"ROLE OF ANCILLARY TECHNIQUES IN IDENTIFYING ETIOLOGY OF GRANULOMATOUS LESIONS IN HISTOPATHOLOGY"

By

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LIST OF ABBREVATIONS USED

AFB	Acid Fast Bacilli
ZN	Ziehl Neelsen
AR	Auramine-Rhodamine
FF	Fite Faraco
RNTCP	Revised National Tuberculosis Control Program
WHO	World Health Organisation
TLR	Toll like receptors
INF	Interferon
PAS	Periodic Acid Schiff
MHC	Major Histocompatibility Complex

ABSTRACT

Background: Granulomatous inflammation is a distinctive pattern of chronic inflammation that is encountered in a limited number of infectious and non-infectious conditions. Recognizing granulomatous lesion and finding the etiology in tissue specimen is important for specific treatment.

Objectives:

1) To study histomorphological patterns in granulomas detected in various granulomatous lesions on tissue specimens.
2) To assess the role of ancillary techniques in finding the etiology of such lesions.

Materials and Methods: Prospective study of 135 cases was carried out in the Department of Pathology, B.L.D.E.(Deemed to be University) Shri B M Patil Medical College, Hospital & Research Centre, Vijayapura between 1st September 2016 and 31st August 2018, fulfilling the inclusion criteria. All granulomatous lesions confirmed on haematoxylin and eosin stained sections were subjected to special stains like Ziehl-Neelsen, Fite Faraco, Periodic acid Schiff, Fluorescent microscopy (Auramine rhodamine) and Polarised microscopy to identify the specific eitiological factors.

Results: In the present study, 114 (84.4%) cases were due to infectious etiology. Tuberculosis was the most common cause for granulomatous inflammation accounting for 64(47.4%) cases, followed by leprosy 38(28.1%). Eight cases (5.9%) were of fungal infection and four cases (3%) of actinomycosis. Foreign body granuloma was seen in 17(12.6%) cases. Two cases were of autoimmune etiology (1.5%) and one each case of Crohn's disease (0.7%) and cholesterol granuloma

(0.7%). Majority of cases were in the age group of 21-30 years, followed by age group of 31-40 years. Male to female ratio was 1.1:1 with slight male preponderance. Among 102 cases which included tubercular and leprosy as etiological agent, 17 (12.6%) cases were positive on ZN staining and 12(8.9%) were positive on FF stain. Also, there were two cases each of Mucormycosis, Madura Mycetoma and Aspergillosis. One cases each of Phaeohypomycosis and Hyalohypomycosis. The most common foreign body material encountered in the present study was suture material that is, 8 cases (47.1%) and vegetative material in 6 cases (35.3%).

Conclusion: Our study showed most common etiology for granulomatous lesion is tuberculosis followed by leprosy. AR stain increased the detection of AFB from 26.56% to 40.62% in cases of tuberculosis and in cases of leprosy from 31.57% to 47.36%. Utilization of these simple ancillary techniques optimizes and improves the accuracy of histopathology reporting in cases having granulomatous lesions.

Key words: Granulomas, Etiology, Special stains

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INTRODUCTION

Granulomatous disorders are a group of disorders with granulomatous inflammatory response leading to common histological denominator of granuloma formation. Granulomatous inflammation was recognized as a distinct entity in early 19th century and has been of continuing interest since then. It is a distinctive pattern of chronic inflammation that is encountered in a limited number of infectious and non-infectious conditions.[1]

Pathologically the granulomatous inflammatory response is a manifestation of many infective, toxic, allergic, autoimmune and neoplastic diseases and also conditions of unknown etiology. Granulomas usually form as a result of the persistence of a non-degradable product or as a result of hypersensitivity responses. Granulomatous inflammation is characterized by collection of activated macrophages (epithelioid cells) often with T lymphocytes and sometimes associated with central necrosis. At times activated macrophages may fuse forming giant cells. Recognition of granulomatous pattern and finding the etiology in a biopsy specimen is very important for diagnosis, specific treatment and there by outcome of the disease.[2]

Histopathology is a tool which can be used for establishing a correct diagnosis like in many other diseases pertaining to various organ systems of the body. Over the past few decades' advances in molecular diagnostic techniques have allowed identification of organisms involved in granulomatous diseases that previously were of unknown etiology.[3]

However, good clinical history, a close histological examination and a clinicopathological correlation are essential in making a final diagnosis. By combining all the available information, one should be able to arrive at a reasonable

differential diagnosis on which to proceed. In a minority of the cases, it will not be possible to make a definitive diagnosis, even with all the clinical information being available. Ancillary techniques like ZN stain, FF stain, AR stain (fluorescent microscopy), fungal stain, PCR, Polarized microscopy, IHC etc help in identifying etiology of granulomatous lesions. The histopathological examination of various granulomatous reactions is mandatory to reach a definitive diagnosis.[3]

OBJECTIVES OF THE STUDY

1) To study histopathological pattern in granulomas detected in various granulomatous lesions on tissue biopsy specimens and to find the etiology of such granulomatous lesions.
2) To assess the role of ancillary techniques in finding the etiology of such lesions.

REVIEW OF LITERATURE

"Chronic inflammation is a response of prolonged duration in which inflammation, tissue injury and attempts at repair coexist, in varying combination". Granulomatous inflammation is a distinctive pattern of chronic inflammation that is encountered in a limited number of infectious and some noninfectious conditions. Granuloma is defined by the presence of mononuclear leukocytes, specifically histiocytes (activated macrophages), which respond to various chemical mediators of cell injury. Examination under light microscopy, these activated histiocytes appear as epithelioid cells with round to oval nuclei, often with irregular contours and abundant granular eosinophilic cytoplasm with indistinct cell borders. They may also coalesce to form multinucleated giant cells. Identification and classification of the granulomatous inflammation pattern can be helpful in narrowing a differential diagnosis.[2,4]

Etiopathogenesis of granulomatous inflammation are –

Tuberculosis-

Tuberculosis was called by different names, one among them is Phthisis (Greek–phthiein–to waste away). Phthisis described tuberculosis as commonest disease and it is fatal. Hippocrates described this disease as "weakness of the lung" associated with cough and fever in his Book 1 of the epidemics (410-400BC). Hippocrates noticed that the tuberculosis disease was more among young adults between the age of eighteen to thirty-five. He also described pulmonary phthisis could be hereditary rather than infectious because it occurs in whole family. [5]

In 1679 Sylvius de la Boe was probably the first one to use the term tubercles in place of phthisis of the lung, which he named as tuberculaglandulosa or glandulous tubercules. He described that these tubercules progress to abscesses, ulcers and empyema. [5]

In 19th century it was named as 'The white death' and 'The great white plague'. Later, so many names evolved for the disease such as the robber of youth, the graveyard cough, the captain of all these men of death and the King's evil. Scrofula or 'the Kings evil' was first described by Aristotle and Cessius Felix which means tuberculosis of lymph nodes of the neck which eventually ulcerated. It was named the kings evil in 17th century because it was believed that it could be cured by the King's touch. [5,6]

In Europe, during 18th and 19th centuries, tuberculosis had an become epidemic. In England during1851-1910 four million died from tuberculosis and more than one third were aged between 15 and 34, hence aptly named as the robber of youth. At that time Oliver Wendall Holmes an American named it as the great white plague and the white death. It was named white because of extreme anemic pallor of affected individuals. [5,6]

Tuberculosis was an important disease during World War I and II due to living and working in closed areas. Later it declined after 19th century but still remains as one of the major public health problems.[6]

A Greek physician Claudius Galen of Pergamum in 174CE described phthisis associated with fever, sweating and coughing of bloody sputum. He also found tubercles in phthisis lungs and named it as phuma.[6]

It was also called as Yakshma in Rigveda. Robert Koch, Nobel laureate identified the specific agent Mycobacterium tuberculosis on 24th March 1882 which

was a major event in the history of medicine and this day is celebrated as World TB day every year by WHO. [7]

Structure of tubercle bacilli

To understand the pathogenesis and as various diagnostic approaches are emerging to demonstrate the presence of bacilli, it is important to know the structure of tubercle bacilli. These belong to the genus Mycobacterium and are aerobic, non-motile, non-spore forming, very thin, slightly curved or straight rods and measures 0.2-0.6x 1-10mm in size. Bacilli can occur in pairs, singly or in small groups. These are described as Gram- positive although these organisms cannot be readily Gram stained. These species have an unusual cell wall structure with a very high lipid content composed of N-glycolylmuramic acid. This creates hydrophobic permeability barrier due to which cell wall structure it is difficult to stain with basic aniline dyes which are used for Gram staining.[8]

These organisms when stained with carbolfuchsin by Zeihl-Neelsen technique or by auramine- rhodamine (fluorescent dyes) they withstand decolourisation by absolute alcohol and 20% Sulphuric acid for ten minutes. This important property of mycobacterium which derives from their cell wall is referred to as acid fastness which is due to unsaponifiable wax (mycolic acid) or semi-permeable membrane around the cell. Staining may be uniform or granular.[9]

Another characteristic feature is that it will grow very slowly than other human pathogenic bacteria due to their hydrophobic cell surface. Because of this hydrophobicity, these tend to clump so that nutrients are not easily allowed into the cell surface. These are resistant to chemical disinfectants. They are also sensitive to formaldehyde and glutaraldehyde. [9]

Epidemiology

Mycobacterium tuberculosis is one of the major public health problem leading in mortality globally from a single infectious agent. It is estimated that 1.7 billion or one-third of the population are infected and 2.9 million deaths annually are because of M tuberculosis. In addition 1.1 million individuals living with HIV are having a co-infection of tuberculosis. It is one of the complicating factors in the management of tuberculosis. [7,9]

Pathogenesis

Mycobacterium tuberculosis is an obligate aerobic organism. These organisms cannot replicate in the environment and therefore isolated to grow in tissues of humans and other warm-blooded animals. In most of the cases the bacilli enter via the respiratory route. As it is an intracellular pathogen it has more tendency towards lung tissue. The infection is initiated when inhaled bacilli are phagocytosed by alveolar macrophages. The spread of bacilli occurs through hematogenous or via lymphatics to other parts of the body. The apex of the lung has high oxygen pressure which favors the bacillary growth. Typically infection causes of the delayed type of hypersensitivity reaction which can be determined by Mantoux skin test or tuberculin test (PPD test). But infection and active diseases cannot be differentiated by Mantoux test. [10]

Binding of M tuberculosis to macrophages

Mycobacterium tuberculosis enters macrophages via endocytosis mediated by mannose-binding lectin and CR3. Other implement receptors (CR1,CR2& CR4) play a vital role in binding of an organism in the process of phagocytosis. This mannose receptors attach to a glycolipid in the bacterial cell wall and helps in phagocytosis. [11]

Replication in macrophages

Inside the macrophage Mycobacterium tuberculosis inhibits maturation of phagosome and prevents the formation of phagolysosome, there by allowing the bacteria to replicate within the vesicle. This prevention of phagolysosome formation is by inhibiting Ca^{++} signals and disrupting recruitment of proteins which helps in phagosome-lysosome formation. Thus, during the stage of primary tuberculosis (i.e <3 weeks) in nonsensitized cases, bacilli replicate in the alveolar macrophages which results in the seeding of multiple sites.[10,11]

Innate and adaptive immune responses to M tuberculosis

After primary infection, a T-helper 1(TH1) cell activates macrophages. Antigens of Mycobacteria which enter the draining lymph nodes bind to TH1 cells which in turn activate macrophages and are presented to T cells. These TH1 cells also produce IL-12. This mycobacterial ligand promotes the production of IL-12 by stimulation of Toll-like receptors (TLR) where in Toll-like receptor-2 is primarily involved in this process. In lymph nodes and lung, this TH1 mediated macrophage produces INF-γ. [10,11]

Actions of INF-γ

1. INF-γ stimulates maturation of the phagolysosome in infected macrophages
2. It helps in the production of nitric oxide (NO). This NO combines with other oxidants to form nitrogen intermediates which play an important role in killing Mycobacterium.
3. It mobilises antimicrobial peptides against the bacteria
4. It stimulates autophagy. [10,11]

Granuloma formation & tissue damage

This is the most important finding of TB and one of the initial host immune response to tuberculosis. Granuloma is a microscopic aggregation of (activated macrophages) epithelioid histiocytes, some of these epithelioid cells fuse to form langhans giant cells. These are surrounded by lymphocytes and plasma cells. There may be with or without central caseous necrosis and foamy macrophages within the granuloma. These are large macrophages filled with lipid-containing bodies. In later stages a tight layer of fibroblasts surrounding the granuloma might be seen.[10,11]

In a study done by Pascale Peyron *et al*, they stated that these lipid droplets are well-known nutrients for persistent bacilli. They also found that these bacilli remain alive within lipid droplets but do not replicate, there by enabling the organism to cause latent infection. Hence, the ZN stained sections demonstrates most of the bacilli located within foamy macrophages. Indicating a strong correlation between their existence with in granulomas. The association between foamy macrophages with necrosis was also documented. Foamy macrophages favor the appearance of necrosis. Association of foamy macrophage and necrosis may be the consequence of the foamy macrophage cleaning process of lipoproteins released into the necrotic tissue as seen in atherosclerosis lesions. Thus mycolic acid present in the cell wall of M TB may be responsible for the development of necrotic lesions due to their ability to induce TNF-α production by foamy macrophages where bacilli can hide and survive. [12]

According to their results for survival of Mycobacterium for long periods in a persistent state there should be oxygenated mycolic acids either free or as constituents of TDM or linked to the cell wall arabinogalactan should be considered a major virulence factor and also there oxygenated mycolic acid could be responsible for the

induction of necrosis within lesions and favoring Mycobacterium tuberculosis dissemination. [11,12]

The diagnosis of tuberculosis depends on demonstrating mycobacteria by using special stains like ZN, AR, AF. If ZN stain is negative for AFB on the section, the histopathological features of caseous necrosis or granulomatous inflammation are considered to be sufficient for initiation of treatment in endemic areas. It helps to avoid unnecessary complications due to surgical interventions and avoids the delay of treatment. Hence identifying granulomatous inflammation is necessary for the diagnosis of tuberculous inflammation. [13]

The detection of AFB by ZN staining technique is the most commonly used method in developing countries due to its cost effectiveness, minimal equipment required and less time involved comparision with other modalities. [14]

The cell wall of mycobacteria has the distinctive ability to bind the fuchsin dye and resists destaining by acid alcohol. This property of AFB forms the basic principle and is helpful in the early diagnosis of infection. The detection of AFB in stained smear is the easiest and most rapid procedure which can be done by ZN (hot stain), Kinyoun (cold stain) and fluorochrome using Auramine O with or without a second fluorochrome, Rhodamine. [15]

For aqueous carbol fuchsin to penetrate through the waxy capsule that surrounds the mycobacterial cell, the capsule should be softened. This is done by ZN procedure. The dye will penetrate into the waxy capsule by heating and binds to the cell wall. The bacterial cells cool down after the heat is removed, the capsule again hardens and protects the bounded dye and cell wall from the acid alcohol decolorizer.[15]

With carbol fuchsin, the AFB stains bright red against either blue or green background depending on the counterstain used in the procedure of staining. [16]

It should be emphasized that Auramine Rhodamine stain is a fluorescent antigen- antibody technique, with a direct physio-chemical binding of the stain to mycolic acid rich cell wall. [15,16]

Leprosy:

Introduction

It is an infective disease caused by Mycobacterium leprae, which is a debilitating disease. Historians of the disease stated that leprosy originated in the Indian subcontinent after that it has spread to Europe in 4th century BC. [17]

Discovery of lepra bacilli

In 1873 Gerhard Henrik Armauer Hansen discovered the lepra bacilli in Norway. He also initiated the preventive means against it. Hansen observed some nonrefractile small rods in unstained tissue sections, later he stained these with Zeihl's method and observed the similarities with Koch's bacillus. He stated three significant differences between these organisms

1. Rods are numerous
2. These rods are arranged in an intracellular collections called globii
3. Rods had various shapes

Depending on these observations he suggested that leprosy was caused by an organism related to Mycobacterium tuberculosis. The first historic mention of leprosy in India dates back to as early as 600 BC, where it is denoted by Sanskrit word "Kusta" which means "eating away". [17]

Leprosy has been held in superstitious dread and the person suffering from leprosy considered unclean and a social outcast. Though this was the first bacterial pathogen of humans to be described, it remains one of the least understood. This is because it has not been possible to grow the bacilli in culture media.[9,18]

Morphology

M. leprae is a straight or slightly curved rod ranging from 1-8 X 0.2-0.5µm in size. Clubbed forms, lateral buds or branching may be observed. It is a gram positive organism and stains readily than the tubercle bacilli. Hence 5% sulfuric acid is used instead of 20% for de-colorisation after staining with carbol fuschin.[19]

We have to differentiate between the live and dead bacilli in the stained smear. Live bacilli appear solid and uniformly stained whereas the dead bacilli appear as fragmented and granular.

