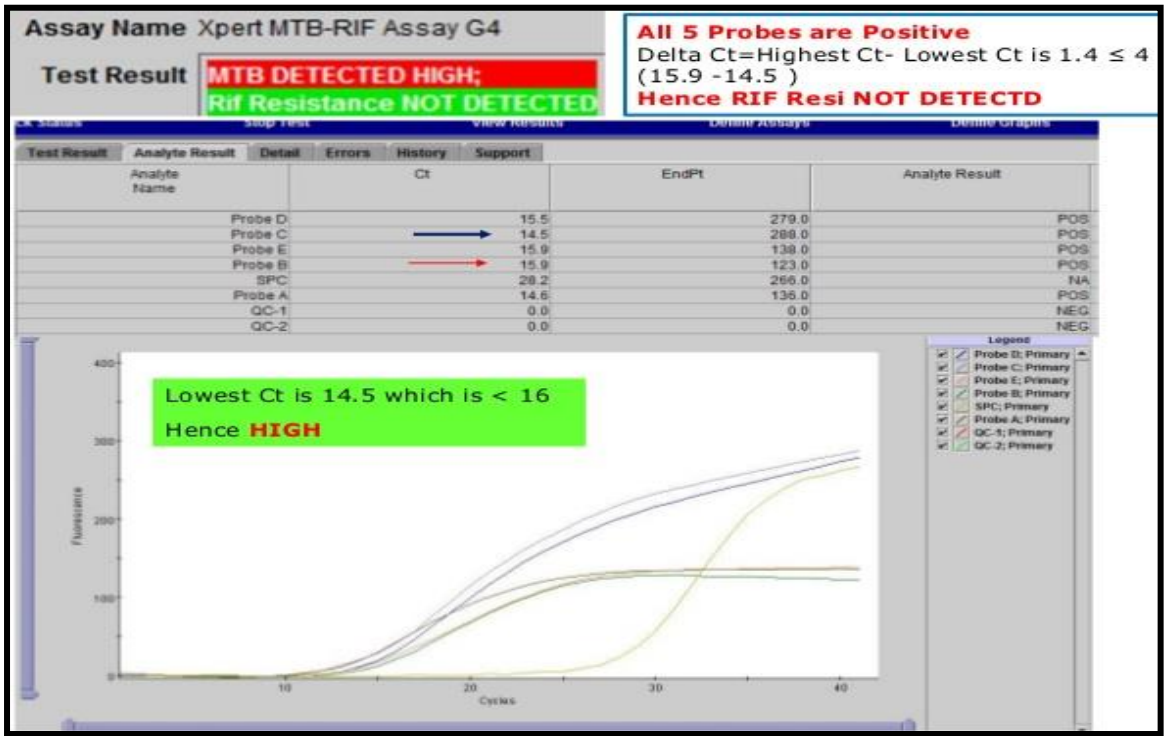


Figure:23- Figure showing Report generated by Gene Xpert MTB/RIF machine for the given sample.(MTB detected, Rifampicin sensitive)

Discussion

Tuberculosis (TB) is with humankind since ancient times and has caused many deaths in the last 200 years. It has caused more deaths when compared to other disease with infective etiology.[50] Worldwide, among the top 10 causes of death, TB is one of it causing many deaths from a single etiology.[1] India has the highest TB burden according to the reports estimated in global TB report 2016.[76]

Due to the infective nature, complicated immune response, chronicity of the disease and need for accurate treatment, Tuberculosis is always considered as a major health burden among the countries with high prevalence of tuberculosis such as developing countries like, India. With the evolution of multidrug resistance forms of Tuberculosis (MDR-TB) and HIV-TB co-infection, again it has become a challenge to the human society.[80]

Multiple organs are affected from this infectious disease and most often lungs. Lymph nodes are the most common organ to be affected in the extra-pulmonary form of TB (EPTB). The diagnosis of the disease is easy when the disease is florid. But the difficulty comes in diagnosis once disease affects extra-pulmonary sites.

Numerous methods have been used for the diagnosis of EPTB. Microscopic demonstration of granulomas, caseous necrosis and Langhan's type of giant cells is diagnostic. Since many viral infections, fungal infections also show similar morphology, further methods for the confirmation of the disease is to be done. Other laboratory methods play a major role in such condition.

Since many years, Ziehl-Neelsen (ZN) stain is in use for the detection of AFB in extra-pulmonary sites because of its easy availability and low cost. But this test has very less sensitivity. Culture is the gold standard for the detection of AFB. Because it is time-consuming, it delays in diagnosis and treatment. In search of newer methods, Auramine-Rhodamine (AR) stain (Fluorescent stain) and Cartridge Based Nucleic Acid Testing (CBNAAT) e.g.,Gene Xpert MTB/RIF assay were explored. Because of accuracy and rapidity, these modalities are helpful in

early detection of TB and the initiation of anti-tubercular treatment especially in developing countries like India. Also Gene Xpert MTB/RIF assay detects Rifampicin resistance strain, thus detecting Rif-resistant TB within no time. ZN stain can detect a minimum of 5000-10000/ml of bacillary load in the FNA sample.[77] Whereas AR stain can detect bacillary load of 500-1000/ml[59] and Gene Xpert MTB/RIF test detects 130-150CFU/ml of bacillary load in the given sample. [66]

Any age group can be affected by tuberculosis. In the present study, youngest patient was 5 months old and oldest patient was 82 years old. The mean age being 30.6 years. (Table 4) Similarly, in the studies conducted by Soumitesh *et al*[64], Lalitharani *et al*[77], Krishna *et al*[57], Brijesh *et al*[2] and Joshi *et al*[78] also showed that incidence of TB was seen most commonly in the 3rd decade of life.

In the present study, slight male preponderance (53.7%) was seen which was not statistically significant (Table 5). Similar result was seen in a study conducted by Soumitesh *et al*[64].

In the present study, cervical region was the most commonly affected site involved in 70.3% (76) of cases (Table 6). Studies by Vikas *et al*[69], Lalitharani *et al*[77], Brijesh *et al*[2], Krishna *et al*[57] and Joshi *et al*[78] also showed cervical region was the most commonly affected site.

The predominant cytomorphological pattern encountered in the current study was found to be granulomatous inflammation comprised of clusters of epithelioid cells with or without Langhan's giant cells, with or without caseous necrosis accounting for 56.5%(56) of cases. This was seen in cordance with the studies conducted by Syed *et al*[72] showing 98.4% and Lalitharani *et al*[77] showing 72.3% of cases with predominantly granulomas as a cytomorphological pattern. (Table 7)

Table-19: Comparison chart of Predominant Cytomorphological pattern in the present study with other studies.

