PREVALENCE OF GENITAL TUBERCULOSIS, ITS RISK FACTORS AND ASSOCIATED CLINICAL FEATURES AMONG THE POPULATION OF NORTHERN PART OF BIHAR

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

in

Clinical Microbiology

By

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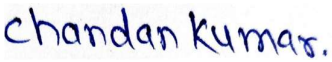
LOVELY PROFESSIONAL UNIVERSITY, PUNJAB
2025

DECLARATION

I, hereby declare that the presented work in the thesis entitled **Prevalence of genital tuberculosis, its risk factors and associated clinical features among the population of northern part of Bihar** in fulfilment of degree of **Doctor of Philosophy (Ph.D)** is outcome of research work carried out by me under the supervision of **Dr. Naresh Kumar,** working as Associate Professor Department of Medical Laboratory Sciences, School of Allied Medical Sciences, Lovely Professional University, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator.

I do also declare that I have made all the corrections as directed and recommended by my external examiners.

This work has not been submitted in part or full to any other University or Institute for the award of any degree.



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CERTIFICATE

This is to certify that the work reported in the Ph.D. thesis entitled "**Prevalence of genital tuberculosis, its risk factors and associated clinical features among the population of northern part of Bihar"** submitted in fulfillment of the requirement for the award of degree of **Doctor of Philosophy (Ph.D.)** in the Clinical Microbiology, is a research work carried out by Chandan Kumar (41800702) is bonafide record of his original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.

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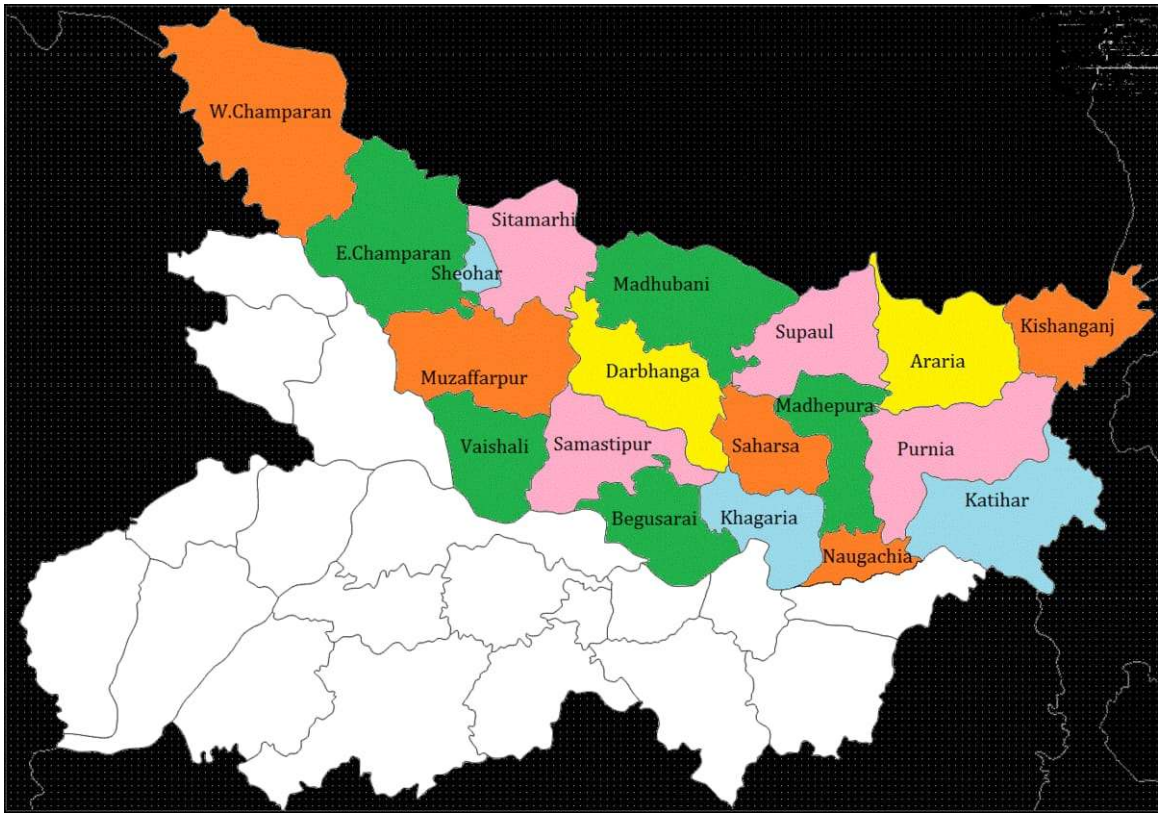