The bacilli are seen singly or in groups, intracellularly or lying free outside the cell. They frequently appear as agglomerate, the bacilli being bound together by a lipid like substance the glia. Masses of bacilli are called globi. In these globi the bacilli are arranged in parallel rows and appear as cigar bundles. The globi appear in Virchow's lepra cells or foamy cells which are large undifferentiated histiocytes. [9,19]

Pathogenesis :

The source of infection and route of transmission are not known. However human respiratory secretions or soil are likely origins of leprosy infection. Similar to *M. tuberculosis*, *M. leprae* also does not secrete any toxins and its virulence is due to its cell wall.

The T-helper lymphocyte response to *M. leprae* determines whether an individual has tuberculoid or lepromatous leprosy. People with tuberculoid leprosy have a TH1 response associated with production of IL-2 and IFN-γ. Interferon γ is mainly responsible for macrophage response in the host. In lepromatous leprosy there will be weak TH 1 response. A relative increase in TH 2 response may be seen in some cases.

Overall result will be weak cell mediated immunity which will be unable to control the bacteria. Antibodies to mycobacterium leprae antigens are commonly produced in lepromatous leprosy which are not protective. But in turn immune complex formed by these antibodies and free antigens can lead to glomerulonephritis, vasculitis and erythema nodosum. [20]

Clinical and immunological spectrum of leprosy

In endemic countries clinically leprosy has been characterized by

1. Paucibacillary- 5 or fewer skin lesions and negative AFB skin split smear
2. Multibacillary - greater than 5 skin lesions and positive AFB skin spit smear

The Ridley Jopling classification system is also used to describe the subtype of disease. This system divides patients into 5 types

1. Tuberculoid (TT)
2. Borderline tuberculoid (BT)
3. Mid- borderline (BB)
4. Borderline lepromatous (BL)

5. Lepromatous leprosy (LL)

   This is based on combination of clinical manifestations, bacillary load and histopathlogical features.[21]

Tuberculoid and lepromatous leprosy patients have stable cell mediated immunity, which means their disease manifestations do not change over time. Patients with BT, BB, BL (borderline disease) has unstable cell mediated immunity and their clinical manifestations may change over time (upgrade or downgrade) towards tuberculoid or lepromatous presentation.[22,23]

Why peripheral nerve damage in leprosy ?

Mycobacterium leprae invades Schwann cells, the glial cells of the peripheral nervous system. "These Schwann cells form a functional unit with peripheral nerve axons surrounded by basal lamina. Mycobacterium leprae specifically interact with the G-domain of the α2 subunit of laminin-2, a neural specific extracellular matrix protein. This domain of laminin-2 can bind simultaneously to mycobacterium lepra and to the schwann cell laminin receptor, α-dystroglycan allowing high-affinity binding of *M lepra* to Schwann cells by using laminin-2 as a bridging molecule."

Laminin-2 also interacts with molecules on the surface of M leprae

1. 21-kDa protein – M. lepraelamini binding protein (ML-LBP21)
2. Glycolipid PGL-1

This 21-kDa protein, termed M. lepraelaminin binding protein (ML-LBP21), interacts with G4 molecule of the α-subunit of laminin-2 and ML-LBP21 is sufficient to mediate invasion of Schwann cells. M. leprae or its PGL-1 is bound and internalized by Schwann cells. It can cause demyelination of peripheral nerves in vitro and in vivo

in the absence of cellular immune response. Demyelination by M. leprae can promote further invasion of Schwann cells by the bacteria.[24]

Histopathology of various types of leprosy

Both in defeat and victory, granuloma formation is the hallmark of leprosy. Though some authors restrict the word granuloma to aggregates of only epitheliod cells, in leprosy the word includes that of macrophages also. Granuloma formation is often non specific and its exact mechanism is not understood completely. Evolution of epitheliod cells is an indication of good cellular immune response. [22,23]

Intermediate leprosy

It is a pregranulomatous stage of disease. Ridley suggested early and late stages of intermediate leprosy. In early lesions there will be occasional AFB either in normal nerve, erector pilorum muscle, hair follicles, sub epidermal zone or in perivascular infiltrate and also varying number of lymphocytes can appreciate in the perineural sheath. Sometimes the nerve fibre in the neurovascular bundle is hardly detected because it is almost replaced by a band of lymphocytes.

Histological changes of intermediate leprosy cases are known to precede the clinical manifestation by 3-6 months. M. leprae in the perivascular macrophages indicates lack of cell mediated immunity. Seventy percent of intermediate leprosy are known to heal spontaneously.[25,26,27]

Tuberculoid leprosy

It is characterised by granuloma consisting of epithelioid cells, giant cells and lymphocytes which varies in number and distribution. Here macrophages captures and kills the bacilli and presents it to major histocompatibility complex II (MHC II) to

CD4+ cells, thereby performing the function as antigen presenting cells. This CD4+ cells receive antigen-MHC and differentiate to T helper 1 (TH1) cells mediated by IL12 and 18 secreted by macrophages. This helps in production of IL-2 and 15 along with INF-γ and TNF-α which are T cell growth factors. Some TH1 cells helps in recruiting fresh macrophages, activate and retain them until the bacilli are destroyed and the disease gets arrested. [28,29]

On histopathology there will be large epithelioid cells arranged in compact granuloma along with dense and diffuse lymphocytic infiltration with absence of Langhans type of giant cells. Dermal nerves may be eroded by lymphocytes or it may be absent. [30]

Mid-Borderline leprosy-

The macrophages are activated uniformly to form epithelioid cells but these epithelioid cells are not organized into distinct granulomas and they contain few lymphocytes. Prominent dermal edema in between the inflammatory infiltrate is noted in these cases.[23,30]

Lepromatous Leprosy:

Macrophage granuloma is a distinct feature of Lepromatous leprosy. Granulomas of active LL is packed with macrophages. The cells are monotonously uniform with brightly stained eosinophilic cytoplasm, due to pressure from accumulating cells the epidermis often becomes thinned out and is separated by a narrow grenz zone of normal collagen. The infiltrate leads to the destruction of the hair follicles, sebaceous glands, other cutaneous appendages and at times involving even the subcutaneous fat. Bacilli get accumulated in macrophages called as lepra cells or Virchow cell.[23,30]

Borderline lepromatous Leprosy:

In this type the lymphocytes are more prominent. There is predilection for macrophages to get activated and form granulomas which are poorly to moderately defined. Perineural fibroblast proliferation forming an onion skin appearance on cross-section, which is a typical feature of BL. [23,30]

Foreign body granulomas

Any exogenous foreign body material or endogenous material which are altered can cause foreign body granuloma. Many multinucleated giant cells, histiocytes and varying number of inflammatory cells surround this material. The giant cells may be foreign body type having multiple nuclei scattered throughout the cytoplasm. Few cases show Langhans type of giant cells.

Endogenous substances causing foreign body reaction are oxalate, urates in gout, keratinous material in pilomatricoma as well as ruptured epidermoid and trichilemmal cysts. A granulomatous reaction to keratin and hair shaft can be seen adjacent to ruptured epidermal, dermoid cyst and other conditions associated with rupture or destruction of hair follicle. On cross section hair shafts are rounded or oval with cortical and medullary layers. They are variably birefringent in polarized light.[22,23]

Exogenous substances like silk or nylon sutures, wood or plant material, paraffin, silicone gel, injected mineral oil and hyaluronic acid , pencil lead, talc, surgical glove starch powder can cause foreign body reaction. Some of these are doubly refractile such as wood, talc, nylon and suture. Pulse granuloma is a reaction to food particles. When the vegetable matter is examined under polarized light they will appear as birefringent refractile particles. The cellulose membrane present in the vegetable matter will appear as light color and starch content will appear dark. The

collagen surrounding these membranes will show very weak birefringence indicating immature collagen fibres. The recent term for this pulse granuloma is hyaline ring granuloma due to the presence of clusters of small to medium sized hyaline rings. They may be seen around the fistulous tract involving the gastrointestinal tract.[23,31]

Immunization with tetanus toxoid may produce a central zone of granular debris containing aluminium and phosphate surrounded by granular histiocytes. There may be lymphoid aggregates and eosinophils at the periphery. This may be confused with the Kimura's disease, and the polarized microscopy will be helpful. Monosodium glutamate in BCG vaccine can also cause foreign body granuloma. Intradermal injection for Mantoux test can lead to granulomas due to implantation of keratin. Silica granulomas can be seen in a tennis player most commonly involving elbow due to repeated fall in the tennis court which is covered by artificial silica.

A histologic conclusion of whether a granuloma is of foreign body type or of allergic- type is not always possible. A granuloma of allergic type is more likely rounded, well- circumscribed collections of epithelioid histiocytes and less common to have multinucleated giant cells. By passing a beam of polarized light on tissue sections one can appreciate various foreign bodies like crystals, hair, suture material etc.[22,23]

Principles

"All the rays vibrate in a single plane in polarized microscopy. This polarizing microscope has 2 accessories 1. Polarizer, 2. analyzer (disc) which are made up of polarizing plastic that allows light vibrating in one plane to pass."

1. Polarizer – placed below the condenser

2. Analyzer – placed in between the objective lens.

"The placement of discs is such that they allow light vibrating in planes perpendicular to each other. Hence, when both the discs are in place no light can pass on to the eyepiece. Therefore through the eyepieces only dark background is seen unless a doubly refractile object is placed in the path of polarized light, in which case the doubly refractile object appears illuminated against a dark background." [32,33,34]

"Polarization characteristics of foreign bodies"

1. Suture material – linear on curvilinear refractile
2. Starch – maltese cross appearance
3. Talc – needle shaped white refringent oval, quadrangular particles
4. Wooden splinters – a refractile honeycomb pattern
5. Silica/glass particles – doubly refractile spicules
6. Zinc – birefringent rhomboidal crystals

By passing a beam of polarized light on tissue sections one can examine the foreign bodies like crystals, hair, suture material etc with the help of polarized microscopy.[35,36]

"Polarization characteristics of Crystals" [37]

1) Monosodium urate – needle shaped doubly refractile structures
2) Hyperglycemia – fine grained particles
3) Hypercreatinemia – dendrite like spherulites
4) Hyperoxalemia – fan type dendrites
5) Fabry's disease – Maltese crosses

Fungal Infections:

Fungi are eukaryotes with cell walls that give them their shape. Fungi can be molds or yeast. Molds are multicellular filaments and they form at room temperature. Yeasts are single cells or chain of cells and form at human body temperature. Reproduction of yeast is by budding.

Some yeast such as Candida albicans exhibits pseudohyphae. These pseudohyphae are buds that are formed but fail to detach and become elongated. Molds exhibit hyphae that grow and divide at their tips. These molds can produce conidia which are round cells. Conidia can easily become airborne leading to spread of the fungus. Diagnosis of fungal infection can be done by histologic examination. But few fungal species require culture for diagnosis.

There are four types of fungal infections

1) Superficial and cutaneous mycoses, limited to the superficial layers of skin, hair, and nails.
2) Subcutaneous mycoses, which involve the skin, subcutaneous tissues, lymphatics and rarely disseminate systemically
3) Endemic mycoses, caused by dimorphic fungi that can produce serious systemic illness in healthy individuals and
4) Opportunistic mycoses, which can cause life-threatening systemic diseases.[10,38]

Mucormycosis (Zygomycosis)

Mucormycosis is caused by the fungal class called Zygomycetes, which include Mucorales and Entomophthorales. Entomophthoromycosis is an uncommon disorder caused by Entomophthorales which affects the skin and subcutaneous tissue.

Mucormycosis is caused by Mucorales infecting the immunocompromised individuals which can be lethal. Based on clinical features and organ involved mucormycosis can be divided into

1. Rhinocerebral
2. Pulmonary
3. Cutaneous
4. Gastrointestinal
5. Disseminated
6. Miscellaneous

Pathogenesis

Their transmission is by asexual spores which are airborne. These inhaled spores produce infection mainly in the lungs and sinuses. Macrophages help in primary defense mechanism by oxidative killing and phagocytosis.[39]

Morphology

These are broad ribbon-like aseptate hyphae branching at the right angle. Hyphal angioinvasion will be noticed in all patients with invasive mucormycosis and associated infarction of surrounding tissues are noted the in majority of cases. Surrounding tissue shows neutrophilic infiltration, necrosis and granulomatous response. Few patients have only granulomatous response.[39]

Phaeohypomycosis

Phaeohypomycosis is a subcutaneous or systemic infection caused by dematiaceous, mycelia forming fungi. Bipolaris, phialophora alternaria and exophiala are fungi responsible for phaeohypomycosis. [40]

These are characterized by pigmented spores without hyphal forms. On microscopy these will be stellate foci of suppurative granulomatous inflammation.

The area of inflammation enlarges and forms a single large cavity of pus associated with granulomatous reaction so called phaeohypomycotic cyst .The organisms are often found within the cavity often with histiocytes. The hyphal forms often have irregularly placed branches and show constrictions around their septae that may resemble pseudohyphae or yeast forms. Mycelia if present will be loosely arranged than the hyphal forms.[41]

Aspergillosis

Aspergillosis is an ubiquitous fungus. There are approximately 200 species of aspergillus but only few of them are pathogenic to humans.

A. Fumigates – is the most common cause of aspergillosis in humans. Other species which cause aspergillosis in humans are A flavus, A niger and A terrens.

Pathogenesis

Transmission of aspergillus species through conidia which are airborne. They enter mainly through the lungs. The spores of A fumigatus are approximately 2-3µm, so this enables to reach them till the alveoli. Hyphae develop from these conidia and invade into tissues. Alveolar macrophages and neutrophils are the main host defenses against these aspergillus. Aspergillus produces many virulence factors consisting of adhesins, enzymes, antioxidants and toxins. Allergic alveolitis can be caused by sensitization to aspergillus spores. Angioinvasive aspergillosis is seen in chronically immune-compromised individuals which is a life threatening fulminant infection. Lower respiratory tract is the most common primary site of infection and associated with respiratory symptoms. Diagnosis is quite difficult in these cases because sputum cultures are often negative. Hence, lung or sinus biopsy can be helpful in arriving at the diagnosis.[39,42]

Microscopy

The hyphal forms are uniform with septa at regular intervals. These grow in parallel fashion and branching is dichotomous and most at often 45-degree angle. These hyphae can be seen in routine H & E sections but better appreciated with PAS, Grocott and GMS stain. Proliferating masses of hyphae form fungus ball, which shows heterogenous staining intensity of alternating zones of growth. These hyphae are surrounded by neutrophils and in chronic lesions these are surrounded by granulomas which contain scattered giant cells, neutrophils and eosinophils. Fruiting bodies or conidial heads are rarely observed in the lesions where the organism exposed to air. [39,42]

Rheumatoid nodules:

Rheumatoid nodules are seen in patients with rheumatoid arthritis. They are most commonly seen over extensor aspects of proximal forearm over the olecranon process, metacarpophalangeal and proximal interphalangeal joints. These are deep seated firm masses. Lung and heart can be their extracutaneous sites. These nodules may be solitary or multiple and vary from few millimeter to 5 centimeter. Rheumatoid factor is almost always positive.[43,44]

On microscopy these nodules can be seen in deep dermis and subcutaneous tissue. Fibrinoid degeneration of collagen bundles is also noted. These are surrounded by histiocytes in a palisading arrangement. In 50% of the biopsies foreign body giant cells will be present. The surrounding stroma will show proliferation of blood vessels with fibrosis and admixture of inflammatory cell infiltrate such as lymphocytes and neutrophils are most common, but mast cells, plasma cells and eosinophils may be noted. [45]

Sarcoidosis:

Sarcoidosis is a multisystem inflammatory disease of unknown etiology. It is characterized by formation of non-caseating granulomas. Clinical evidence of sarcoidosis is necessary to confirm the diagnosis. It can present with various clinical patterns but most commonly presents with bilateral hilar lymphadenopathy. Other clinical presentation may be fever, iritis, polyarthritis which will be migratory in nature and skin lesions in the form of papules, plaques or nodules. When papules or plaques are present over the cheek, nose, and earlobes it is termed as lupus pernio. This should be differentiated from other granulomatous inflammation such as foreign body reaction, as polarisable crystals may be present in the lesion sometimes. Rarely caseation can be seen in the centre of the granuloma which will be confused with tuberculosis and tuberculoid leprosy. [46,47]

Pathogenesis

Although it is benign condition, it can be fatal in about 5% of cases. The etiology of sarcoidosis is unknown and pathogenesis varies in all individuals. It can be due to alterations in immunological factors or could be due to several genetic associations. The important cytokines involved are IL-2and INF-γ secreting helper T cells and other cytokines TH-1 and TH-17. The IL-2 causes T cell expansion and INF-γ causes macrophage activation. So this increases level of the cytokines and causes recruitment of T cells and monocytes which leads to granuloma formation.