Different Studies	Cases (Percentage, %)	Predominant Cytomorphological Pattern
Syed *et al*[72]	123 (98.4%)	Granulomatous inflammation
Lalitharani *et al*[77]	128 (72.3%)	Granulomatous inflammation
Krishna *et al*[57]	75 (85.2%)	Granulomatous inflammation
Brijesh *et al*[2]	90 (62.1%)	Caseating necrotizing
Present study	**61 (56.5%)**	**Granulomatous inflammation**

In the study conducted by Brijesh *et al*[2], it was mentioned that predominant fine needle aspirate material was blood mixed (51.1%) and rest cases showed pus aspirate. Similarly in the present study, most common fine needle aspirate was blood mixed (54.6%) followed by pus (32.4%) and blood mixed pus aspirate (13%) (Table 19). Among ZN stain, AR stain and Gene Xpert MTB/RIF test positive cases, predominant aspirate was pus (ZN stain-65%, AR stain-55.8%, GX-42.6%) followed by blood mixed (ZN stain-20%, AR stain-26.4%, GX-37.7%) and blood mixed pus (ZN stain- 15%, AR stain-17.6%, GX-19.6%). Type of FN aspirate also plays an important role. Present study showed that when the aspirate was pus or pus-like material, rate of detection of TB increased. (Table 9 and Figure 5)

Kumar *et al*[79] studied fine needle aspirate of 226 cases which were suspicious for TB. They examined both ZN stained and AR stained smears for acid-fast bacilli. AFB positivity for ZN stain was found to be 33.5% and for AR stain it was found to be 45.5%. They found that AR stain was superior to conventional ZN stain in detection of AFB. Also in the study conducted by Krishna *et al*[57], it was mentioned that smear positivity for AFB on ZN stain, AR stain and AF stain was 37.5%, 81.8% and 86.3% respectively.

Similarly in the present study, 18.5% (20) of cases were positive for both ZN stain and AR stain. Whereas an additional 13%(14) of cases were positive only for AR stain which was failed to get picked up by ZN stain. Thus ZN stain showed sensitivity of 58.8% and Negative predictive value of 84% when compared to AR stain. This concludes that AR stain is superior to ZN stain. In other studies conducted by Brijesh *et al*[2], Vamseedhar *et al*[58], Vikas *et al*[69] and Roma *et al*[55], concluded that AR stain has higher sensitivity when compared to conventional ZN stain as mentioned in the table 20.

Table-20:Comparison chart of AR stain and ZN stain in the present study with other studies.

Different Studies	**ZN stain**	**AR stain**
Kumar *et al*[79]	33.5%	45.5%
Krishna *et al*[57]	37.5%	81.82%
Brijesh *et al*[2]	26.7%	34.4%
Vamseedhar *et al*[58]	44.1%	81.37%
Vikas *et al*[69]	36.5%	51.3%
Roma *et al*[55]	7.4%	14.69%
Present study	**18.5%**	**31.5%**

In a study conducted by Mespa *et al*[63], 43 cases suspicious for TB was studied. Out of 43, only 2.3%(1) of case was positive on ZN stain, 23.3%(10) of cases was positive for Gene Xpert MTB/RIF test and 58.1%(25) of cases were detected on cytomorphology. This showed that ZN stain carried the least sensitivity of 4.9% when compared to Gene Xpert MTB/RIF which showed a sensitivity of 25.6%.

Similarly in the present study, we examined 108 cases suspicious for TB. Out of which 18.5% (20) of cases showed positive for ZN stain and 56.5% (61) of cases were positive for Gene Xpert test (Table 12 and 14). All cases were rifampicin sensitive on Gene Xpert assay. ZN stain showed sensitivity of 32.7% and a negative predictive value of 53.41%.

When AR stain and Gene Xpert test was combined, 31.4% (34) cases were positive for both AR stain and Gene Xpert test. 25% (27) of additional cases which were AR stain negative showed positive for Gene Xpert test. With this, AR stain showed sensitivity of 32.8% and negative predictive value of 53.41%. Thus more cases were picked up by Gene Xpert MTB/RIF assay making it most sensitive. On combining ZN stain, AR stain and Gene Xpert assay, definitely the number of true positive cases were increased.

In the present study, we found that 9.2% (10/108) cases showing granulomas were found to be negative for ZN stain, AR stain and Gene Xpert MTB/RIF test. This could be because of faulty FNAC technique yielding scant aspirate or due to hemorrhagic aspirate or due to very low TB bacillary load. Presence of blood components in the FNA aspirate may act as inhibitors for nucleic acid amplification which may be the reason for false-negative Gene Xpert results. Similarly, in a study conducted by Sunil *et al*[70], 7.9% (23/146) cases did not correlate with FNAC and Gene Xpert MTB/RIF test.

Recommendations

Based on the above facts and observations, following points are recommended for the diagnosis of tuberculosis in the patients having suspicious of EPTB.

1. FNAC technique should be used as an initial, essential tool in diagnosis of EPTB cases.
2. Adequate fine needle aspirate material should be obtained for the accurate result and for the evidence of tuberculosis either in cytomorphological feature or in demonstration of TB bacilli.
3. Blood free aspirate should be preferred over blood-mixed/contaminated aspirate as it may interfere with CBNAAT results, because of possible inhibitors.
4. Judicial use of FNA material should be done for ZN stain, AR stain and Gene Xpert MTB/RIF assay
5. Gene Xpert MTB/RIF assay is the best method for the rapid and accurate detection of TB bacilli and should be used where facility is available, followed by AR stain and ZN stain.

CONCLUSION

Diagnosis of Tuberculosis (TB) solely on the history, clinical examination and radiological evaluation is difficult. It requires cytomorphological demonstration of granulomas, with or without giant cells, with or without caseous necrosis and demonstration of tubercle bacilli by special methods.

The morphologic spectrum in Extrapulmonary Tuberculosis (EPTB) differs based on the stage of the disease and immunity of the host. It varies from well-formed granulomas to scattered epithelioid cells. In such cases, giving a definite diagnosis would be difficult.

In developing countries like India, where the burden of TB is high, early diagnosis and rapid initiation of treatment will be of high priority. Fine needle aspiration cytology (FNAC) being a rapid and OPD procedure, its application in the diagnosis of EPTB has made convenient and easy. Since TB lymphadenitis is the most common form of EPTB, problem arises when the aspirate shows polymorphous population of lymphocytes with singly scattered epithelioid cells in the absence of giant cells and caseous necrosis. In such conditions, diagnosis of TB infection becomes difficult.