The State of Bihar

Begusarai and adjoining districts where the study population was taken

ABSTRACT

Tuberculosis is one of the top five fatal diseases mostly occur in reproductive age group. But it is also capable to produce disease in all age group people. Its different form, its ability to cause disease in every organ of the body and its capacity to escape from host immunity makes it one of the most dangerous deaths causing disease. Despite the continuous development in medical science there is not a single test to detect this disease at its early stages. Among all type tubercle infection, genital tuberculosis is responsible for irreversible changes in genital organs leads to infertility.

Methods: The study was carried out during January 22 to August 24 upon 342 subjects selected by inclusion and exclusion criteria. This study is based on classification of patient on their symptoms, clinical history and their pathological examinations.

Results: Clinical evaluation, pathological investigations, radiological examinations, and advanced diagnostic methods such as GeneXpert and interferon-gamma assays were employed. The study found that 9 patients (2.6%) were confirmed positive for GTB, with a higher prevalence among females (5.18%) than males (0.96%). GTB was most common in the 21–30 years age group, accounting for 55.6% of all positive cases. Key findings included significant associations between GTB positivity and prior TB contact history, symptomatic presentation, ESR, and abnormal leucocyte counts like particularly neutrophilia and lymphocytosis. Among 12 cases of infertility (3.5%), 4 female cases (1.16%) were directly linked to GTB. Additionally, 63 individuals presented with clinical and pathological features highly suggestive of TB but tested negative in confirmatory assays, highlighting the limitations of current diagnostic modalities. The study highlights the urgent need for improved diagnostic strategies, increased awareness, and better reproductive health support in low-resource settings. Public health interventions focusing on early detection and community education are vital for reducing the burden of genital tuberculosis and preventing its long-term complications, including infertility. In our study 45 (13.1 %) patients out of 342 are found tuberculosis positive. In which male and female positive cases are 5.8 % and 7.2 % respectively. We found that there is lacking of awareness due to illiteracy and inadequate ground level awareness campaign.

Conclusion: This study reveals that numerous hidden cases contribute to the spread of tuberculosis (TB), particularly genital TB, which often remains undiagnosed due to public ignorance and the unavailability of accurate diagnostic tools. Factors such as poverty, illiteracy,

overcrowded living conditions, early marriage, and poor personal hygiene significantly contribute to the prevalence and transmission of TB in the region. Although some cases were detected through clinical symptoms and pathological investigations, many remain undiagnosed, especially in asymptomatic individuals. The study highlights the lack of a single diagnostic test capable of rapidly and accurately detecting *Mycobacterium tuberculosis s* (MTB) in all cases. Notably, the misuse of anti-TB medications by untrained medical practitioners contributes to the rise of multi-drug-resistant TB (MDR-TB). Furthermore, the study emphasizes that children of TB-infected individuals have compromised immunity, placing them at higher risk. Efforts to raise awareness, improve diagnostic access, and ensure proper treatment protocols are essential to combat the spread of genital TB and its related complications, including infertility, especially among women.