HLA-DQB1 and HLA-DRB1 are strongly associated with sarcoidosis having good prognosis. Some studies have suggested mycobacterium tuberculosis as the cause and others have suggested atypical mycobacteria, propionibacterium and ricketsiae. But there is lack of supporting evidence to prove the infectious etiology. [48,49]

Histopathology

Cutaneous lesions contain well circumscribed non caseating granulomas consisting of epithelioid cells and occasional giant cells and these giant cells may be large and irregular in shape. In few cases giant cells may contain asteroid and Schaumann bodies. However, these are not specific for sarcoidosis and it can be seen in other granulomatous conditions like tuberculosis, foreign body reaction and necrobiotic xanthogranuloma also. These granulomas may be present in superficial dermis, deep dermis or subcutaneous tissue. Asteroid bodies are star shaped eosinophilic structures with radiating spikes and Schaumann bodies are round to oval darkly stained lamellated calcified structures. Darkly stained appearance is due to presence of calcium. These are also called naked granulomas due to presence of scant lymphocytic infiltrate around the epithelioid cells.

The diagnosis of sarcoidosis is made by clinical signs and symptoms, biopsy findings and by excluding other causes of granulomatous inflammation. [50,51]

Williams GT *et al* in a review article of granulomatous inflammation concluded that granulomatous inflammation represents a distinctive tissue reaction to an irritant in which the central cell is the mononuclear phagocyte cell, but which can be modified by other phenomena, especially hypersensitivity. If the destructive properties of granulomas can be reduced while the beneficial functions are amplified, there will be immense scope for preventing the long-term complications, especially fibrosis, of many infective granulomatous diseases and for improving host defence, especially against neoplasia.[52]

MATERIALS AND METHODS

SOURCE OF DATA

Specimen received for histopathological examination in the department of pathology, BLDE (Deemed to be University), Shri. B. M. Patil Medical College, hospital and research centre, Vijayapura was taken.

Study period: 1st September 2016 to 31st August 2018.

INCLUSION CRITERIA:

All the cases with granulomas identified in histopathological examination due to any etiology.

EXCLUSION CRITERIA:

Nil.

METHOD OF COLLECTION OF DATA

It is a prospective study. One hundred thirty five samples with granulomatous lesion identified in the histopathological section between the period of 1st September 2016 to 31st August 2018, referred to the Department of Pathology, BLDE (Deemed to be University) Shri B M Patil Medical College, Hospital and Research Centre, Vijayapura was included in the study.

A detailed history of the patient and laboratory investigations was collected from the in-patient case records. Specimens received for histopathological examination were processed using routine tissue processing protocol using semi automated tissue processor. For histopathological examination, the specimens were fixed in 10% neutral buffered formalin. After passing the tissue dehydration in graded alcohol for 2 hours each in three changes, clearing was done with two changes of xylene for each hour. Followed by impregnation and embedding in paraffin blocks, 3-5µ sections were cut.

These slides were stained with haematoxylin and eosin, dried and mounted using DPX. The cases showing granulomatous inflammation from all sites were included. Special stains like Ziehl-Neelsen stain, Fite Faracco stain, Periodic Acid Schiff, Auramine Rhodamine stain under fluorescent microscope using the blue excitation filter and Polarized microscopy were used to find the etiology of granulomatous lesions on a case to case basis.

HEMATOXYLIN AND EOSIN STAIN:

H & E stain or hematoxylin and eosin stain is the most widely used stain in histopathological diagnosis. The staining method involves application of hemalum, which is a complex formed from aluminium ions and oxidized hematoxylin. Hematoxylin stains the cell nuclei blue black and shows good intranuclear detail. Hematoxyline also demonstrates intracellular substances (eg. Chromosomes, keratohyalin), extracellular substances (eg. elastin), ground substance (eg.cement lines in bone) and minerals (eg. Calcium, Copper). The nuclear staining is followed by counterstaining with an aqueous or alcoholic solution of eosin Y, which stains cell cytoplasm and most connective tissue fibres in varying shades of red, pink and orange. Eosin is the most suitable stain that combines with an alum hematoxylin. It demonstrates the general histological architecture of a tissue. It can distinguish between the cytoplasm of different types of connective tissue fibres and matrices, by staining them differing shades of red and pink.

Staining Procedure:

1) Deparaffinize sections, 2 changes of xylene, 10 minutes each.
2) Re-hydrate in 2 changes of absolute alcohol, 5 minutes each.
3) 95% alcohol for 2 minutes and 70% alcohol for 2 minutes.
4) Immerse in water for 5min.
5) Stain in hematoxylin solution for 8-10 minutes.
6) Differentiate in 1% acid alcohol for 30 seconds.
7) Rinse in running tap water.
8) Bluing in 0.2% ammonia water or saturated lithium carbonate solution for 30 seconds to 1 minute.
9) Counterstain in eosin-phloxine solution for 30 seconds to 1 minute.

10) Dehydrate through 95% alcohol, 2 changes of absolute alcohol, 5 minutes each.
11) Clear in 2 changes of xylene.
12) Mount in DPX

Interpretation:

Nuclei stains-Blue

Cytoplasm stains- Pink to red.

SPECIAL STAINS USED IN PRESENT STUDY

Suspected cases of tuberculosis were stained with ZN stain to find the Acid fast bacilli in the tissue section.

ZIEHL-NEELSEN STAINING PROCEDURE –

1) Bring section to water.
2) Cover the slide with Ziehl's carbol fuschin working solution.
3) Heat the slide from underneath with the flame of Bunsen burner until vapour start to rise.
4) Rinse the slide gently with water to remove excess carbol fuschin. Drain excess water.
5) Add 20% sulfuric acid drop by drop till the pale pink color appears- approximately 5 to 10min.
6) Rinse the slide gently with water to remove excess sulfuric acid. Drain excess water.

7) Cover the slide with 0.3% methylene blue counterstaining solution and allow standing for 1 minute.
8) Dehydrate, clear and mount.

Interpretation - Acid fast bacilli- Red

Background – Light blue

PERIODIC ACID-SCHIFF (PAS) STAINING PROCEDURE:

1) Bring sections to water
2) Oxidize for 5 min in 1% aqueous periodic acid.
3) Wash in running water for 5 min and rinse
4) Treat with Schiff reagent for 8 to 15 min
5) Wash in running water for 10 min
6) Counter stain with hematoxylin
7) Dehydrate ,clear and mount

Interpretation:

PAS Positive substances: Bright Red/ Magenta

Nuclei: Blue

FITE'S ACID FAST STAINING PROCEDURE - LEPROSY:

1. Deparaffinize in xylene/peanut oil mixture, 2 changes, and 12 minutes each.
2. Drain slides, wipe off excess oil and blot opacity.
3. Add Carbol-fuchsin, 20-30 minutes
4. Wash in tap water.
5. Add 1% acid alcohol until the section is faint pink- 1min.

6. Wash in tap water.
7. Counterstain in working methylene blue, 30 seconds.
8. Wash in tap water.
9. Dehydrate clear and mount.

Interpretation: Acid-fast bacilli -Red

Background- Light Blue

Negative sections were subjected for additional six sections and stained with FF stain before declaring them as Negative

AURAMINE RHODAMINE STAINING PROCEDURE:

Staining was done according to the procedure of Kuper and May.

1. Deparaffinize was performed with 1part of peanut oil and 3parts xylene mixture, two changes of 10minutes each and then blotted carefully.
2. Flood the slide with auramine rhodamine stain and allow to stain for 20min
3. Rinse the slide with water
4. Decolorization was performed in 0.5% hydrochloric acid in 70% ethanol for 2minutes.
5. Rinse the slide with water
6. Counter stain with 0.5% aqueous Potassium Permanganate for 2min
7. Wash the slide with water.

   Interpretation- Acid fast bacilli- Bright yellow

AR stain was taken as a reference standard and AR stained smears were scanned under fluorescence microscope (ZEISS microscope of wavelength 450nm). Acid fast bacilli appear as bright yellow fluorescence emitted by the bacilli.

ZN and FF stained smears were examined for AFB using a bright field microscope in oil immersion (Labomed® 300i). Appropriate control slides were stained simultaneously along with the test slides in all the cases.

POLARIZED MICROSCOPY STAINING PROCEDURE –

Suspected foreign body granulomas on H & E, were examined under polarized microscopy.

1) Deparaffinize sections, 2 changes of xylene, 10 minutes each.
2) Re-hydrate in 2 changes of absolute alcohol, 5 minutes each.
3) 95% alcohol for 2 minutes and 70% alcohol for 2 minutes.
4) Stain in eosin-phloxine solution for 30 seconds to 1 minute.
5) Dehydrate through 95% alcohol, 2 changes of absolute alcohol, and 5 minutes each.
6) Clear in 2 changes of xylene.
7) Mount in DPX

Note: No nuclear stain required.

A polarizing microscope has two accessories one of them is polarizer and another is analyzer. Polarizer is placed below the condenser and analyzer is placed in the top part of the microscope (Labomed Lx300i). Foreign body material was observed in 10X and 40X objectives.

Figure 1: Brightfield microscope (Labomed Lx 300i)

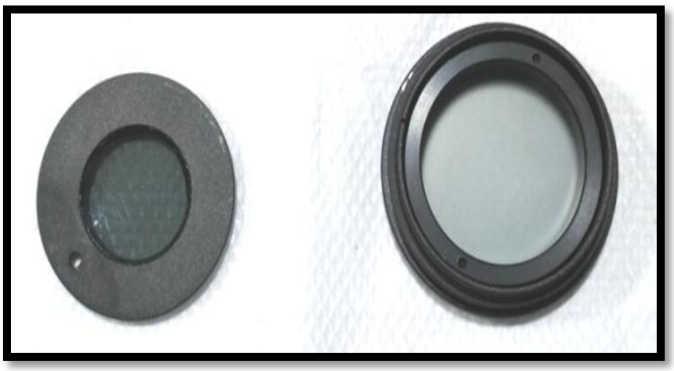


Figure 2: Picture showing analyzer and polarizer

STATISTICAL ANALYSIS

All characteristics were summarized descriptively. For continuous variables, the summary statistics of mean, standard deviation (SD) were used. For categorical data, the number and percentage were used in the data summaries. Chi-square (χ^2)/ Freeman-Halton Fisher exact test was employed to determine the significance of differences between groups for categorical data. Sensitivity- specificity was done to check relative efficiency. If the p-value was < 0.05, then the results were considered to be statistically significant otherwise it was considered as not statistically significant. Data were analyzed using SPSS software v.23.0 and Microsoft office.

RESULTS

In this prospective study of 2 years duration, 135 cases with granulomatous lesion identified in the histopathological section from all the sites were included. Age of the patients ranged from 5 to 65 years with a mean age of 23years. Majority of cases were in the age group of 21-30 years followed by 31-40 years. Male to female ratio was 1.11:1 with slight male 71(52.6%) preponderance. The detailed distribution of age group and the male female distribution of the cases are represented in the Table (1, 2) and Figure (3, 4).

Table 1: Distribution of cases according to age

AGE	**N**	**%**
≤10	7	5.2
11-20	16	11.9
21-30	39	28.9
31-40	33	24.4
41-50	18	13.3
>50	22	16.3
Total	135	100

Figure 3: Bar diagram showing distribution of cases according to age

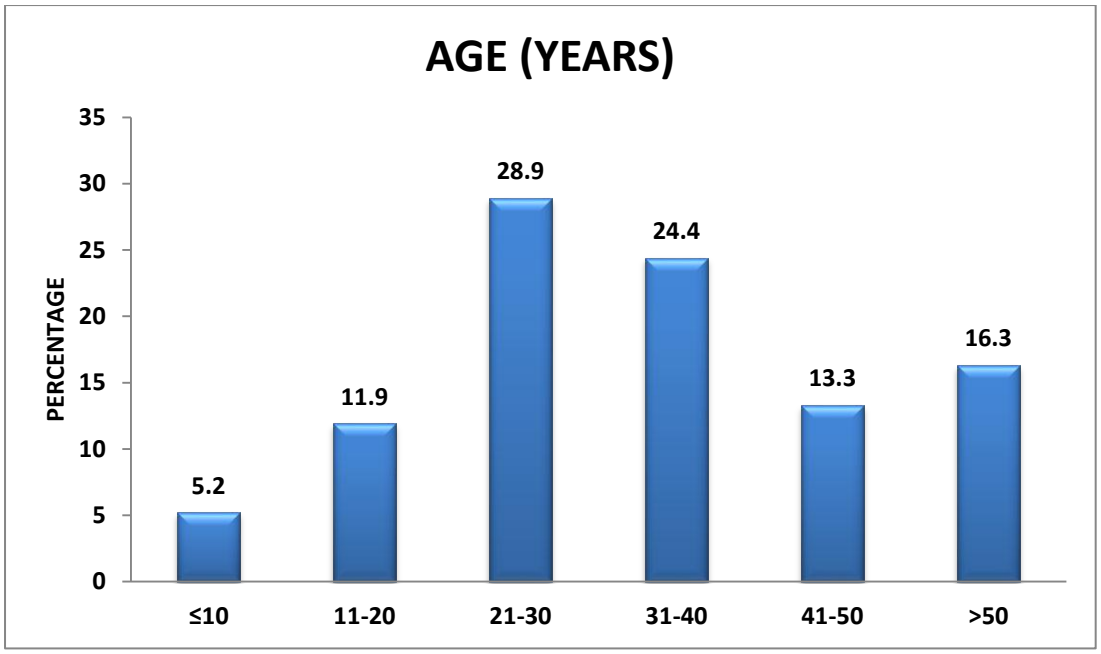
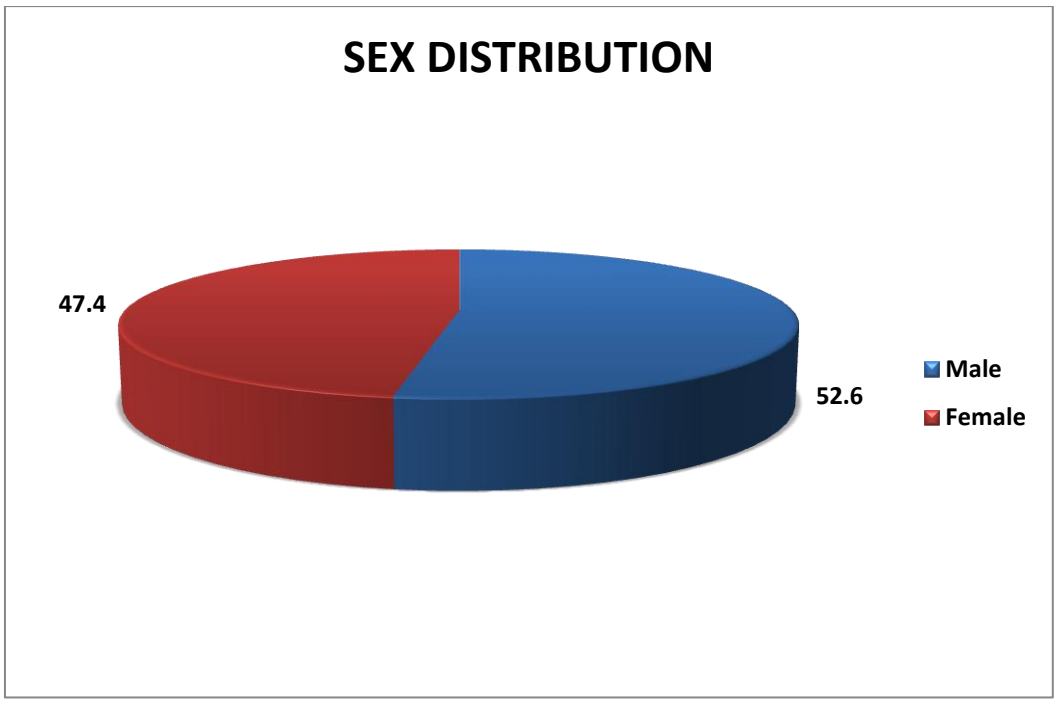


Table 2: Distribution of Cases According to Sex

SEX	N	%
Male	71	52.6
Female	64	47.4
Total	135	100

MALE TO FEMALE RATIO 1.11:1

Figure 4: Pie Chart Showing Distribution of Cases According to Sex



The cases were stratified based on the etiology into broad categories like neoplastic, non-neoplastic and inflammatory lesions. Predominant category was inflammatory. Among them infections etiology was most common with 114 (84.4%) cases. Amongst the infectious lesions, upon further refinement of the diagnosis, tuberculosis followed by leprosy and fungal infection formed the major groups of

cases with 47.4%, 28.1% and 5.9% respectively. Four cases (3%) were due to actinomycosis. Remaining 20(14.8%) cases were due to foreign body, autoimmune cause or cholesterol granuloma. There were no neoplastic causes in the study group. A single case (0.7%) of non-neoplastic lesion due to Crohns disease (0.7%) as etiology was seen.

All causes are represented in the Table (3) and as a graphical format in the Figure (5) with the number of cases in each group as a histogram.