Routinely done Ziehl-Neelsen(ZN) stain can demonstrate acid-fast bacilli (AFB), but carries low sensitivity. It requires minimum of 10^4 bacilli/ml to be visible in the ZN stained smears. Culture is the gold standard test for the isolation of the bacilli. But it takes longer time. Thus there is a need for advanced test which is rapid and accurate in demonstrating the tubercle bacilli.

Hence in the present study, we used AR stain and Gene Xpert MTB/RIF test simultaneously on fine needle aspirate. Auramine- Rhodamine (AR) stain is a fluorescent stain which can detect AFB when the load is less. Problem arises when cytomorphology showing only necrosis. In such scenarios, AR stain plays an important role in picking up the AFB. On AR stain, bacilli are easily detectable under low power view and at the same time, large area can be covered in a short duration of time. Thus AR stain is considered superior to ZN stain.

Gene Xpert MTB/RIF test, as recommended by WHO, it is considered as an initial diagnostic tool in patients suspected to have TB. In the current study, the remnant of FNA was subjected for the Gene Xpert MTB/RIF assay. This test detects live as well as dead bacilli and with the minimum bacillary load of 130-150cfu/ml. Gene Xpert MTB/RIF is a CBNAAT machine takes about 4 hours to obtain the result. This test not only gives information about the presence of Mycobacteria tuberculosis bacilli, but also gives information about rifampicin-resistant strain which helps in the treatment management.

Thus with the combined application of AR stain and Gene Xpert MTB/RIF assay, we can detect TB at the earliest and can help in the initiation of treatment. Inadequate FNA material, hemorrhagic material and low TB bacillary load can give false-negative results. Utmost care should be taken to overcome these disadvantages by proper technique of FNA procedure, and judicial use of aspirate material.

SUMMARY

Tuberculosis is seen affecting any part of the body. Extrapulmonary tuberculosis poses many difficulties in its diagnosis. Careful examination and proper use of laboratory tests are necessary for the diagnosis of EPTB. In view of this, the present study was undertaken in Department of Pathology at BLDE (Deemed to be University), Shri B M Patil Medical College, Hospital and Research Centre, Vijayapura from 1st December 2017 – 30th June 2019.

A total of 4641 FNAC's were done, among which 108 cases was found to be suspicious for tuberculosis. Out of 108 cases, predominant age group involved in the present study was 20-30 years (30.6%), followed by 30-40 years (23.1%). Slight male preponderance was seen in the study (Male-53.7%, Female-46.3%). The most common site involved in this study was cervical region accounting for 70.3%. Predominantly, aspirated FNA material was blood mixed (54.6%), followed by pus (32.4%) and blood mixed pus (13%).

Out of 108 cases, 61 (56.4%) cases showed cytomorphological pattern of granulomatous inflammation followed by 29 (26.8%) cases showing reactive lymphadenitis. 9(8.3%) cases showed suppurative inflammation and 9 (8.3%) cases showed caseous necrosis.

A total of 20 (18.5%) cases was diagnosed as TB on ZN stain, 34 (31.4%) cases on AR stain and 61 (56.4%) cases on Gene Xpert MTB/RIF assay. Thus, ZN stain showed less sensitivity of 32.7%. Combined use of AR stain and Gene Xpert MTB/RIF assay was able to pick up 31.4% (34) more cases of TB which was not found on conventional ZN stain. Gene Xpert assay picked up 25% additional cases of TB which were negative on AR stain.

BIBLIOGRAPHY

1. World Health Organization. Global tuberculosis report 2018. Geneva, Switzerland: World Health Organization; 2018. https://www.who.int/tb/publications/global_report/en/external icon.
2. Thakur B, Mehrotra R, Nigam J. Correlation of Various Techniques in Diagnosis of Tuberculous Lymphadenitis on Fine Needle Aspiration Cytology. Pathology Research International. 2013;2013(6):1- 4.
3. Chandrappa N, Rastogi A, Bhatnagar A. Cartridge based nucleic acid amplification test is superior in diagnosing lymphnode tuberculosis. Indian Journal of Tuberculosis. 2019;66(3):402-406.
4. Mittal P, Handa U, Mohan H, Gupta V. Comparative evaluation of fine needle aspiration cytology, culture, and PCR in diagnosis of tuberculous lymphadenitis. Diagnostic Cytopathology. 2010;39(11):822-826.
5. Damor P, Thakor N, Baranda U, Gadhavi R, Patel N, Thakkar D *et al*. Human immunodeficiency virus associated tuberculous lymphadenitis: a clinical study of 50 cases of Saurastra region of Gujarat, India. International Journal of Advances in Medicine. 2015;2(2):100.
6. Daniel TM. Rapid diagnosis of tuberculosis: Laboratory techniques applicable in developing countries. Rev Infect Dis 1989;2:471-8.
7. Balows A, Hausler WJ, Herrmann KL, Shadomy HJ. In: Manual of clinical Microbiology. 5th ed. American Society for Microbiology. Washington: D.C: 1991. p. 308-11.
8. Daniel T. Rapid Diagnosis of Tuberculosis: Laboratory Techniques Applicable in Developing Countries. Clinical Infectious Diseases. 1989;11(Supplement 2):S471-S478.
9. Githui W, Matu S, Muthami L, Juma E. Improved diagnosis of Ziehl-Neelsen smear negative tuberculosis using sodium hypochlorite sedementation method. East African Medical Journal. 2008;84(10):455-459.