Key words- genital organs, tuberculosis, infertility, AFB, genital tuberculosis

ACKNOWLEDGEMENT

All praises to my parents for giving me the strength and patience for completing this research work.I thank to Almighty for providing ways and opportunities and enabling me to overcome difficulties. I experienced a roller coaster of emotions during this whole period of research time.

I would like to acknowledge my indebtedness, render my warmest thanks and express my sincere gratitude to my esteemed supervisor **Dr. Naresh Kumar**, Associate Professor Department of Medical Laboratory Sciences, School of Allied Medical Sciences, Lovely Professional University, for his attentive guidance, constructive criticism, immense knowledge, scientific suggestions and full support in completion of this thesis successfully. It has been a great pleasure and honor to honor him as my supervisor.

I would like to thank, **Mrs. Rachna Sinha Director Ganga Diagnostic Center**, **Dr Umesh Kumar, Dr Manish Kumar MD Path** Ganga Diagnostic Centre Begusarai for generously sharing the time, equipment and knowledge in conducting my research work and for his tireless efforts in accomplishing it withgreat success.

I prolong my heartfelt thanks to chief medical officer, department of pathology, Sadar hospital Khagaria, **Md Kamran Ekbal** (Senior lab technician Supervisor) Department of Tuberculosis, RNTCP Sadar Hospital Begusarai and **Mr. Gurinder Singh**, Head of Department of Medical Laboratory Sciences and all other faculty members of the School of Allied Medical Sciences, Lovely Professional University for providing me the facilities to carry out my research work and for their support throughout my work.

I take this opportunity to express warm thanks to **Dr. Ashok Mittal (Honorable Chancellor, LPU), Mrs. Rashmi Mittal (Worthy Pro-Chancellor, LPU), Dr. Preeti Bajaj (Honorable Vice Chancellor, LPU), Dr. Monica Gulati (Registrar and Head of the Faculty of Applied Medical Sciences)** for providing an opportunity to work in such a prestigious university.

I also want to give my special appreciation to our lab technician, **Mr. Harsh Ranjan**, **Mr. Saquib Alam, Mr. Ramu Kumar, Rishi Kumar,** and lab assistant**, Mr. Guddu Kumar** for providing support required at every stage of my research work.

I would like to thank, Late **Dr. Amrita MD Gynecologist** Begusarai for generously sharing the time, and knowledge in conducting my research work and for her tireless efforts in accomplishing it with great success.

Throughout this journey, I cannot forget the encouragement, support and help of my friends and colleagues Mr. Ankur Kumar,

To **My parents and In-laws**. I owe more than words can express. I would like to dedicate this thesis to myparents, my dear mother, **Rekha Sinha** and my dear father, **SN Sinha,** who were constantly willing to provide everything I needed during the journey of this research and their endless sacrifice have helped me reach where I stand today. I am forever thankful toGod for giving me such parents who are always supportive of my dreams and for their unconditional love. My dear parents, thanks for believing in me, you are my role models. I couldn't have blossomed without you.

My children, Pratik and Aarav always suffered my impatience and low moods with unusual understanding for which I admire them ever so much.

I would like to give special thanks to the Backbone of my journey, my wife **Mrs. Rachna Sinha**. I cannot forget the encouragement, emotional support, and help she given meduring this journey and outside of this journey. Thanks for always being there.

A very special gratitude goes to my uncle "**Mr. Vijay Kumar Sinha, Mr. Sanjay Kumar Sinha and Rajendra Kumar Sinha**". You will always have a special place in my heart. Thanks for your love and prayers. Lastly, I would like to thank all whom I have not mentioned by name, but who directly or indirectly helped me to complete my experimental work and thesis.