Table 3- Distribution of cases according to type of lesions

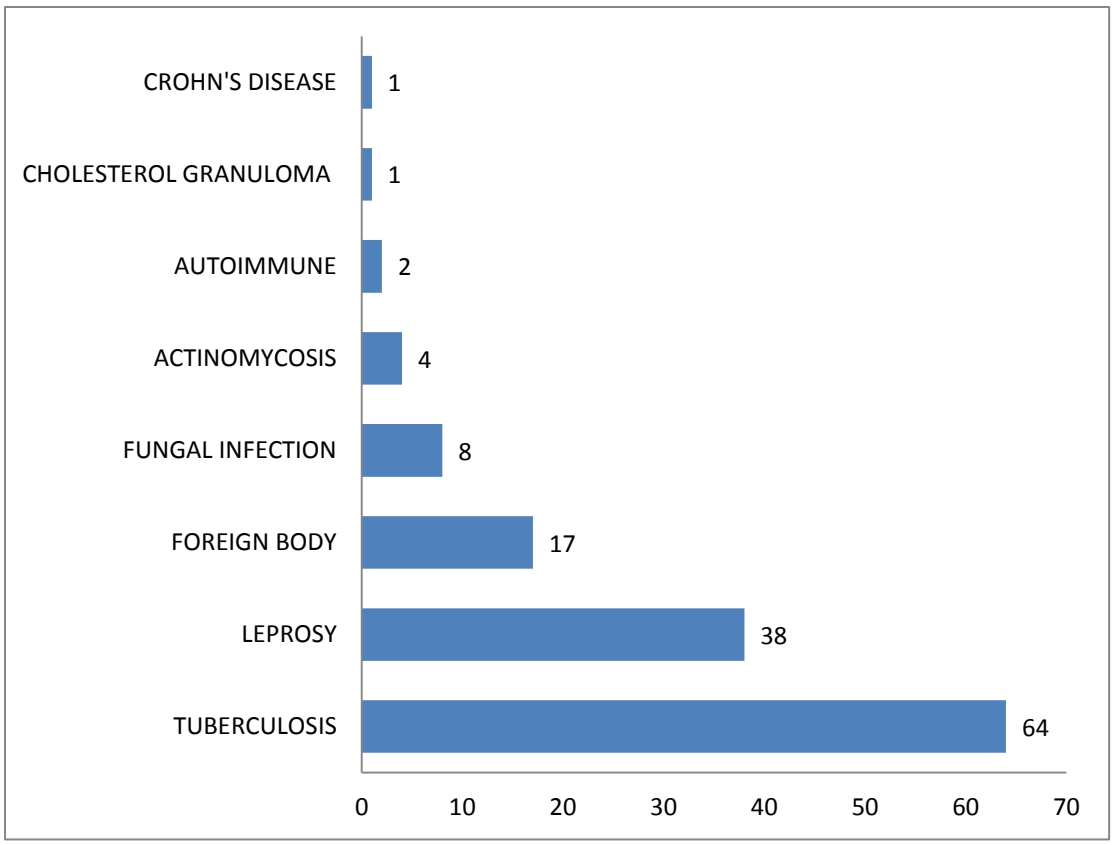
Etiology	Number of cases
Neoplastic	0
Non –neoplastic	1 (0.7%)
Inflammatory 1. Infectious 2. Non infectious	114(84.4%) 20(14.8%)
Total	135

Table 4: Distribution of cases according to etiology

ETIOLOGY	N	%
Tuberculosis	64	47.4
Leprosy	38	28.1
Foreign body	17	12.6
Fungal infection	8	5.9
Actinomycosis	4	3.0
Autoimmune	2	1.5
Cholesterol granuloma	1	0.7
Crohn's disease	1	0.7
Total	135	100

In our study 114 (84.4%) cases were due to infectious etiology, Tuberculosis with 64(47.4%) cases was the most common cause for granulomatous inflammation followed by leprosy 38(28.1%). Eight cases (5.9%) were of fungal infection and four cases (3%) of actinomycosis. Foreign body granuloma was seen in 17(12.6%) cases. Two cases were of autoimmune etiology (1.5%) and one case each of Crohn's disease (0.7%) and cholesterol granuloma (0.7%). All causes were represented in the Table (4) and as a graphical format in the Figure (5) with the number of cases in each group as a histogram.

Figure 5: Bar diagram showing distribution of cases according to etiology



In the present study we had specimens from a wide number of organs and organ systems affected with tuberculosis. Among them lymphnodes were the most commonly affected in 22 (34.4%) cases. Other organs affected and their number is depicted in Table (5) and Figure (6).

Table 5: Distribution of cases of tuberculosis according to organs involved

Tuberculosis according to organ involved	**N**	**%**
Lymphnode	22	34.4
Skin	10	15.6
Abdomen	13	20.3
Synovial tissue	3	4.7
Skeletal vertebra	5	7.8
Kidney	2	3.1
Testis	3	4.7
Lungs	1	1.6
Ovary	1	1.6
Fallopian tube	1	1.6
Soft tissue	2	3.1
Submandibular	1	1.6
Total	64	100

Figure 6: Bar Diagram Showing Distribution of cases of tuberculosis according to organs involved

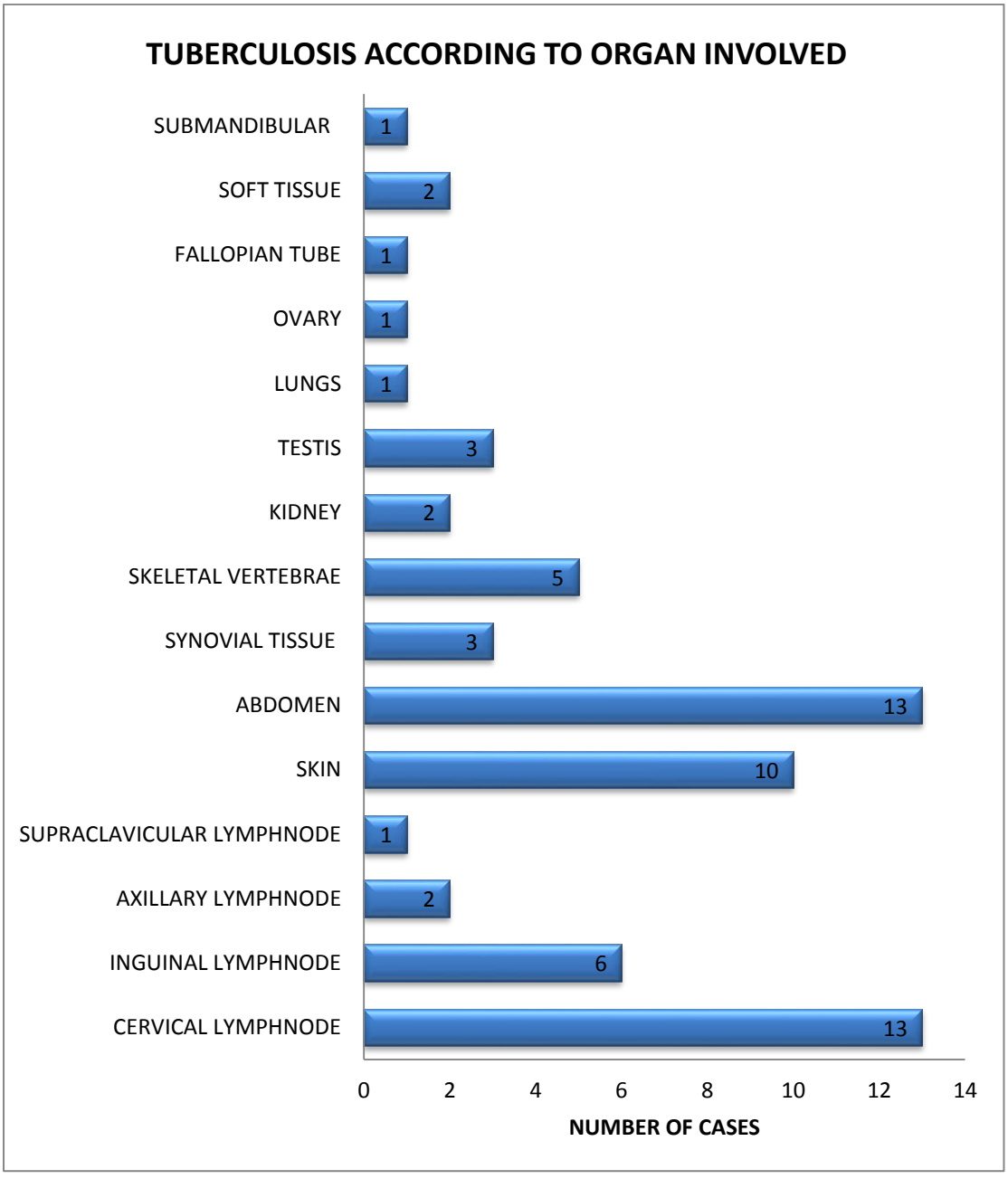


Table 6: Distribution of cases of tuberculosis according to sites of lymphnode involved

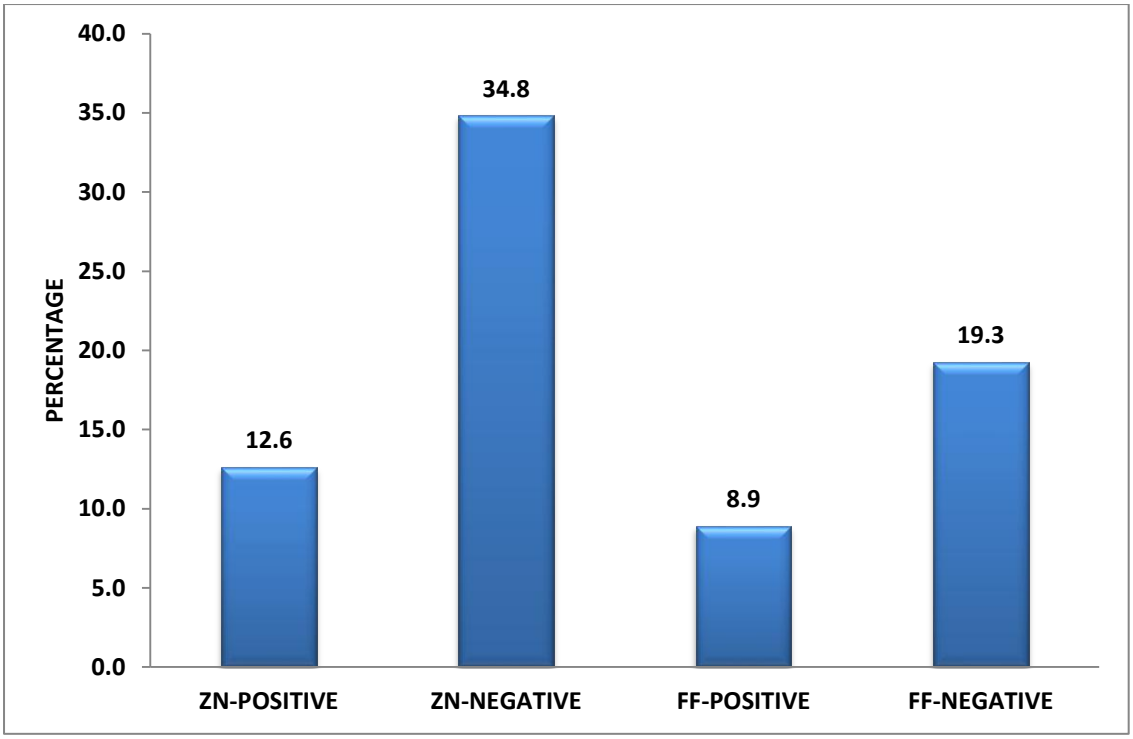
Tuberculosis according to sites of lymphnode involved	N	%
Cervical	13	20.3
Inguinal	6	9.4
Axillary	2	3.1
Supraclavicular	1	1.6

Cervical lymph nodes were the most commonly affected group seen in 13 (20.3%) cases. The other groups of lymph nodes involved were inguinal, axillary and supraclavicular.

Table 7: Distribution of cases according to Ziehl Neelsen and Fite Faraco stain

SPECIAL STAIN	N	%
ZN-POSITIVE	17	12.6
ZN-NEGATIVE	47	34.8
FF-POSITIVE	12	8.9
FF-NEGATIVE	26	19.3

Figure 7: Bar Diagram Showing Distribution of cases according to Ziehl Neelsen and Fite Faraco stain



Among 102 cases which included tubercular and leprosy as etiological agent, 17 (12.6%) cases were positive on ZN staining and 12(8.9%) were positive on FF stain. Majority of the cases, that is 73 (57.8%) were negative on either ZN or FF stains.

All the ZN positive cases showed positive result with the AR stain with 100% correlation. Among 47 (73.43%) ZN negative cases, additional 9 (14.06%) cases showed positivity result with the AR stain. On the other hand, for all the leprosy cases FF stain and AR stain were done. FF positive was seen in 12 (31.57%) cases and negative in 26 (68.42%) cases. All the FF positive cases showed positive result with the AR stain with100 % correlation. Among 26 (68.42%) FF negative cases, additional 6 (15.78%) cases showed positive result with the AR stain. The association of ZN stain and FF stain with AR stain is represented in the Table (8) and as a graphical format in the Figure (8).

Table 8: Association of ZN stain and FF stain with AR stain

Special stain	Cases	AR-Negative		AR-Positive		p value
		N	%	N	%	
ZN-Positive	17	0	0.0	17	38.6	<0.001*
ZN-Negative	47	38	65.5	9	20.5	
FF-Positive	12	0	0.0	12	27.3	
FF-Negative	26	20	34.5	6	13.6	
Total	102	58	100.0	44	100.0	

Note: * means significant association between AR staining and special staining with p value <0.05.

Figure 8: Bar diagram showing Association of ZN stain and FF stain with AR stain

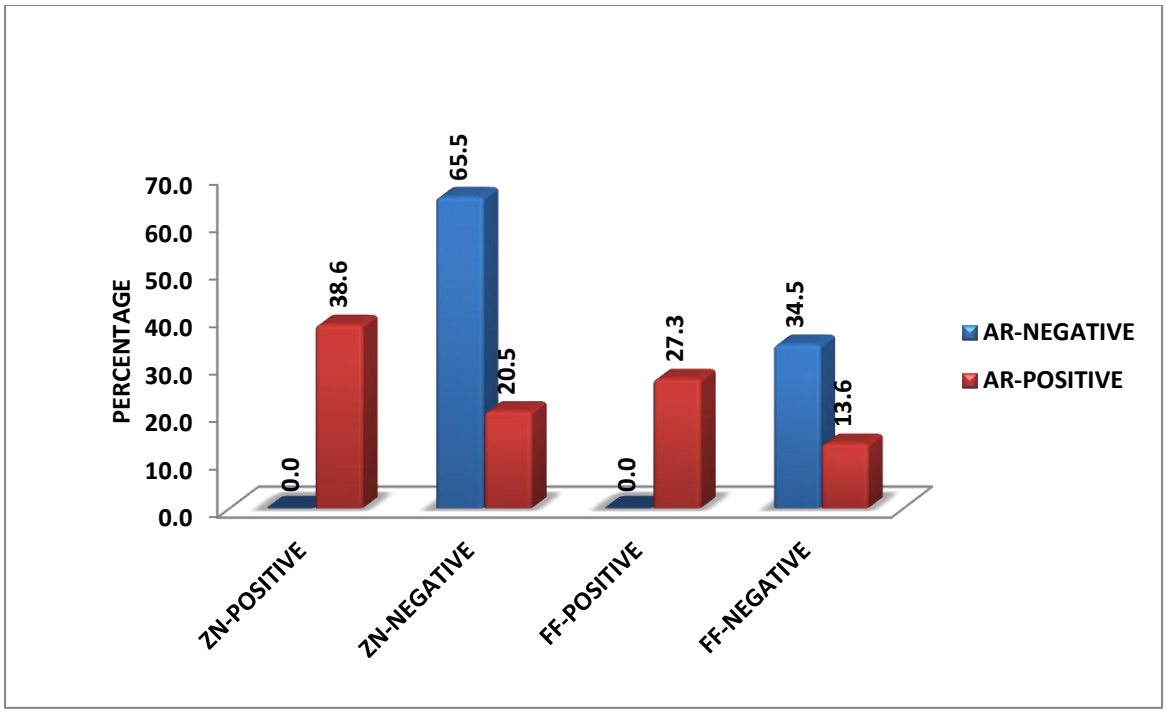


Table 9: Sensitivity analysis of FF stain

TP (true positive)	12
FN (false negative)	6
FP (false positive)	0
TN (true negative)	20

Sensitivity	66.7%
Specificity	100.0%
PPV	100.0%
NPV	76.9%
Accuracy	84.2%

Taking AR stain as reference standard, the FF showed a Sensitivity of 66.7%, Specificity of 100%, Positive predictive value (PPV) of 100% and Negative predictive value (NPV) of 76.9% and Accuracy of 84.2%.