10. Seth V, Kabra S. History of Tuberculosis. In: Seth V, Kabra SK. Essentials of tuberculosis in children. 4th ed. New Delhi: Jaypee Bros. Medical Publishers; 2011.p.3-6.
11. Iseman MD. Extra-pulmonary tuberculosis in adults. In: Iseman MD, ed. A clinician's guide to tuberculosis. Philadelphia: Lippincott Williams & Wilkins, 2000: 145-197.
12. Grzybowski S, Allen EA. History and importance of scrofula. Lancet 1995; 346: 1472-4.
13. Sharma SK, Mohan A. Extra pulmonary tuberculosis. Indian J Med Research 2004; 120:316-53.
14. Friedland JS. Tuberculosis. In: Armstrong D, Cohlen J, editors. Infectious Diseases. 1st ed. Philadelphia: Mosby; 1999.p.2.30.1-2.30.16.
15. Set R, Bankar S, Sharma D, Shah D, Shastri J. Performance of Xpert MTB/RIF for detection of Mycobacterium tuberculosis and rifampicin resistance in pus aspirates. Indian Journal of Tuberculosis. 2018.
16. Brown H, Abbitt P, Wilkinson E. Diagnosis of Clinically Unsuspected Extrapulmonary Tuberculosis by Fine Needle Aspiration. Acta Cytologica. 2001;45(6):1032-1036.
17. Lee J. Diagnosis and Treatment of Extrapulmonary Tuberculosis. Tuberculosis and Respiratory Diseases. 2015;78(2):47.
18. Gupta P R. Difficulties in managing lymph node tuberculosis. Lung India 2004;21:50-3
19. Alamelu Raja. Immunology of tuberculosis. Indian J Med Res. 2004 Oct;120(4):213-32.
20. Robbins S, Cotran R, Kumar V, Abbas A, Aster J. Infectious diseases. In: McAdam AJ, Milner DA, Sharpe AH, editors. Pathologic basis of disease. 9th ed. Philadelphia, PA: Saunders Elsevier; 2015.p.371-7.
21. Rathan A, Singhal S, Sood R, Rao M. Essentials of Tuberculosis in children. 2nd ed. New Delhi; Jaypee brothers 2001; 41-9.
22. Seth V, Kabra S. Immunology of Tuberculosis: Basic Aspects and Relevance for Immunodiagnostic tests. In: Syre H, Grewal MS. Essentials of tuberculosis in children. 4th ed. New Delhi: Jaypee Bros. Medical Publishers; 2011.p.66-83.

23. Robbins S, Cotran R, Kumar V, Abbas A, Aster J. Infectious diseases. In: McAdam AJ, Milner DA, Sharpe AH, editors. Pathologic basis of disease. 9th ed. Philadelphia, PA: Saunders Elsevier; 2015.p.371.
24. Grosset J, Chaisson R. Handbook of Tuberculosis. Cham: Springer International Publishing; 2017.p.20-3.
25. Davies P, Gordon S, Davies G. Clinical tuberculosis. 5th ed. Boca Raton: CRC Press; 2014: p.63.
26. Davies P, Gordon S, Davies G. Clinical tuberculosis. 5th ed. Boca Raton: CRC Press; 2014: p.134-6.
27. Davies P, Gordon S, Davies G. Clinical tuberculosis. 5th ed. Boca Raton: CRC Press; 2014: p.169-170.
28. Garg R, Somvanshi D. Spinal tuberculosis: A review. The Journal of Spinal Cord Medicine. 2011;34(5):440-454.
29. Davies P, Gordon S, Davies G. Clinical tuberculosis. 5th ed. Boca Raton: CRC Press; 2014: p.170-3.
30. Jones P, Campbell P. Tuberculous lymphadenitis in childhood: The significance of anonymous mycobacteria. British Journal of Surgery. 1962;50(221):302-314.
31. Raviglione M. Tuberculosis : The essentials. 4th ed. Florida: CRC Press; 2010:p.146-7.
32. Walker N, Meintjes G, Wilkinson R. HIV-1 and the immune response to TB. Future Virology. 2013;8(1):57-80.
33. Jagirdar J and Zagzag D. Pathology and insight into pathogenesis of tuberculosis: Clinical microbiology 1996; 467-491.
34. Orell S, Sterrett GF. Introduction. In: Orell S, Sterrett GF, editors. Orell and Sterrett's Fine Needle Aspiration Cytology . 5th ed. U.K, London: Elsevier; 2012.p.1-3.

35. Shapiro A, Pincus R. Fine-Needle Aspiration of Diffuse Cervical Lymphadenopathy in Patients with Acquired Immunodeficiency Syndrome. Otolaryngology–Head and Neck Surgery. 1991;105(3):419-421.
36. Gupta R, Naran S, Lallu S, Fauck R. The diagnostic value of fine needle aspiration cytology (FNAC) in the assessment of palpable supraclavicular lymph nodes: a study of 218 cases. Cytopathology. 2003;14(4):201-207.
37. Das D. Fine-Needle Aspiration Cytology in the Diagnosis of Tuberculous Lesions. Laboratory Medicine. 2000;31(11):625-632.
38. Sulis G, Roggi A, Matteelli A, Raviglione M. Tuberculosis: Epidemiology And Control. Mediterranean Journal of Hematology and Infectious Diseases. 2014;6(1):e2014070.
39. Shahabuddin MD, Raghuveer CV. Role of fine needle aspiration cytology in detecting extra pulmonary tuberculosis. J of cytology 2003; 77-8.
40. Moore D, Evans C, Gilman R, Caviedes L, Coronel J, Vivar A *et al*. Microscopic-Observation Drug-Susceptibility Assay for the Diagnosis of TB. New England Journal of Medicine. 2006;355(15):1539-1550.
41. WHO monitoring of Xpert MTB/RIF roll-out [Internet]. World Health Organization. 2017 [cited 9 October 2017]. Available from: http://www.who.int/tb/areas-of-work/laboratory/mtb-rif-rollout/en/
42. Minion J, Sohn H, Pai M. Light-emitting diode technologies for TB diagnosis: what is on the market?. Expert Review of Medical Devices. 2009;6(4):341-345.
43. Lau S, Wei W, Hsu C, Engzell U. Efficacy of fine needle aspiration cytology in the diagnosis of tuberculous cervical lymphadenopathy. The Journal of Laryngology & Otology. 1990;104(1):24-27.
44. Hemalatha A, Shruti P, Kumar M, Bhaskaran A. Cytomorphological patterns of tubercular lymphadenitis revisited. Annals of Medical and Health Sciences Research. 2014;4(3):393-6.