Table 10: Sensitivity analysis of ZN stain

TP (true positive)	17
FN (false negative)	9
FP (false positive)	0
TN (true negative)	38

Sensitivity	65.4%
Specificity	100.0%
PPV	100.0%
NPV	80.9%
Accuracy	85.9%

Taking AR stain as reference standard, the ZN stain showed a Sensitivity of 65.4%, Specificity of 100%, Positive predictive value (PPV) of 100% and Negative predictive value (NPV) of 80.9% and Accuracy of 85.9%

Table 11: Distribution of cases according to age in foreign body granuloma

AGE	N	%
≤10	0	0.0
11-20	0	0.0
21-30	4	23.5
31-40	6	35.3
41-50	2	11.8
>50	5	29.4
Total	17	100.0

Seventeen cases of foreign body granuloma were seen in the present study and most of the cases were in between 21-30 years of age with male predominance. The detailed distribution of age group and the male- female distribution of the cases are represented in the Table (11 and 12) and Figure (9 and 10). For the detection of foreign body, polarizing microscope has been used.

Figure 9: Bar diagram showing Distribution of cases according to age in foreign body granuloma

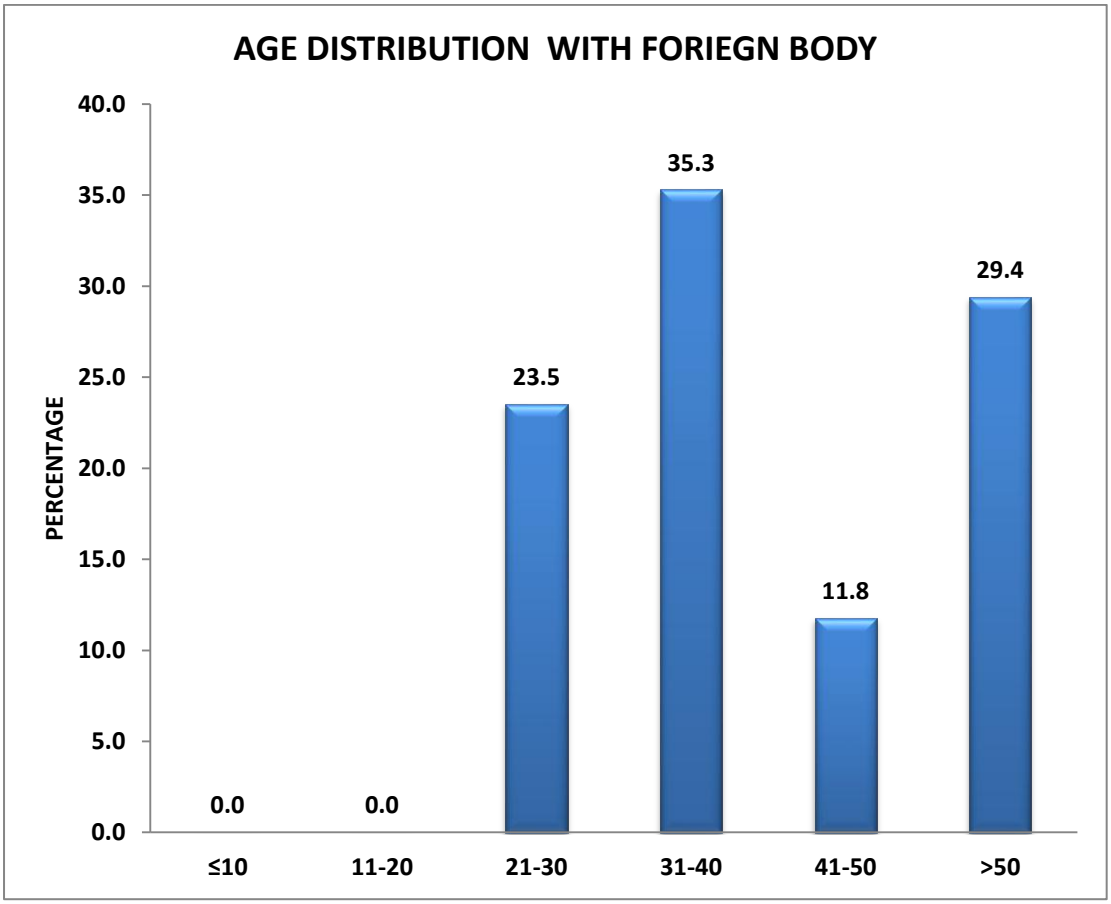
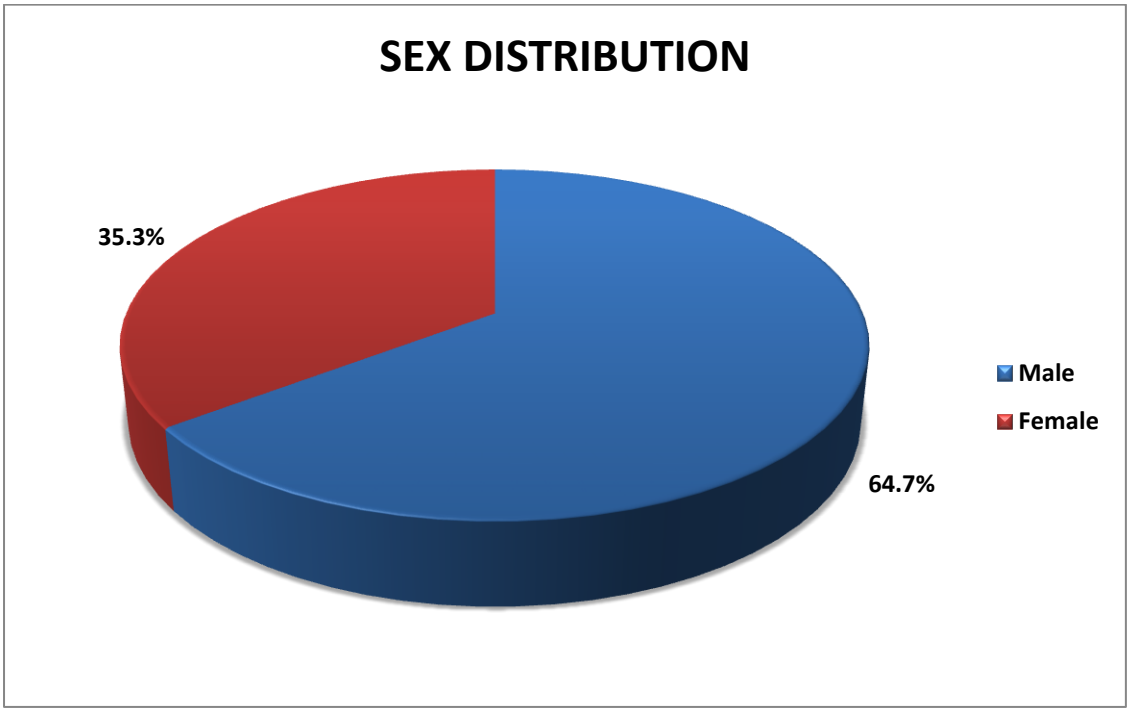


Table 12: Distribution of cases according to sex in foreign body granuloma

SEX	N	%
Male	11	64.7
Female	6	35.3
Total	17	100.0

Figure 10: Pie diagram showing Distribution of cases according to sex in foreign body granuloma



The most common foreign body material encountered in this study was suture material that is, 8 (47.1%) cases and vegetative material in 6 (35.3%) cases. The detailed distribution of which is represented in the Table (13) and Figure (11).

Table 13: Distribution of cases according to foreign body material identified in the granuloma

TYPE OF MATERIAL	N	%
Suture material	8	47.1
Vegetative material	6	35.3
Ruptured cyst	3	17.6
Total	17	100.0

Figure 11: Pie diagram showing distribution of cases according foreign body material identified in the granuloma

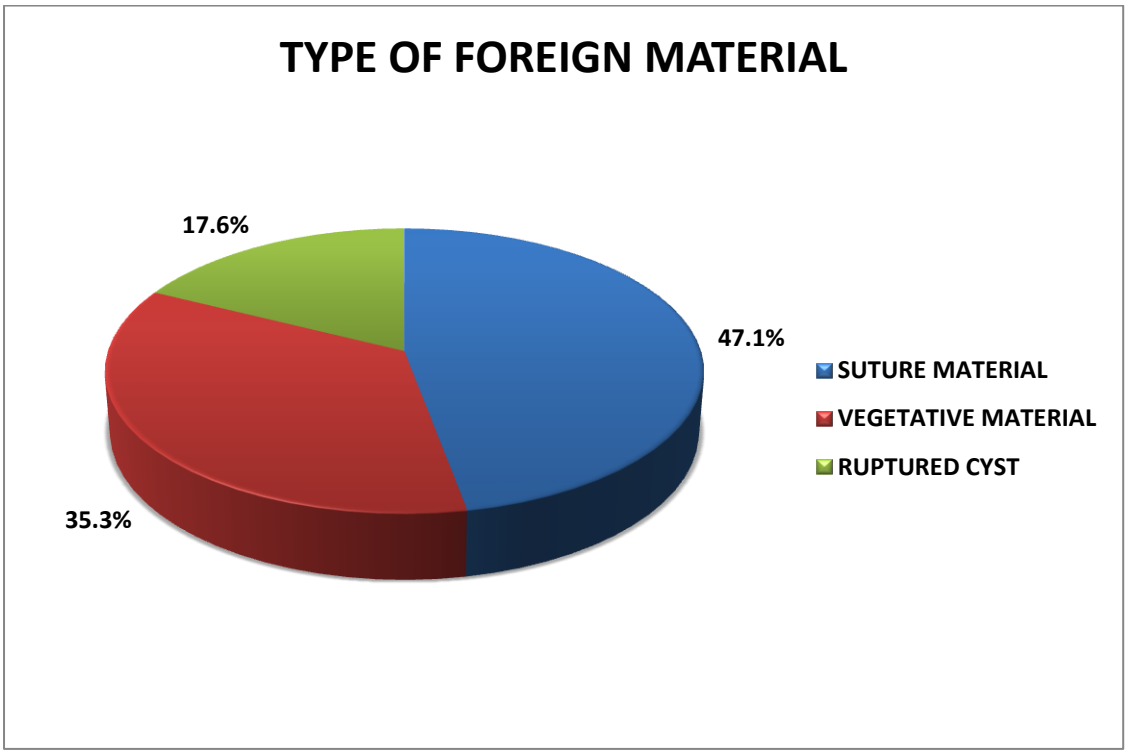
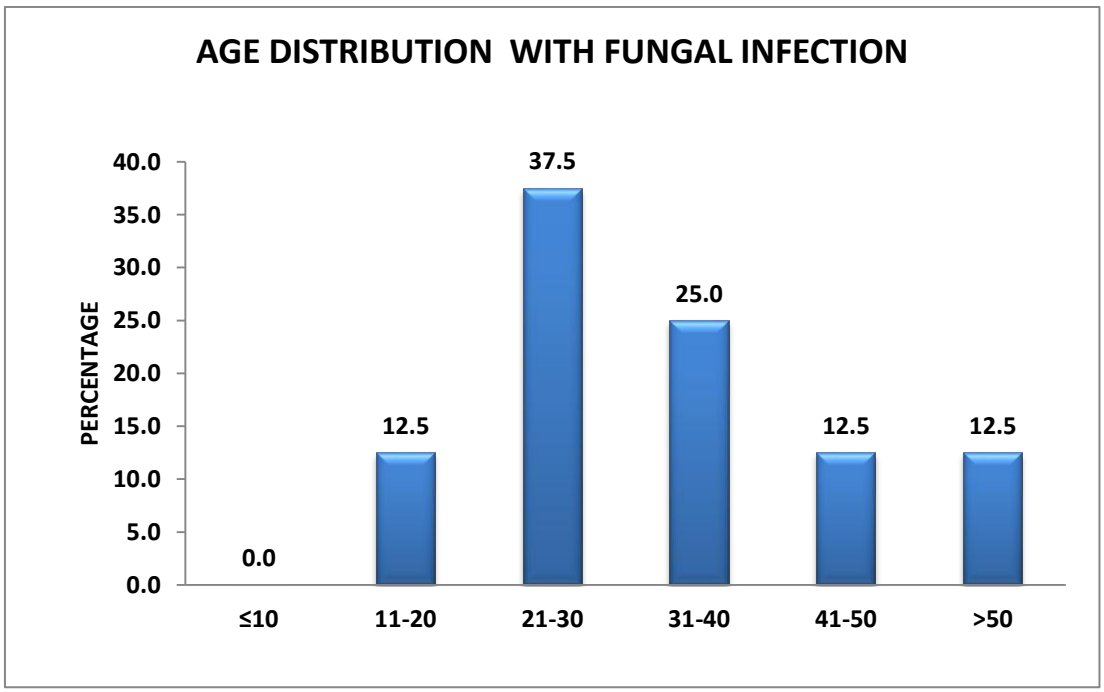


Table 14: Distribution of cases according to age in fungal infections

AGE	N	%
≤10	0	0.0
11-20	1	12.5
21-30	3	37.5
31-40	2	25.0
41-50	1	12.5
>50	1	12.5
Total	8	100.0

Figure 12: Pie diagram showing distribution of cases according to age in fungal infections



Total 8(5.9%) cases of fungal infection were seen in present study. Majority of cases were in the age group of 21-30 years age followed by 31-40 years with slight male 7(87.5%) preponderance. The detailed distribution of age group and the male female distribution of the cases are represented in the Table (14 and 15) and Figure (12 and 13).

Table 15: Distribution of cases according to sex in fungal infections

SEX	N	%
Male	7	87.5
Female	1	12.5
Total	8	100.0

Figure 13: Pie Diagram Showing Distribution of cases according to sex in fungal infections

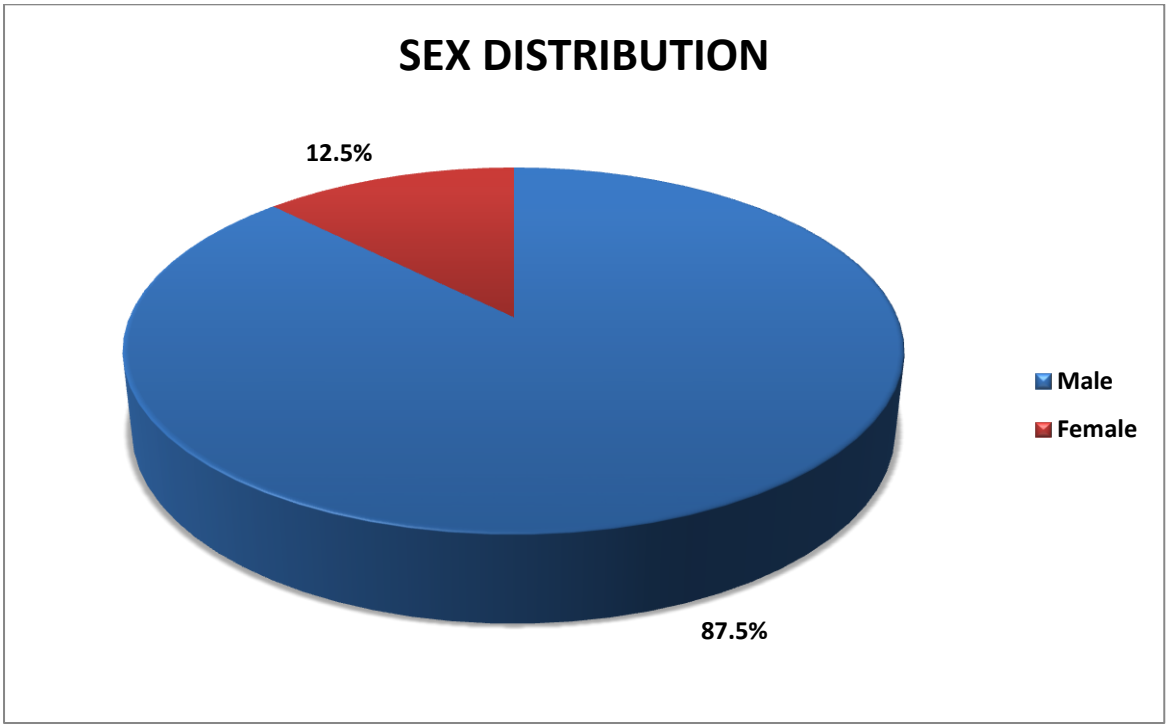
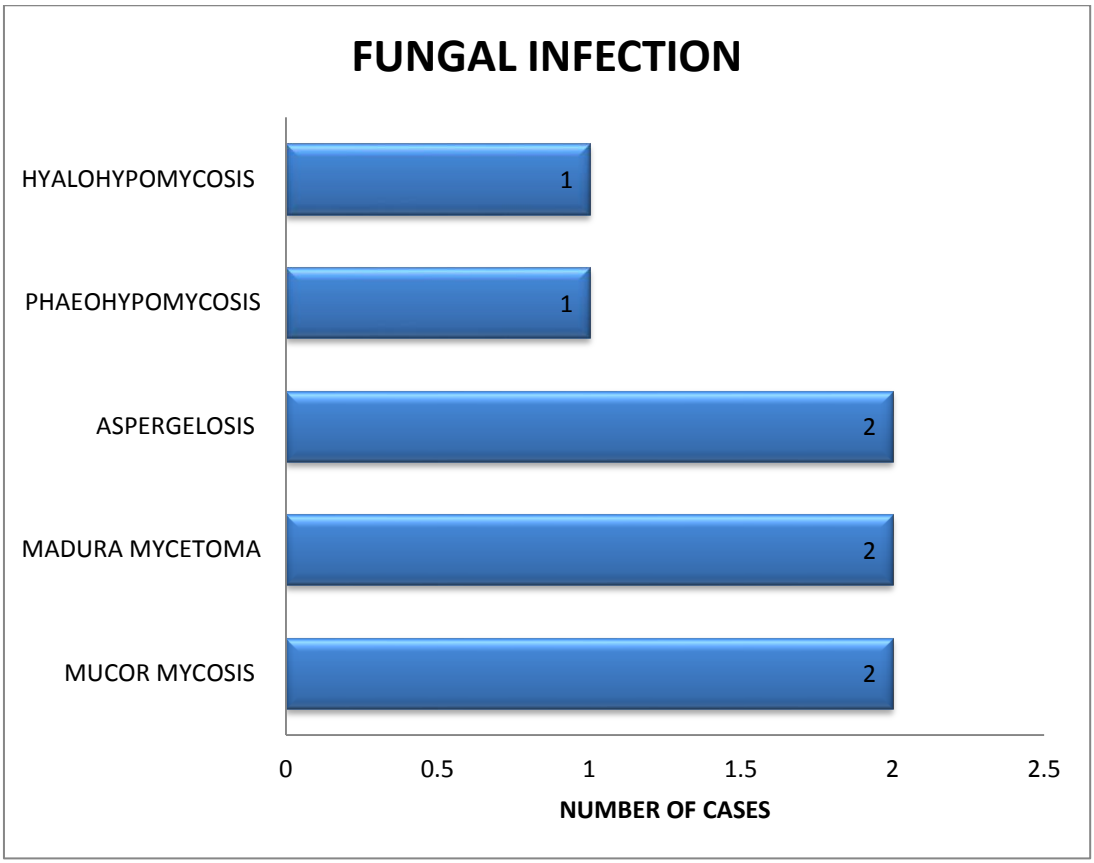


Table 16: Distribution of cases according to site and type of Fungal Infection.

UPPER LIMB	Fore arm	Phaeohypomycosis(1 case)
		Hyalohypomycosis(1 case)
	Right finger	Madura mycetoma(1 case)
LOWER LIMB	Foot	Madura mycetoma(1 case)
HEAD AND NECK	Nose and paranasal sinus	Mucor mycosis (2 cases)
	Nasal polyp	Aspergilosis(1 case)
	Ear	Aspergilosis(1 case)

The most commonly involved site was nose and paranasal sinus infected by mucormycosis. Madura mycetoma was seen in foot and in right finger. The rare cases of phaeohypomycosis and hyalohypomycosis were seen in forearm and cases of aspergillosis seen in nasal polyp and in ear. All the cases were confirmed by PAS stain. The detailed distribution of site of involvement is represented in Table (16) and Figure (14)

Figure 14: Distribution of cases according to fungal infection



In our study there were two cases each of Mucormycosis, Madura Mycetoma and Aspergillosis. One cases each of Phaeohypomycosis and Hyalohypomycosis.

PHOTOMICROGRAPHS

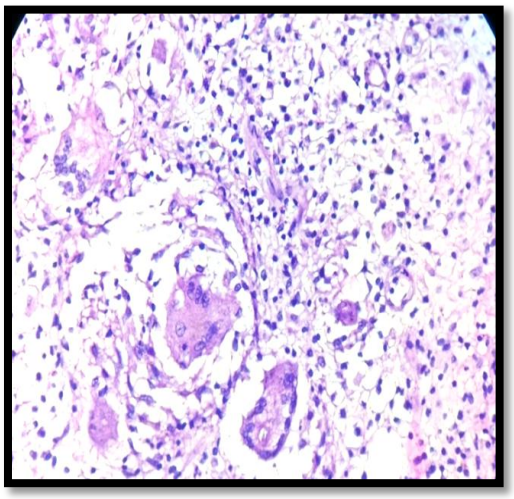


Figure 15: Microphotograph showing well formed granuloma with aggregates of epithelioid cell, lymphocytes and giant cells on H & E stain (400X).

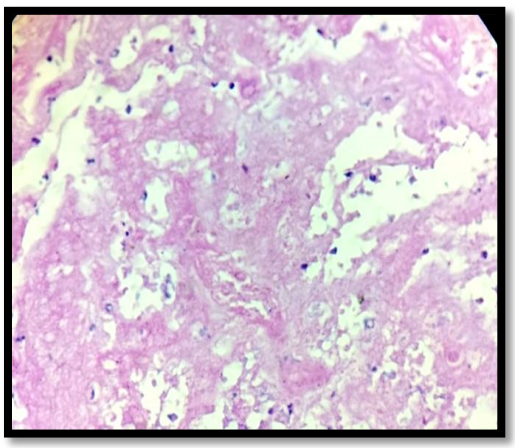
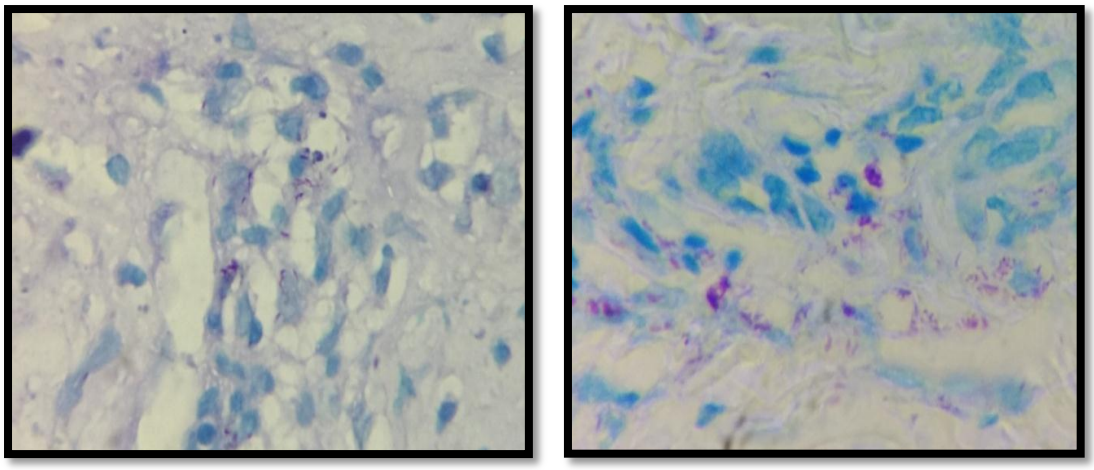


Figure 16: Microphotograph showing Large areas of caseous necrosis in case of tuberculosis on H & E stain (400X).



A **B**

Figure 17: Microphotograph showing AFB-Positive by conventional ZN stain (A) and FF stain (B) (1000X)

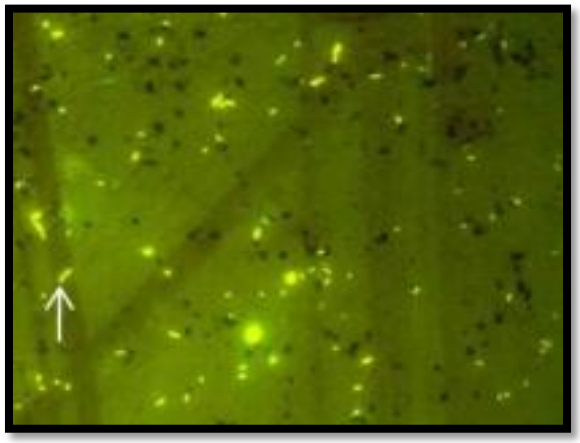


Figure 18: Microphotograph showing Plenty of slender yellow to orange rod shaped bacilli on AR (Auramine- Rhodamine, 400X)

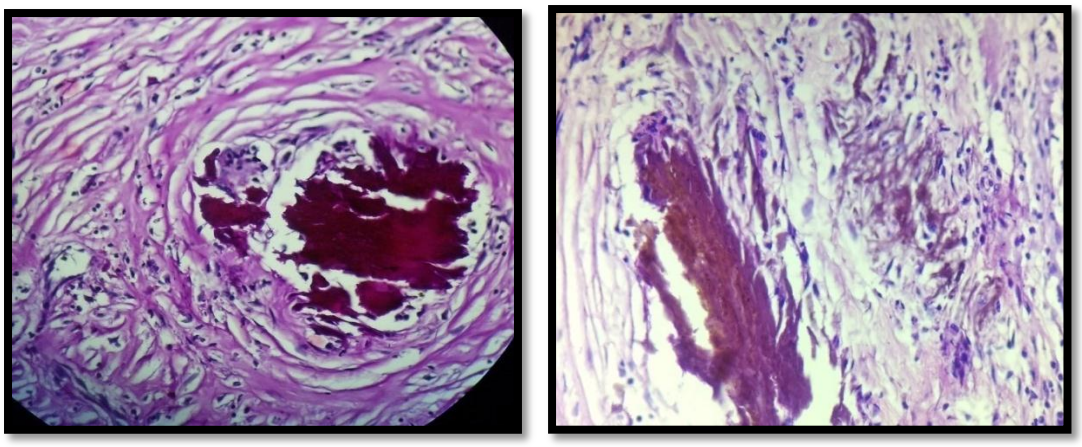


Figure 19: Microphotograph of Phaeohypomycosis on PAS stain (400X)

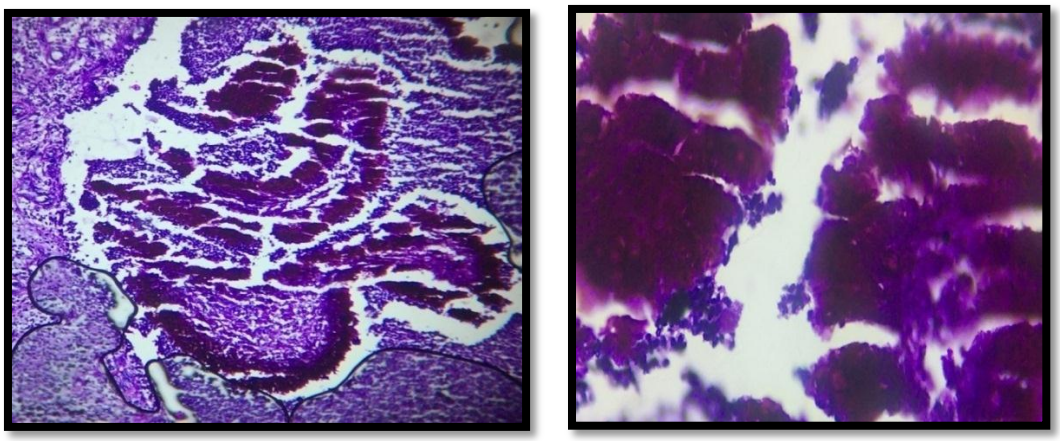


Figure20: Microphotograph of Madurella Myecetomatis on PAS stain (400X)

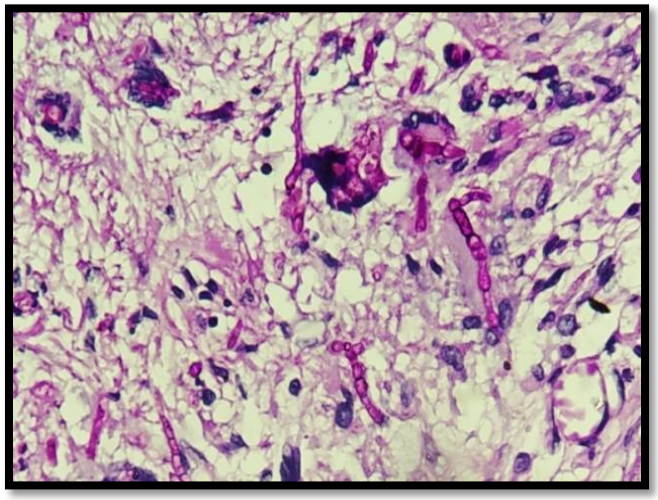


Figure21: Microphotograph of Hyalohypomycosis on PAS stain (400X)

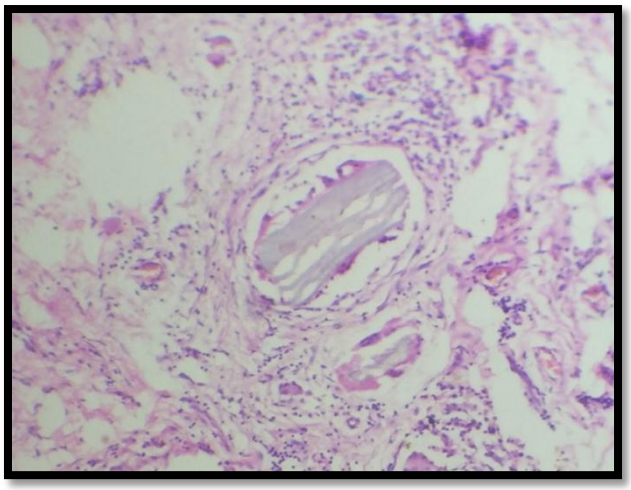


Figure 22: Microphotograph of suspected foreign body on H & E stain (400X)

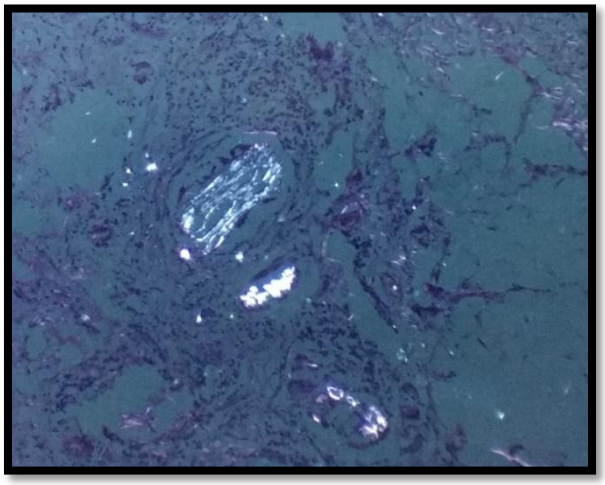


Figure 23: Microphotograph of foreign body on polarized microscope (400X)

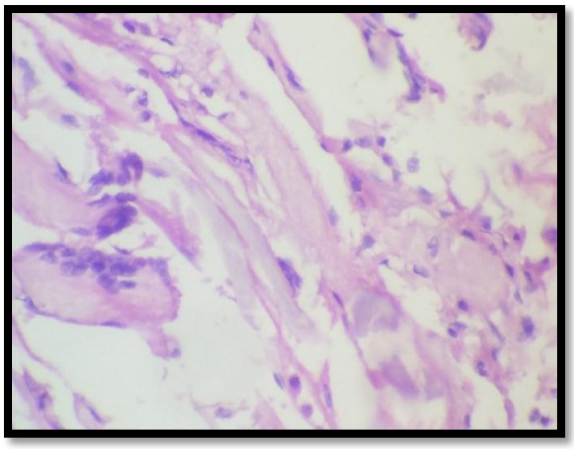


Figure 24: Microphotograph of suspected foreign body on H & E stain (400X)

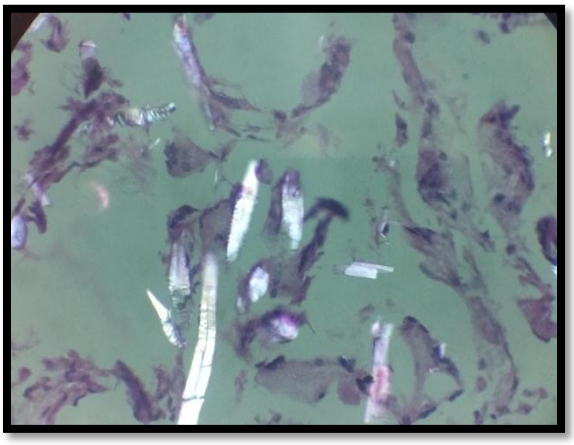


Figure 25: Microphotograph of foreign body on polarized microscope (400X)

DISCUSSION

Granulomatous inflammation is a distinctive pattern of chronic inflammation encountered in a limited number of infectious and non-infectious conditions. Recognition of this pattern in a histopathology biopsy sections is important because of the significance of the diagnosis associated with it. The arrival at an appropriate etiological diagnosis helps in the better management of the case. The morphologic pattern in various granulomatous diseases may be sufficiently different to allow reasonable accurate diagnosis by the pathologist. However, offering a definitive diagnosis on morphological examination alone is not possible, even with all the clinical information being available.

At times the diagnostic approach to granulomatous inflammation is not straight forward. In such cases, special stains form an adjunct to the morphological examination and aids in reaching a diagnosis, incorporating fluorescent stains, polarization microscopy and fluorescent microscopy into the diagnostic routine may further refine the diagnosis in ascertaining the etiology. However, in few cases, no definitive diagnosis can be given other than that of granulomatous inflammation even after utilizing all the available techniques.