45. Rajpal S, Dhingra VK and Aggarwal JK. Sputum grading as predictor of treatment outcome in pulmonary tuberculosis. J of cytology 2003; 77-8.
46. Handa U, Mohan H, Bal A. Role of fine needle aspiration cytology in evaluation of paediatric lymphadenopathy. Cytopathology. 2003;14(2):66-69.
47. Prasoon D. Tuberculosis of the Intercostal Lymph Nodes. Acta Cytologica. 2003;47(1):51-55.
48. Narang S, Solanki A, Kashyap S, Rani L. Utility of fine needle aspiration cytology to comprehend the pathogenesis of extrapulmonary tuberculosis. Diagnostic Cytopathology. 2015;44(2):98-102.
49. Kochhar K, Patel B, Shah M. Pattern of Lymphadenopathy on Fine Needle Aspiration Cytology of Superficial Lymph Nodes (A Study of 150 Cases). Journal of Advance Researches in Biological Sciences. 2012; 4: 288-292.
50. Caws M, Marais B, Heemskerk D, Farrar J. Tuberculosis in Adults and Children. New York: Springer; 2015.pg.27-31.
51. Winn WC, Allen SD, Janda WM, Koneman E, Schreckenberger PC. Koneman's color atlas and textbook of diagnostic microbiology. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2006:1065-124.
52. RNTCP at a Glance | Tuberculosis | Public Health [Internet]. Scribd. 2019. Available from: https://www.scribd.com/doc/53225954/RNTCP-at-a-Glance.
53. Khabnani H, Munjal K. Application of bleach method in diagnosis of extra-pulmonary tuberculosis. Indian J Pathol Microbiol 2005; 546-50.
54. Chattopadhyay S, Ghosh T, Saha S, Mondai SK, Ghosh AK. Tuberculous lymphadenitis - A changing face. J of cytology 2006;156-7.
55. Goyal R, Kumar A. A Comparison of Ziehl-Neelsen Staining and Fluorescent Microscopy for Diagnosis of Pulmonary Tuberculosis. IOSR Journal of Dental and Medical Sciences. 2013;8(5):05-08.

56. Laifangbam S, Singh H, Singh N, Devi K, Singh N. A comparative study of fluorescent microscopy with Ziehl-Neelsen staining and culture for the diagnosis of pulmonary tuberculosis. Kathmandu University Medical Journal. 1970;7(3):226-230.
57. Krishna M, Kumar A. Tuberculous mycobacteria bacilli fluorescence and compare with Ziehl-Neelsen stain in fine-needle aspiration cytology of tubercular lymphnode. Int J Otorhinolaryngol Head Neck Surg 2016;2:66-9.
58. Annam V, Kulkarni M, Puranik R. Comparison of the modified fluorescent method and conventional Ziehl-Neelsen method in the detection of acidfast bacilli in lymphnode aspirates. CytoJournal. 2009;6(1):13.
59. Mamilla R, Suhasini S. Sensitivity of FM Staining Versus Z-N Staining In Diagnosing Sputum Smear Positive PTB. IOSR Journal of Dental and Medical Science. 2015;14(3):29-33.
60. Dzodanu E, Afrifa J, Acheampong D, Dadzie I. Diagnostic Yield of Fluorescence and Ziehl-Neelsen Staining Techniques in the Diagnosis of Pulmonary Tuberculosis: A Comparative Study in a District Health Facility. Tuberculosis Research and Treatment. 2019;2019:1-6.
61. Lawn S, Nicol M. Xpert®MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. Future Microbiology. 2011;6(9):1067-1082.
62. World Health Organization. Global Tuberculosis Report 2014.Geneva.World Health Organization.2014
63. Manyepa M, Rogena E, Oyugi J, Rioki J, Ngayo M. Utility of GeneXpert and Cytomorphology in the Diagnosis of Extrapulmonary Tuberculosis at Kenyatta National Hospital in Kenya. 2019.
64. Chakravorty S, Sen M, Tyagi J. Diagnosis of Extrapulmonary Tuberculosis by Smear, Culture, and PCR Using Universal Sample Processing Technology. Journal of Clinical Microbiology. 2005;43(9):4357-4362.
65. Sahana K, Prabhu A, Saldanha P. Usage of Cartridge Based Nucleic Acid Amplification Test (CB-NAAT/GeneXpert) test as diagnostic modality for pediatric tuberculosis; case series from

Mangalore, South India. Journal of Clinical Tuberculosis and Other Mycobacterial Diseases. 2018;11:7-9.

66. M Agrawal, A Bajaj, V Bhatia, *et al.*Comparative study of Gene Xpert with ZN stain and culture in samples of suspected pulmonary tuberculosis. J Clin Diagn Res. 2016; 10 (5): DC09-DC12
67. Xpert MTB/RIF implementation manual: technical and operational 'how-to': practical considerations. WHO. 2014.p.04. http://apps.who.int/iris/bitstream/10665/112469/1/9789241506700_eng.pdf
68. Xpert MTB/RIF implementation manual: technical and operational 'how-to': practical considerations. WHO. 2014. http://apps.who.int/iris/bitstream/10665/112469/1/9789241506700_eng.pdf
69. Dagar V, Heda S, Barsagade A, Mahore S, Ambhore N, Karyakarte R *et al*. Comparision of ZN Staining and Fluorescent Microscopy in Detection of Acid Fast Bacilli in Fine Needle Aspiration Smears. IOSR Journal of Dental and Medical Sciences. 2016;15(08):79-84.
70. Komanapalli S, Prasad U, Atla B, Nammi V, Yendluri D. Role of CB-NAAT in diagnosing extra pulmonary tuberculosis in correlation with FNA in a tertiary care center. International Journal of Research in Medical Sciences. 2018;6(12):4039.
71. Maynard-Smith L, Larke N, Peters J, Lawn S. Diagnostic accuracy of the Xpert MTB/RIF assay for extrapulmonary and pulmonary tuberculosis when testing non-respiratory samples: a systematic review. BMC Infectious Diseases. 2014;14(1):709.
72. Yasin S, Khan S, Khan F. Utility of Fine Needle Aspiration Cytology in the Diagnosis of Extrapulmonary Tuberculosis: Study at a Tertiary Care Centre of Kashmir Valley. International Journal of Contemporary Medical Research [IJCMR]. 2018;5(4):D8-D10.
73. Momin M, G A, Aluri A, S S. Comparative Analysis of Gene Expert Assay In Addition To Z-N Microscopy for Detection of Extra -Pulmonary TB in A Fine Needle Aspiration Samples. Scholars Journal of Applied Medical Sciences (SJAMS). 2017;5(7D):2803-8.

74. Ghariani A, Jaouadi T, Smaoui S, Mehiri E, Marouane C, Kammoun S *et al*. Diagnosis of lymph node Tuberculosis using the GeneXpert MTB/RIF in Tunisia. International Journal of Mycobacteriology. 2015;4(4):270-275.

75. Biadglegne F, Mulu A, Rodloff AC, Sack U. Diagnostic performance of the Xpert MTB/RIF assay for Tuberculous Lymphadenitis on Fine Needle Aspirates from Ethiopia. Tuberculosis. 2014;94(5):502-505.

76. India M. TB India 2017 :: Central TB Division [Internet]. Tbcindia.gov.in. 2017 [cited 21 September 2019]. Available from: https://tbcindia.gov.in/index1.php?lang=1&level=2&sublinkid=4728&lid=3275

77. N Lalitharani, V Bagiyalakshmi. Incidence of Tuberculosis among cases of peripheral lymphadenopathies and techniques useful in the diagnosis of Tuberculous lymphadenitis by FNAC- Our experience from a tertiary health care centre. Indian Journal Of Applied Research. 2017;7(6).