In the present study, the most common etiology for granulomatous inflammation was Tuberculosis in nearly half of the cases 47.4% (64 cases). Amongst these, 26.56% (17cases) were positive for AFB on ZN staining. The ZN positivity is varied as the detection of AFB is having a wide range for sensitivity and specificity among the study groups. Fluorescent microscopy has been made available in various centers by the Revised National Tuberculosis Control Program (RNTCP) initiative wherein the equipment and the stains like Auramine Rhodamine (AR) are utilized. This

technique is simple, faster, cost-effective and has improved the detection rates thereby making a difference in the management of Tuberculosis in developing countries. In the present study, with the help of this AR stain, an additional 14.06% (9cases) demonstrated AFB in case of Tuberculosis, thus detection of AFB had increased from 26.56% to 40.62%. Similarly in leprosy, an additional 15.78% (6 cases) has been detected, and the detection of lepra bacilli has increased from 31.57% to 47.36%.

With the improvements and refinements in molecular techniques, investigations such as polymerase chain reaction, which made the procedure of the sample collection and processing a simple process by providing Ready to Use specimen collection containers, are making inroads into the developing countries. However , is still an expensive process and is not available at all the centers as of now.

The demonstration of the organisms was not possible in case of 73.43% (47 cases) even after performing ZN and AR, this is probably due to low bacillary load and large areas of caseous necrosis. Based on the clinical presentation and classical morphological appearance on the bright field, H& E staining these cases were offered a diagnosis of Tuberculosis and in leprosy depending on past history, histomorphology patterns and leprosy spectrum rest cases were diagnosed as Leprosy.

Table17- Comparison of etiology with the other studies

ETIOLOGY	Harish *et al*[53]	Jayashree *et al*[54]	Babaria *et al*[1]	Present study
Tuberculosis	47.26%	49.41%	56.33%	47.4%
Leprosy	12.72%	17.65%	17.67%	28.1%
Foreign body	8.36%	14.12%	12.67%	12.6%
Actinomycosis	8.73%	5.88%	1.33%	3%
Autoimmune	1.45%	1.18%	0.33%	1.5%
Fungal infection	1.10%	-	-	5.9%
Cholesterol granuloma	-	-	-	0.7%
Crohn's disease	-	-	-	0.7%
Unknown etiology	8%	-	11.34%	-
Rhinoscleroma	5.10%	11.76%	-	-
Total number of cases	275	170	300	135

The studies done by Harish *et al*[53] and Jayashree *et al*[54] had nearly half of the cases caused by Tuberculosis in concordance with the present study. Babaria *et al*[1] had 56.33% of tuberculosis cases in their study with a sample size of 300 cases. In developing countries, the diagnosis of TB is made on symptoms based algorithms. Initially the diagnosis was made based on certain patterns like Granulomatous inflammation, Caseous necrosis, Langhan's type of giant cells. But many viral infections, bacterial infections can also simulate the same histomorphological patterns. So laboratory test plays an important role to establish the cause of such granulomatous inflammation because the prognosis and treatment will differ. In the present study the youngest patient with TB granulomatous inflammation was 5years

old and oldest was 66years old. Majority of patients were in 21-30years of age with definitive diagnosis and morphological appearance.

Next in order, In the present study with 28.1%, was leprosy which was on the higher side in comparison to data reported by Harish *et al* [53](12.72%), Jayashree *et al* [54](17.65%) and Babaria *et al*[1](17.67%). This can be attributed to the reason that our institution is a referral center with a specialty Leprosy clinic and geographical variation of incidence.

The present study showed male predominance with Male: Female ratio of 1.11:1 and it correlated well with the studies done by Harish *et al*[53], Jayashree *et al*[54], Gautam *et al*[55] and Babaria *et al*[1] with the ratio of 1.09:1, 1.18:1, 1.7:1 and 1.25:1 respectively.

Table18: Comparison of results of ZN stain in tuberculosis for the demonstration of AFB in the present study with other studies.

	Babaria *et al*[1]	**Harish *et al*[53]**	**Jayashree *et al*[54]**	**Present study**
Positive	20.71%	20.62%	22.62%	26.56%
Negative	79.23%	77.38%	77.38%	73.43%

The present study correlated well with the study done by Babaria *et al*[1], Harish *et al*[53] and Jayashree *et al*[54] where ZN positivity was 20.71%, 20.62% and 22.62% respectively. Staining of ZN stain was done on thin sections (3µ) of the block, complete deparaffinization and positive control slides were taken for every batch. Extensive search for the bacilli was a prerequisite before reporting it as negative, which may give a false negative result.

Table19: Comparison of FF stain with other studies

	Positive	**Negative**
Babaria *et al*[1]	11(20.75%)	42(79.25%)
Harish *et al*[53]	09(25.74%)	26(74.28%)
Present study	11(28.94%)	27(71.05%)

Among the leprosy cases, the present study correlated well with the study done by Babaria *et al*[1] and Harish *et al*[53] where FF positivity was 20.75% and 25.74% respectively. The positivity of FF stain was comparatively high in the present study, this could be due to, as a part of protocol, minimum of six sections were examined before declaring them negative. According to Elder DE *et al* [23], there should be 1,000 bacilli per cubic centimeter of tissue in order to detect 1 bacillus in a section.

Table 20: Showing comparison of Auramine Rhodamine and ZN stain in tissue sections in the present study with other study

ZN stain	**Jayashree Pawal *et al*[54]**			**Present study**		
	AR +ve	**AR -ve**	**Total**	**AR +ve**	**AR -ve**	**Total**
ZN+ve	01(4%)	00	01(4%)	17(26.56%)	00	17
ZN –ve	19(76%)	05(20%)	24(96%)	09(14.06%)	38(59.37%)	47
Total	20(80%)	05(20%)	25	26(40.62%)	38(59.37%)	64
%	80	20		40.6	59.3	

In the present study, Sixty four cases of tuberculosis were diagnosed on the H&E stain. All the cases were stained with ZN along with a positive control, wherein ZN stain was positive in 26.56% (17cases) and rest 73.43% (47 cases) was negative. The AR stain was positive in all the cases of ZN positive cases and in ZN negative cases AR was positive in 14.06% (9 cases), thus the detection of AFB with the help of AR stain has increased to 40.62 % (26 cases). In the study done by Jayashree Pawal *et al*[54], 25 cases were randomly selected for Auramine Rhodamine staining, out of these, 24 cases were negative for the ZN stain and 1 case was positive for ZN stain, 19 cases showed positive with AR stain and 5 cases were negative out of 24 negative ZN stained cases, One case which is positive for ZN stain showed positivity in AR stain too. The disparity in the detection of ZN positive and AR positive cases is probably due to the random selection of cases for AR staining in the study done by Jayashree Pawal *et al*[54].

In the present study, among 102 cases including tubercular and leprosy, 28.43% (29 cases) were positive in ZN and FF stain. AR stain increased the detection of AFB to 49.01% (50 cases).

In the present study, two cases each of Mucormycosis, Madura Mycetoma and Aspergillosis were noted in the infectious causes along with very rare cases like Phaeohypomycosis and Hyalohypomycosis. In all of these cases special stain PAS was done, which has shown the positivity. In the study done by Jayashree Pawal *et al*[54], 88% (10cases) of fungal lesions were identified, with the majority being Maduramycosis (3cases) followed by P. boydii and Rhinosporidiosis of two cases each.

In both the cases of Mucormycosis, angioinvasion and extensive areas of necrosis were noted which have a significance in the management of the case. As these cases are usually seen in immunocompromised individuals, a look out for co-infection by another organism should be considered. PAS stain helped in distinguishing morphological features of mucormycosis.

These phaeohypomycosis fungi (Fig:19) are pigmented spores. The organisms are found within the cavity associated with histiocytes. The hyphae forms often have irregularly placed branches and show constrictions around their septae that may resemble pseudohyphae or yeast forms.

Hyalohypomycosis (Fig:21) are hyaline septae fungal hyphae. In that Fusarium species causes a broad spectrum of infection. These fungal hyphae are difficult to appreciate on H and E stain, these appear similar to those of aspergillus species, with hyaline septa and branching at right angles. But the presence of sporulation and the presence of hyphae and yeast-like structures together suggest fusarium. To demonstrate the morphology of the fungus, PAS stain is needed.

All cases of actinomycosis showed suppurative granulomas having central actinomycotic colonies, which contains characteristic sulfur granules and radiating eosinophilic deposits (Splendor-Hoeppli phenomenon). Two cases of autoimmune etiology were noted, it was diagnosed based on morphology and serology. One each of cholesterol granuloma and crohns were diagnosed based on morphology.

In the present study, a total of 25 cases were suspected to have foreign body material on H and E stain, out of which 17 cases had shown to have a foreign body on polarized microscopy. Most commonly encountered foreign material was suture material followed by vegetative matter. Suture material mostly was observed in the

fallopian tube. The vegetative matter was demonstrated in cases of fistula in ano. In a study done by Manjunatha BS *et al* [31], they stated that the vegetable matter examined under polarized light appeared as a birefringent refractile matter. The appearance of such material was characteristic on polarized microscopy as described in figure (23).

The foreign body material is sometimes very difficult to appreciate on the routine H and E stain, the tissue sections will show granulomas with many giant cells, which are nonspecific findings where a definite etiology cannot be ascribed without utilizing the polarized microscopy and special stains.

On histomorphological examination, three cases showed thin fragments of foreign material on tissue sections stained with H and E stain. However, due to the lack of contrast and dense and diffuse inflammation on H & E examination their morphology was not appreciated. With the aid of polarized microscopy, foreign body material was appreciated with certainty by observing the birefringence and the characteristic appearance which was specific to few of the materials such as suture material appeared as linear or curvilinear refractile material and vegetative matter as birefringent refractile matter.

In majority of the cases, the most common location of the foreign body material was within the cytoplasm of the giant cells or in the center of the granulomas. So by using this simple polarized filters to the routine microscope, we can establish a confirmatory diagnosis in cases of suspected foreign body granuloma.

The morphologic spectrum in granulomatous lesion varies depending on the etiology of the disease. Tuberculosis in the developing countries such as India continued to be the most common cause of granuloma formation.

Routinely performed ZN staining has low sensitivity as it rarely detects AFB when there is a low bacillary load. However, in the present study, we used AR stain

for detection of AFB. It is observed that whenever there is scant AFB positivity, searching for them is a tedious process on conventional ZN and FF stain as compared to AR stain. On AR, AFB are easily detectable under low power view, the large area of the section can be screened within a short period of time, which reduces the turnaround time. Although the PCR technique was not done in the present study it gives positive result even with a low bacillary load. In a study conducted by Yoo Jin Lee *et al* [56], higher sensitivity of PCR was seen in specimens containing necrosis. They also stated that long duration of formalin exposure causes DNA fragmentation and decreases DNA's quality. Two to four hours of formalin exposure gives successful amplification. They also correlated PCR with histologic findings and noted that its sensitivity was higher in necrotizing granulomatous inflammation and lower in non specific inflammation.

Polarized microscopy helps in confirming as well as detecting foreign body previously which were of unknown etiology. This is the simple and cost-effective method for detecting suspected foreign body granulomas.

H & E stain does not differentiate the morphology of fungus clearly. Hence PAS stains will help for diagnosis and confirmation of fungal etiology. Cooperation between the clinician and the pathologist is more important to derive the greatest benefit from the biopsy.

Based on the observation in the present study following recommendations have been proposed:

1) In suspected cases of Tuberculosis/ Leprosy on HPR, we recommend to do AR stain under fluorescence microscope.
2) AR negative with histopathology showing suspected morphology, then subject the tissue for PCR.
3) Simple use of polarized lenses on routine microscopy will aid in the identifying the foreign body.
4) PAS stain and other fungal stain should be routinely practiced in suspected cause of fungal infections.

CONCLUSION

Histopathological examination of granulomatous lesions and finding the etiology in the biopsy specimen is very important for specific treatment. An aid of ancillary techniques is very helpful in finding out the etiology of granulomatous inflammation which is previously of unknown etiology. The present study was undertaken to determine the utility of ancillary techniques like special stain, fluorescence microscopy and polarized microscopy in identifying etiology of the granulomatous lesion on histopathology.

AR stain can be used as a supplementary tool in detecting acid fast bacilli where the bacillary load is scant. AR stain helps in rapid detection of bacilli and helps to improve turnaround time. In this study for all the cases of suspected Tuberculosis ZN stain and AR stain were done and ZN was positive in 26.56% (17 cases) and negative in 73.43% (47 cases). All the ZN positive cases showed positive result with the AR stain with 100% correlation. Among 73.43% (47 cases) ZN negative cases, additional 14.06% (9 cases) showed positive with the AR stain. Thus, AR stain increased the detection of AFB from 26.56% to 40.62%.

On the other hand, for all the leprosy cases FF stain and AR stain were done. FF positive was seen in 31.57% (12 cases) and negative in 68.42% (26 cases). All the FF positive cases showed positive result with the AR stain with 100 % correlation. Among 68.42% (26 cases) FF negative cases, additional 15.78% (6 cases) showed positive with the AR stain. Thus, AR stain increased the detection of lepra bacilli from 31.57% to 47.36%.

Polarizing filters are simple attachment used for the routine microscope, which helps in detection and identification of foreign body material. PAS stain helps in identifying the morphology of fungus.

Utilizing these simple ancillary techniques optimizes and improves the accuracy of histopathology reporting in granulomatous disorders.

SUMMARY

A prospective study of Role of Ancillary Techniques in Identifying Etiology of Granulomatous Lesions in Histopathology was undertaken, during 1st September 2016 to 31st August 2018 in the Department of Pathology, B.L.D.E.(Deemed to be University) Shri. B. M. Patil Medical College, Hospital & Research Centre, Vijayapura.

The salient features observed in this study are –

A total of 135 cases were included in the present study which are showing granulomatous inflammation with age ranged from 5 to 65 years and mean age is 23years. Increased incidence was observed in 2nd to 3rd decade with slightly male preponderance. Male to Female ration is 1.11: 1. Most common cause of granuloma was tuberculosis (64 cases,47.4%) followed by leprosy (38cases ,28.1%), foreign body granulomas (17 cases,12.6%), fungal infection (8 cases,5.9%) , actinomycosis (4cases ,3%), autoimmune cause (2cases,2%) ,cholesterol granuloma (1case ,0.7%) and Crohn's disease (1case,0.7%).

The most common organs involved in tuberculosis were lymph node (22 cases, 34.4%) , followed by abdominal organs (13cases, 20.3%), skin(10cases,15.6%) , musculoskeletal system (5cases,7.8%), male genital system 4.7% ,kidney (2cases,3.1%), female genital tract (2cases, 3.1%) and each case in lung and submandibular gland (1.6%).

In this study for all the cases of suspected Tuberculosis ZN stain and AR stain were done and ZN was positive in 26.56% (17 cases) and negative in 73.43% (47 cases). All the ZN positive cases showed positive result with the AR stain with 100%

correlation. Among 73.43% (47 cases) ZN negative cases, additional 14.06% (9 cases) showed positive with the AR stain. Thus, AR stain increased the detection of AFB from 26.56% to 40.62%.

On the other hand for all the leprosy cases FF stain and AR stain were done. FF positive was seen in 31.57% (12 cases) and negative in 68.42% (26 cases). All the FF positive cases showed positive result with the AR stain with100 % correlation. Among 68.42% (26 cases) FF negative cases, additional 15.78% (6 cases) showed positive with the AR stain. Thus, AR stain increased the detection of lepra bacilli from 31.57% to 47.36%.

Foreign body granuloma was seen in 12.6% (17cases) out of which most common foreign body encountered was suture material 47.1% (8 cases), followed by vegetative material 35.3% (6 cases) and 17.6% (3 cases) of ruptured cyst. All these foreign material was confirmed by using polarized microscopy with characteristic morphological features.

Two cases each of Mucormycosis, Madura Mycetoma and Aspergillosis. One case each of Phaeohypomycosis and Hyalohypomycosis were note. All these were confirmed by PAS stain.

All the cases of Actinomycosis showed suppurative granulomas having central actinomycotic colonies with splendore hoeppli phenomenon.

With introducing simple ancillary techniques like ZN, FF, AR (Flurosent microscopy), polarized microscopy and special stains helped in accessing the more convincing etiology.

In Tuberculosis only with ZN stain the detection of AFB was 26.56% and with the help of AR stain the detection rate has increased up to 40.62%.

In leprosy, FF stain alone detected 31.57% of lepra bacilli. The AR stain has increased the detection rate up to 47.36%.

In foreign body granuloma polarized microscopy helped in detecting characteristic features and identification of particular foreign body material.

In fungal infection, PAS stain helped in identification of fungal elements.

BIBLIOGRAPHY

1. Babaria K, Agravat A, Dhruva G. Granulomatous reaction - A Histopathological study: a retrospective and prospective study of 5 years. Int J Res Med Sci 2015;1(3):201-6.
2. Kumar V, Abbas AK, Aster JC. Inflammation and Repair. In: *Robbins and Cotran Pathologic Basis of disease*. 9th Ed. New Delhi: Elsevier; 2014. p 93-97.
3. Blessing K. Mini-symposium: Inflammatory skin pathology: cutaneous granulomatous inflammation. Cur Diag Pathol 2005;11(4):219–35.
4. Shah KK, Pritt BS, Alexander MP. Histopathologic review of granulomatous inflammation. Journal of Clinical Tuberculosis and Other Mycobacterial Diseases. 2017 May 1;7:1-2.
5. H Herzog B. History of tuberculosis. Respiration. 1998; 65(1):5-15.
6. Frith J. History of tuberculosis. Part 1-phthisis, consumption and the white plague. Journal of Military and Veterans Health. 2014 Jun;22 (2):29.
7. Seth V, Seth R. History of tuberculosis. Seth V, Kabra SK (eds). *Essentials of tuberculosis in children*. 2nd ed. New Delhi: Jaypee brothers. 2001: p3-6.
8. Tille PM. Mycobacteria. In: Bailey and Scotts Diagnostic Microbiology. 14th Ed. St.Louis, Missouri; 2017.p524-26.
9. Anantnarayan R, Paniker J. Mycobacterium tuberculosis. In : *Textbook of microbiology*. 13th Ed. Universities press (India) Pvt Ltd; 2017. p351-53.
10. McAdam AJ, Milner DA, Sharpe AH. Infectious Diseases. In: Kumar V, Abbas AK, Aster JC(eds) *Robbins and Cotran Pathologic Basis of disease*. 9th Ed. New Delhi: Elsevier; 2014. p 341-402.

11. Russell DG, Cardona PJ, Kim MJ, Allain S, Altare F. Foamy macrophages and the progression of the human tuberculosis granuloma. Nature immunology. 2009 Sep;10(9):943.
12. Peyron P, Vaubourgeix J, Poquet Y, Levillain F, Botanch C, Bardou F et al. Foamy Macrophages from Tuberculous Patients' Granulomas Constitute a Nutrient-Rich Reservoir for M. tuberculosis Persistence. PLoS Pathogens. 2008;4(11):e1000204.
13. Rajpal S, Dhingra VK, Aggarwal JK. Sputum grading as predictor of treatment outcome in pulmonary tuberculosis. Indian Journal of Tuberculosis. 2002;49(3):139-42.
14. Prasoon D. Acid fast bacilli in fine needle aspiration smears from tuberculous lymphnodes: where to look for them. Acta cytol 2000;44(3):297-300.
15. Winn Jr WC, Allen SD, Janda WM, Koneman EW, Schreckenberger PC, et al. *Kinsman's color atlas and textbook of diagnostic microbiology*. 6th Ed.Philadelphia: Lipincott Williams and Wilkins 2006;1065-124.
16. Chattopadhyay S, Ghosh T, Saha S, Mondal SK, Ghosh AK. Tuberculosis lymphadenitis-A changing face. J of cytology 2006;156-67.
17. Robbins G, Tripathy VM, Misra VN, Mohanty RK, Shinde VS, Gray KM,et al. (2009) Ancient Skeletal Evidence for Leprosy in India (2000 B.C.). PLoS ONE 4(5): e5669.
18. Rastogi N, Rastogi RC. Leprosy in ancient India. International journal of leprosy and other mycobacterial diseases: official organ of the International Leprosy Association. 1984 Dec;52(4):541.
19. Fite GL, Cambre PJ, Turner MH. Procedure for demonstrating lepra bacilli in paraffin sections. Arch. Pathol.1947;43(6):624-5.

20. Ottenhoff TH, Verreck FA, Hoeve MA, vande Vosse E. Erratum to “control of human host immunity to mycobacteria”: Tuberculosis. 2006 Jan 1;86(1):74-5.
21. Ridéey D, Jopling WH. Classification of leprosy according to immunity. A five-group system. International journal of leprosy. 1966;34(3):255-73.
22. Ko CJ, Glusac EJ, Shapiro PE. Noninfectious granuloma. In: Elder DE (ed) *Lever’s Histopathology of skin*. 10th Ed. Philadelphia, USA: Lippincott Williams and Wilkins; 2009. p361-87.
23. Johnston RB. Granulomatous reaction pattern. In: *Weedon’s Skin Pathology Essentials*. 2nd Ed. USA: Elsevier; 2017. p137-62.
24. Nath I, Chaduvula M. Immunological Aspects.In: Kumar B, Kar Hk (eds) *IAP Textbook of Leprosy*. 2nd Ed. New Delhi ; Jaypee Brothers Medical Publishers (P)Ltd;2016.p113-16.
25. Turk JL. Leprosy as a model of subacute and chronic immunologic diseases. Journal of Investigative Dermatology. 1976 Sep 1;67(3):457-63.
26. Azulay RD. Histopathology of skin lesions in leprosy. International journal of leprosy and other mycobacterial diseases: official organ of the International Leprosy Association. 1971;39(2):244.
27. Lara CB, Nolasco JO. Self-healing, or abortive, and residual forms of childhood leprosy and their probable significance. Int. J. Lep. 1956 Jul; 24.
28. Yamamura M, Wang XH, Ohmen JD, Uyemura K, Rea TH, Bloom BR, Modlin RL. Cytokine patterns of immunologically mediated tissue damage. The Journal of Immunology. 1992 Aug 15;149(4):1470-5.
29. Jullien D, Sieling PA, Uyemura K, Mar ND, Rea TH, Modlin RL. IL-15, an immunomodulator of T cell responses in intracellular infection. The Journal of Immunology. 1997 Jan 15;158(2):800-6.

30. Lucas S. Bacterial Diseases. In: Elder DE (ed) *Lever's Histopathology of skin*. 10th Ed. Philadelphia, USA: Lippincott Williams and Wilkins; 2009. p549-67.
31. Manjunatha BS, Kumar GS, Raghunath V. Histochemical and polarization microscopic study of two cases of vegetable/pulse granuloma. Indian Journal of Dental Research. 2008 Jan 1;19(1):74.
32. Mowry RG, Sams WM, Caulfield JB. Cutaneous silica granuloma: a rare entity or rarely diagnosed? Report of two cases with review of the literature. Archives of dermatology. 1991 May 1;127(5):692-4.
33. Kasper CS, Chandler PJ. Talc deposition in skin and tissues surrounding silicone gel-containing prosthetic devices. Arch Dermatol .1994 Jan 1;130(1):48-53.
34. Leonard DD. Starch granulomas. Arch Dermatol.1973;107:101-3.
35. Nygaard F, Mondragon G, King DF, King LA. The appropriate processing of tophi for microscopy. The American Journal of Dermatopathology. 1982 Jun 1;4(3):239-40.
36. Savina LV, Pavlishchuk SA, Samsygin V, Bolotova EV, Gotovtseva LP, Chekmareva SE,et al. Polarization microscopy in diagnosis of metabolic disorders. Klin Lab Diagn 2003;3:11-4.
37. Dongre A, Bhisey P, Khopkar U. Polarized light microscopy. Indian Journal of Dermatology, Venereology, and Leprology. 2007 May 1;73(3):206.
38. Vander MS, Hossain MA, Ghannoum MA. Cutaneous infections dermatophytosis, onychomycosis, and tinea versicolor. Infectious disease clinics of North America. 2003 Mar;17(1):87-112.

39. Grand CNP, Lobo AZC, Mihm MC. Skin Infections. In: Kradin RL(ed)*Diagnostic pathology of infectious disease*.9th Ed. Philadelphia: Elsevier;2010.p576-77.
40. Fothergill AW. Identification of dematiaceous fungi and their role in human disease. Clinical infectious diseases. 1996 May 1;22 (Supplement_2):S179-84.
41. Ziefer A, Connor DH. Phaeomycotic cyst. The American journal of tropical medicine and hygiene. 1980 Sep 1;29(5):901-11.
42. Kauffman HF. Immunopathogenesis of allergic bronchopulmonary aspergillosis and airway remodeling. Front Biosci. 2003 Jan 1;8:e190-6.
43. Moore CP, Willkens RF. The subcutaneous nodule: its significance in the diagnosis of rheumatic disease. In: Seminars in arthritis and rheumatism. WB Saunders. 1977 Aug 1 (7). p. 63-79.
44. Veys EM, Keyser FD. Rheumatoid nodules: differential diagnosis and immunohistological findings. Annals of the rheumatic diseases. 1993 Sep;52(9):625.
45. Patterson JW. Rheumatoid nodule and subcutaneous granuloma annulare. A comparative histologic study. The American journal of dermatopathology. 1988 Feb;10(1):1-8.
46. James WD, Berger TG, Elston DM. Macrophage/ Monocyte disorder. In: Andrews' Diseases of Skin. 12th Ed. Philadelphia,2016:p706-708.
47. Ikonomopoulos JA, Gorgoulis VG, Zacharatos PV, Manolis EN, Kanavaros P, Rassidakis A, et al. Multiplex polymerase chain reaction for the detection of mycobacterial DNA in cases of tuberculosis and sarcoidosis. Modern pathology: an official journal of the United States and Canadian Academy of Pathology, Inc. 1999 Sep;12(9):854-62.

48. Nilsson K, Pahlson C, Lukinius A, Eriksson L, Nilsson L, Lindquist O. Presence of Rickettsia helvetica in granulomatous tissue from patients with sarcoidosis. The Journal of infectious diseases. 2002 Apr 15;185(8):1128-38.

49. Li N, Bajoghli A, Kubba A, Bhawan J. Identification of mycobacterial DNA in cutaneous lesions of sarcoidosis. Journal of cutaneous pathology. 1999 Jul;26(6):271-8.

50. Tchernev G, Patterson JW, Nenoff P, Horn LC. Sarcoidosis of the skin–a dermatological puzzle: important differential diagnostic aspects and guidelines for clinical and histopathological recognition. Journal of the European Academy of Dermatology and Venereology. 2010 Feb;24(2):125-37.

51. Ziegenhagen MW, Müller-Quernheim J. The cytokine network in sarcoidosis and its clinical relevance. Journal of internal medicine. 2003 Jan;253(1):18-30.

52. Williams GT, Williams WJ. Granulomatous inflammation--A review. J Clin Pathol 1983;36(7):723–33.

53. Permi HS, Shetty J, Padma SK, Teerthanath S, Mathias M, Kumar S. A histopathological study of granulomatous inflammation. NUJHS. 2012;2(1):15-9.

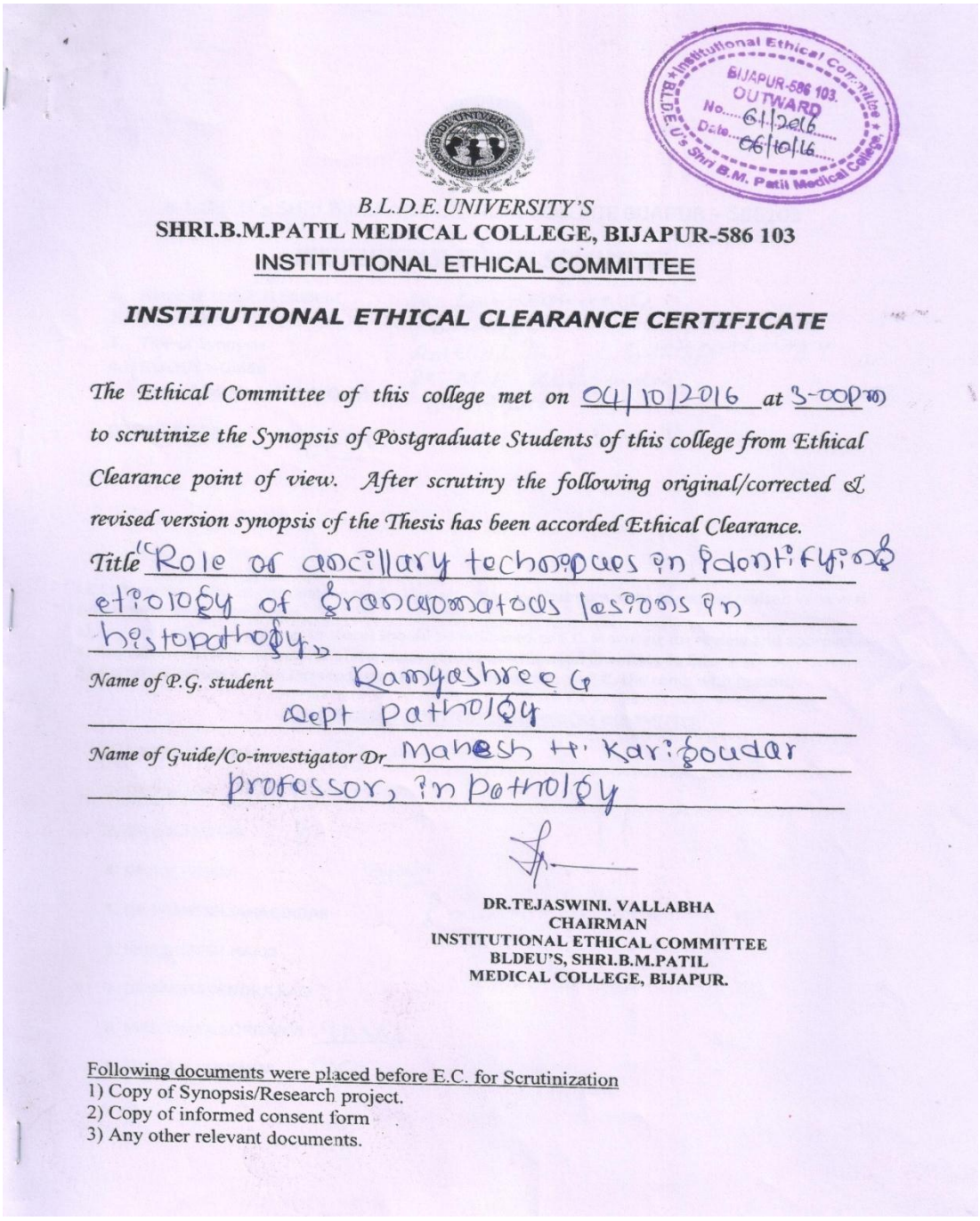
54. Pawale JS, Puranik R, Kulkarni MH. A histopathological study of granulomatous inflammations with an attempt to find the aetiology. J Clin Diagn Res 2011;5:301-6.

55. Gautam K, Pai RR, Bhat S. Granulomatous lesions of the skin. Journal of Pathology of Nepal 2011;1:81-86.

56. Lee YJ, Kim S, Kang Y, Jung J, Lee E, Kim JY, et.al., Does polymerase chain reaction of tissue specimens aid in the diagnosis of tuberculosis?. Journal of pathology and translational medicine. 2016 Nov;50(6):451.

ANNEXURES

ETHICAL CLEARANCE CERTIFICATE



B.L.D.E. UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103
INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 04/10/2016 at 3-00pm to scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis has been accorded Ethical Clearance.

Title "Role of ancillary techniques in identifying etiology of granulomatous lesions in histopathology"

Name of P.G. student Ramyashree G
Dept Pathology

Name of Guide/Co-investigator Dr Mahesh H. Karigoudar
Professor, in Pathology

DR.TEJASWINI. VALLABHA
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

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

BLDE (DEEMED TO BE UNIVERSITY)

Shri. B. M. Patil Medical College, Hospital & R.C

Vijayapura, Karnataka

INFORMED CONSENT FOR PARTICIPATION IN DISSERTATION/RESEARCH

I, the undersigned, _____, D/O W/O, _____ aged _____years, ordinarily resident of do hereby state/declare that Dr. RAMYASHREE. G of _____Hospital Shri B M Patil Medical College, Hospital & R.C Vijayapura, Karnataka 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 Dr. RAMYASHREE.G informed me that she is conducting dissertation/research titled "**ROLE OF ANCILLARY TECHNIQUES IN IDENTIFYING ETIOLOGY OF GRANULOMATOUS LESIONS IN HISTOPATHOLOGY**" under the guidance of Dr. MAHESH H KARIGOUDAR 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.

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 me by the investigator will be kept secret and not assessed 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 my participation in 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:

PROFORMA FOR STUDY

Demographic details:

Name:

Age:

Sex: M/F

Occupation:

Residence:

OPD/IP NO:

HPR. No.:

Chief complaints:

History of present illness:

Past history:

Family history:

General physical examination:

Operative findings :

Tissue :

Site of biopsy :

Clinical diagnosis :

Macroscopy :

Microscopy :

- Granulomatous lesion +/-
- Single/ multiple
- Necrosis – caseous/fibrinoid/others

Special stain used :

Others : Polarized microscopy (if any)

Final diagnosis:

Comment:

KEY TO MASTER CHART

ZN	- Ziehl Neelsen stain
FF	- Fite Faraco stain
PAS	- Periodic Acid Schiff
AR	- Auramine Rhodamine
Positive	- 1
Negative	- 2
HPR	- Histopathology
L	- Leprosy
TB	- Tuberculosis
FB	- Foreign body
F	- Fungus
AI	- Autoimmune
LV	- lupus vulgaris
LN	- Lymphnode
BT	- Borderline tuberculosis
BL	- Borderline lepromatous leprosy
MM	- Madurella mycetomatis
PH	- Phaeohypomycosis