78. Joshi P, Singh M, Bhargava A, Singh M, Mehrotra R. Autofluorescence-an important ancillary technique for the detection ofMycobacterium tuberculosis: Revisited. Diagnostic Cytopathology. 2012;41(4):330-334.

79. Kumar N, Tiwari MC, Verma K. AFB staining in cytodiagnosis of tuberculosis without classical features: a comparison of Ziehl-Neelsen and fluorescent methods. Cytopathology.1998;9:208-14.

80. Barberis I, Bragazzi NL, Galluzzo L, Martini M. The history of tuberculosis: from the first historical records to the isolation of Koch's bacillus. J Prev Med Hyg. 2017;58:E9-12.

Annexure – I

INSTITUTIONAL ETHICAL COMMITTEE CLEARANCE CERTIFICATE

Annexure - II

SHRI B.M.PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTER, VIJAYAPURA-586103

INFORMED CONSENT FOR PARTICIPATION IN DISSERTATION/RESEARCH

I, the undersigned, S/O D/O W/O ----------, aged ------years, ordinarily resident of ------ do hereby state/declare that _____ of Shri B. M. Patil Medical college, Hospital & Research Centre has examined me thoroughly on at (place) and it has been explained to me in my own language that I am suffering from disease (condition) and this disease/condition mimic following diseases . Further Doctor informed me that she is conducting dissertation/research titled "**Utility of Auramine-Rhodamine Stain and Gene Xpert PCR in Diagnosis of Tuberculosis during FNAC procedure**" under the guidance of _____ requesting my participation in the study. Apart from routine treatment procedure the pre-operative, operative, post-operative and follow-up observations will be utilized for the study as reference data.

Doctor has also informed me that during conduct of this procedure__like adverse results may be encountered. Among the procedure related complications most of them are treatable but are not anticipated. Further Doctor has informed me that my participation in this study help in evaluation of the results of the study which is useful reference to treatment of other similar cases in near future, and also I may be benefited in getting relieved of suffering or cure of the disease I am suffering.

The Doctor has also informed me that information given by me, observations made/ photographs/ video graphs taken upon by the investigator will be kept secret and not accessed by the person other than me or my legal hirer except for academic purposes.

The Doctor did inform me that though my participation is purely voluntary, based on information given by me, I can ask any clarification during the course of treatment / study related to diagnosis, procedure of treatment, result of treatment or prognosis. At the same time I have been informed that I can withdraw from this study at any time if I want or the investigator can terminate me from the study at any time from the study but not the procedure of treatment and follow-up unless I request to be discharged.

After understanding the nature of dissertation or research, diagnosis made, mode of treatment, I the undersigned Shri/Smt_____under my full conscious state of mind agree to participate in the said research/dissertation.

Signature of patient:

Signature of doctor:

Witness: 1.

2.

Date:

Place

Annexure – III

PROFORMA FOR STUDY:

Demographic details:

Name:

Age:

Sex: M/F

Occupation:

Residence:

Contact no:

OPD/IP NO:

FNAC. No.:

Chief complaints:

Clinical findings:

Local examination:

Laboratory investigations:

a. Hematological-

b. Microscopy- FNAC

i)ZN stain for AFB-

ii)Auramine Rhodamine stain-

c. Special investigation- Gene Expert:

d. Rifampicin resistance- if present:

Morphological diagnosis:

Molecular diagnosis:

KEY TO MASTERCHART

SL No. - Serial Number

OP No. - Out Patient Number

IP No. - In Patient Number

M – Male; F – Female; Y – Years; M- Months

FNAC no- Fine Needle Aspiration number

FN aspirate- Fine needle aspirate

ZN stain- Zeihl-Neelsen stain

AR stain- Auramine-Rhodamine stain

PCR- Polymerase Chain Reaction

RL- Reactive lymphadenitis

GI s/o TB- Granulomatous inflammation suggestive of Tuberculosis

GL- Granulomatous Lymphadenitis

NL- Necrotising Lymphadenitis

NGL- Necrotising Granulomatous Lymphadenitis

NI- Necrotising inflammation

NGI- Necrotising granulomatous inflammation

TL- Tubercular lymphadenitis

SI- Suppurative inflammation

NSI- Necrotising suppurative inflammation

GM- Granulomatous mastitis

TI- Tubercular inflammation

MASTERCHART

Sl no	OP/IP no	FNAC no	Age		Sex	Site of lesion	Type of FN aspirate	Cytomorphological diagnosis	Granuloma	Necrosis	ZN stain	AR stain	Gene Xpert (PCR)
1	433195	4193/17	40	Y	F	Lt cervical	Pus like	RL	Not seen	Not seen	Negative	Negative	Negative
2	425216	4194/17	25	Y	F	Lt cervical	Blood mixed	RL	Not seen	Not seen	Negative	Negative	Negative
3	1991	012/18	7	Y	M	Lt cervical	Blood mxed	RL	Not seen	Not seen	Negative	Negative	Negative
4	147	011/18	23	Y	F	Lt inguinal	Pus like	GL	Seen	Seen	Negative	Negative	Positive
5	4508	35/18	16	Y	F	Lt cervical	Blood mixed	RL	Not seen	Not seen	Negative	Negative	Negative
6	6271	59/18	25	Y	F	Lt breast	Pus	GI s/o TB	Seen	Not seen	Negative	Positive	Positive
7	100	61/18	82	Y	M	Lt supraclavicular	Blood mixed	RL	Not seen	Not seen	Negative	Negative	Negative
8	9562	82/18	56	Y	F	Cervical	Blood mixed	RL	Not seen	Not seen	Negative	Negative	Negative
9	17618	138/18	25	Y	F	Rt cervical	Blood mixed	RL	Not seen	Not seen	Negative	Negative	Negative
10	23716	219/18	45	Y	F	Cervical	Blood mixed	RL	Not seen	Not seen	Negative	Negative	Negative
11	49215	358/18	40	Y	M	Rt cervical	Blood mixed	GL s/o TB	Seen	Not seen	Negative	Negative	Negative
12	50672	359/18	45	Y	M	Rt cervical	Blood mixed	GL s/o TB	Seen	Not seen	Negative	Negative	Positive
13	63712	431/18	30	Y	F	Rt cervical	Blood mixed pus	GL s/o TB	Seen	Not seen	Negative	Negative	Positive
14	65830	438/18	32	Y	M	Rt cervical	Blood mixed pus	GL	Seen	Not seen	Negative	Negative	Negative
15	72261	473/18	24	Y	F	Lt cervical	Blood mixed	GL	Seen	Not seen	Negative	Negative	Positive
16	8055	573/18	22	Y	M	Rt cervical	Blood mixed pus	NL	Not seen	Seen	Positive	Positive	Positive
17	93702	575/18	50	Y	F	Rt breast	Blood mixed	GI	Seen	Not seen	Negative	Negative	Negative
18	96505	586/18	25	Y	M	Rt cervical	Blood mixed pus	NGL	Seen	Seen	Negative	Positive	Positive
19	99983	606/18	17	Y	F	Rt axilla	Blood mixed	GL s/o TB	Seen	Not seen	Negative	Positive	Positive
20	105081	627/18	23	Y	M	Rt inguinal	Pus	NI	Not seen	Seen	Negative	Positive	Positive
21	10292	694/18	20	Y	M	Cervical	Blood mixed pus	GL	Seen	Not seen	Negative	Negative	Positive
22	125102	739/18	35	Y	M	Cervical	Blood mixed pus	NGI	Seen	Seen	Negative	Negative	Negative
23	127705	768/18	26	Y	M	Rt cervical	Blood mixed	RL	Not seen	Not seen	Negative	Negative	Negative
24	130387	779/18	31	Y	M	Rt cervical	Blood mixed pus	GL s/o TB	Seen	Not seen	Negative	Negative	Positive
25	134245	809/18	7	Y	M	Lt submandibular	Blood mixed pus	GL s/o TB	Seen	Seen	Negative	Negative	Positive

26	161054	978/18	39	Y	M	Cervical	Pus	TL	Not seen	Seen	Positive	Positive	Positive
27	177604	1082/18	3	Y	F	Rt axilla	Hemorrhagic	SI	Not seen	Not seen	Positive	Positive	Positive
28	183211	1132/18	30	Y	M	Lt axilla	Pus	TL	Not seen	Seen	Positive	Positive	Positive
29	183312	1168/18	21	Y	F	Lt supraclavicular	Pus	NSI s/o TB	Seen	Seen	Positive	Positive	Positive
30	194619	1192/18	30	Y	M	Lt cervical	Pus	TL	Seen	Not seen	Positive	Positive	Positive
31	203244	1241/18	27	Y	M	Lt cervical	Hemorrhagic	TL	Seen	Seen	Positive	Positive	Positive
32	205532	1261/18	23	Y	F	Rt cervical	Pus	NL s/o TB	Seen	Seen	Negative	Negative	Positive
33	221990	1352/18	25	Y	F	Rt breast	Blood mixed	GM	Seen	Not seen	Negative	Negative	Negative
34	226504	1386/18	24	Y	F	Cervical	Blood mixed	RL	Not seen	Not seen	Negative	Negative	Negative
35	236609	1438/18	55	Y	M	Rt axillary	Pus	SI	Not seen	Seen	Negative	Negative	Negative
36	243351	1481/18	16	Y	M	Rt cervical	Blood mixed	RL	Not seen	Not seen	Negative	Negative	Negative
37	251944	1534/18	58	Y	M	Rt cervical	Blood mixed	RL	Not seen	Not seen	Negative	Negative	Negative
38	267933	1632/18	5	Y	M	Rt submandibular	Pus	SI	Not seen	Not seen	Negative	Negative	Negative
39	26635	1674/18	65	Y	F	Rt submandibular	Blood mixed	GL	Seen	Not seen	Negative	Negative	Positive
40	275772	1685/18	45	Y	M	Rt cervical	Pus	GL s/o TB	Seen	Seen	Negative	Negative	Positive
41	294742	1784/18	47	Y	M	Lt inguinal	Blood mixed	GL	Seen	Not seen	Negative	Negative	Positive
42	298583	1808/18	17	Y	M	Lt cervical	Blood mixed	NGL s/o TB	Seen	Seen	Negative	Negative	Positive
43	301244	1827/18	40	Y	F	Lt cervical	Blood mixed	GL s/o TB	Seen	Not seen	Negative	Negative	Positive
44	32549	2014/18	25	Y	F	Lt cervical	Blood mixed pus	TL	Seen	Seen	Positive	Positive	Positive
45	32854	2041/18	45	Y	F	Rt inguinal	Blood mixed	SI	Not seen	Not seen	Negative	Negative	Negative
46	369464	2209/18	36	Y	F	Rt cervical	Blood mixed	RL	Not seen	Not seen	Negative	Negative	Negative
47	388119	2311/18	64	Y	M	Rt cervical	Pus	SI	Not seen	Not seen	Negative	Negative	Negative
48	38858	2360/18	38	Y	M	Rt cervical	Blood mixed pus	GL s/o TB	Seen	Seen	Positive	Positive	Positive
49	403758	2387/18	32	Y	M	Lt supraclavicular	Blood mixed	RL	Not seen	Not seen	Negative	Negative	Negative
50	416761	2444/18	22	Y	M	Cervical	Blood mixed	RL	Not seen	Not seen	Negative	Negative	Negative
51	438454	2560/18	32	Y	M	Rt cervical	Pus	TL	Seen	Seen	Positive	Positive	Positive
52	438381	2561/18	32	Y	F	Cervical	Blood mixed	GL	Seen	Not seen	Negative	Negative	Positive
53	452616	2624/18	12	Y	M	Rt cervical	Pus	NGL s/o TB	Seen	Seen	Positive	Positive	Positive

54	4812	34/19	70	Y	F	Lt cervical	Pus	SI	Not seen	Not seen	Negative	Negative	Negative
55	11805	70/19	28	Y	F	Rt cervical	Pus	GL	Seen	Seen	Positive	Positive	Positive
56	13373	77/19	21	Y	F	Rt cervical	Hemorrhagic	GL s/o TB	Seen	Not seen	Negative	Negative	Positive
57	13659	78/19	32	Y	F	Ant chest wall	Pus	NI	Not seen	Seen	Positive	Positive	Positive
58	16772	93/19	30	Y	F	Rt cervical	Blood mixed	GL s/o TB	Seen	Not seen	Negative	Negative	Positive
59	2068	126/19	35	Y	M	Rt cervical	Pus	TL	Not seen	Seen	Positive	Positive	Positive
60	2445	158/19	21	Y	M	Submandibular	Pus	TI	Seen	Seen	Positive	Positive	Positive
61	36207	200/19	5	M	M	Lt supraclavicular	Pus	GL s/o TB	Seen	Seen	Negative	Positive	Positive
62	38573	209/19	30	Y	M	Cervical	Pus	TL	Seen	Seen	Positive	Positive	Positive
63	45738	259/19	22	Y	F	Lt cervical	Pus	TL	Seen	Seen	Positive	Positive	Positive
64	58391	344/19	40	Y	M	Rt cervical	Blood mixed	TL	Seen	Not seen	Positive	Positive	Positive
65	7685	565/19	55	Y	F	Rt breast	Pus	GM	Seen	Not seen	Negative	Negative	Negative
66	92971	588/19	40	Y	M	Rt supraclavicular	Blood mixed	RL	Not seen	Not seen	Negative	Negative	Negative
67	112668	754/19	14	Y	F	Rt cervical	Blood mixed	GL	Seen	Not seen	Negative	Negative	Positive
68	113297	755/19	45	Y	F	Lt supraclavicular	Blood mixed	GL s/o TB	Seen	Seen	Positive	Positive	Positive
69	113483	756/19	6	Y	F	Rt cervical	Blood mixed	GL	Seen	Not seen	Negative	Positive	Positive
70	123424	829/19	39	Y	M	Rt cervical	Blood mixed	GL s/o TB	Seen	Seen	Negative	Positive	Positive
71	123487	830/19	52	Y	F	Lt breast	Blood mixed	SI	Not seen	Not seen	Negative	Negative	Negative
72	133916	914/19	18	Y	M	Cervical	Blood mixed	RL	Not seen	Not seen	Negative	Negative	Negative
73	140077	948/19	45	Y	F	Rt cervical	Blood mixed	RL	Not seen	Not seen	Negative	Negative	Negative
74	149075	1006/19	35	Y	F	Rt cervical	Blood mixed	RL	Not seen	Not seen	Negative	Negative	Negative
75	160175	1064/19	16	Y	M	Submental	Hemorrhagic	RL	Not seen	Not seen	Negative	Negative	Negative
76	168958	1109/19	3	Y	F	Cervical	Pus like	Acute suppurative	Not seen	Not seen	Negative	Negative	Negative
77	169070	1110/19	12	Y	M	Rt cervical	Blood mixed	RL	Not seen	Not seen	Negative	Negative	Negative
78	15168	1114/19	25	Y	M	Left epididymis	Pus like	GL s/o TB	Seen	Seen	Negative	Positive	Positive
79	171656	1127/19	32	Y	F	Rt preauricular+cervical	Blood mixed pus	GL s/o TB	Seen	Not seen	Negative	Positive	Positive
80	178326	1159/19	19	Y	M	Rt mandibular	Pus	GL s/o TB	Seen	Seen	Negative	Negative	Positive
81	180620	1184/19	9	Y	M	Lt cervical	Blood mixed	RL	Not seen	Not seen	Negative	Negative	Negative

82	185253	1188/19	34	Y	M	Rt cervical	Blood mixed	GL s/o TB	Seen	Not seen	Negative	Negative	Positive
83	188660	1204/19	42	Y	M	Lt axillary	Blood mixed	GL s/o TB	Seen	Not seen	Negative	Negative	Negative
84	194346	1232/19	16	Y	M	Lt cervical	Blood mixed	RL	Not seen	Not seen	Negative	Negative	Negative
85	197242	1246/19	28	Y	F	Lt cervical	Blood mixed pus	GL s/o TB	Seen	Not seen	Negative	Positive	Positive
86	199010	1254/19	75	Y	M	Lt cervical	Blood mixed	RL	Not seen	Not seen	Negative	Negative	Negative
87	205393	1286/19	29	Y	F	Rt cervical	Blood mixed	GL s/o TB	Seen	Not seen	Negative	Negative	Positive
88	206828	1295/19	15	Y	M	Rt cervical	Blood mixed	GL s/o TB	Seen	Not seen	Negative	Negative	Positive
89	18708	1301/19	2	Y	M	Lt axillary	Pus	RL with suppuration	Not seen	Not seen	Negative	Negative	Negative
90	209665	1314/19	15	Y	M	Lt inguinal	Blood mixed	RL	Not seen	Not seen	Negative	Positive	Positive
91	225836	1397/19	20	Y	F	Cervical	Pus	GL s/o TB	Seen	Not seen	Negative	Positive	Positive
92	21038	1423/19	20	Y	F	Rt cervical	Blood mixed	RL	Not seen	Not seen	Negative	Negative	Negative
93	231354	1432/19	28	Y	F	Lt breast	Pus	Granulomatous mastitis	Seen	Not seen	Negative	Negative	Positive
94	233927	1441/19	23	Y	M	Cervical	Pus	GL s/o TB	Seen	Seen	Negative	Negative	Positive
95	237355	1461/19	35	Y	F	Lt postauricular	Blood mixed	RL	Not seen	Not seen	Negative	Negative	Negative
96	239687	1475/19	20	Y	M	Rt cervical	Pus	TL	Not seen	Seen	Positive	Positive	Positive
97	242830	1503/19	23	Y	F	Lt cervical	Blood mixed	NGL s/o TB	Seen	Seen	Negative	Negative	Positive
98	243968	1505/19	23	Y	F	Cervical	Blood mixed pus	NGL s/o TB	Seen	Seen	Negative	Negative	Positive
99	246226	1523/19	14	Y	M	Lt cervical	Blood mixed	RL	Not seen	Not seen	Negative	Negative	Negative
100	249669	1563/19	32	Y	M	Cervical	Pus	NGL s/o TB	Seen	Not seen	Negative	Negative	Positive
101	256034	1614/19	54	Y	M	Lt cervical	Pus	TL	Seen	Seen	Negative	Positive	Positive
102	259032	1647/19	32	Y	F	Rt cervical	Blood mixed	RL	Not seen	Not seen	Negative	Negative	Negative
103	24110	1649/19	60	Y	M	Rt supraclavicular	Blood mixed	GL	Seen	Not seen	Negative	Negative	Negative
104	24302	1663/19	50	Y	F	Rt cervical	Blood mixed	GL s/o TB	Seen	Seen	Negative	Positive	Positive
105	262301	1668/19	35	Y	M	Rt cervical	Blood mixed	GL s/o TB	Seen	Seen	Negative	Negative	Negative
106	262387	1672/19	20	Y	F	Rt breast	Blood mixed	GM	Seen	Not seen	Negative	Negative	Negative
107	24473	1701/19	35	Y	F	Lt postauricular	Pus	NL	Not seen	Seen	Negative	Negative	Negative
108	24908	1731/19	21	Y	M	Rt postauricular	Blood mixed pus	NL	Not seen	Seen	Negative	Negative	Positive