Chandan Kumar

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List of Abbreviations

Abbreviations	Full form
ABC	ATP binding cassette
AFB	Acid Fast Bacilli
AG	Arabinogalactans
AIDS	Acquired Immune Deficiency Syndrome
ART	Anti-Retro Viral Therapy
ATT	Anti-Tuberculosis Treatment
AUB	Abnormal Uterine Bleeding
BCG	Bacillus Calmette Guerin
CB NAAT	Cartridge-Based Nucleic Acid Amplification Test
CD	Cluster of Differentiation
CFP	Culture Filtrate Protein
CLR	C type lectin receptor
CpGDNA	Cytidine-Phosphate guanosine DNA
CRD_3	Carbon hydrate domain
CRs	Complement Receptor
CSF	Cerebral Spinal Fluid
CT	Computed Tomographic
DNA	Deoxy Ribonucleic Acid
DOT	Direct Observe and Treatment
DST	Drug Susceptibility Testing
EBA	Early Bacterial Activity
ELISPOT	Enzyme Linked Immunosorbent spot
ESAT-6	Early Secreted Antigenic Target 6 kDa
FcRs	Fe receptors of immunoglobulin
FDA	Food and drug administration
FGTB	Female Genital Tuberculosis
FL-LPA	First Line Probe Assay
FNAC	Fine Needle Aspiration Cytology
GADD45A	Growth Arrest and DNA Damage-inducible 45
GTB	Genital Tuberculosis
GUTB	Genital Urinary Tuberculosis
HIV	Human Immunodeficiency Virus

HRQoL	Health Related Quality of Life
HSG	Hysterosalpingography
ICMR	The Indian Council of Medical Research
IFNs	Interferon
IPT	Isoniazid Prevention Treatment
IQCs	Internal Quality Controls
IQOLA	International Quality of Life Assessment
IVU	Intravenous Urogram
LAM	Lipoarabinomannan
LJ Medium	Lowenstein Jensen Media
LPS	Lipopolysaccharide
MA	Mycolic acid
MATE	Multidrug and toxic compound extrusion
MCE	Mammalian cell entry
MCS	Mental Component Summary
MD	Myeloid differentiation protein 1
MDP	Muramyl dipeptide
MDR TB	Multi Drug Resistance Tuberculosis
MFS	Major Facilitator Superfamily
MGIT	Mycobacteria Growth Indicator Tube
MRs	Mannose receptors
MTB	Mycobacterium Tuberculosis
MyD_{88}	Myeloid differentiation factor
NBS	Norm Based Scoring
NFHS	National Family Health Survey
NF-kβ	Nuclear factor kappa-light-chain-enhancer
NLR	Nod like receptors
NTEP	National Tuberculosis Elimination Programme
OD	Optical Density
PAMPs	Pathogen associated molecular patterns
PCC	Probe Check Control
PCR	Polymerase Chain Reaction
PCS	Physical Component Summary
PG	Peptidoglycan

PID	Pelvic Inflammatory Disease
PIM	Phosphatidylinositol Mannose
POD	Pouch of Douglas
PtpA	Protein tyrosine phosphatase
RD	Region of Difference
RND	Resistance Nodulation Division
RNF	Ring Finger Protein
RNTCP	Revised National Tuberculosis Control Programme
RPM	Rotation per minute
RRTB	Rifampicin Resistance Tuberculosis
SF 36	Short form 36
SL-LPA	Second Line Probe Assay
SMR	Small Multidrug Resistance
SNPs	Single Nucleotide Polymorphism
SPC	Sample Processing Control
TB	Tuberculosis
TDR	Total Drug Resistance
Th1	Type 1 T helper
TLR	Toll Like Receptor
TMD	Trehalose-6,6'dimycolate
TNFRSF	Tumor necrosis factor receptor superfamily
TNFα	Tumor Necrosis Factor Alpha
TPE	Tuberculosis Pleural Effusion
TST	Tuberculin Skin Test
UNIDS	United Nations Joint Programme on HIV/AIDS
USG	Ultrasonography
WHO	World Health Organization
XDR	Extensively Drug Resistance Tuberculosis
ZN STAIN	Ziehl Neelsen Stain

SUMMATION OF WORK IN ONE PAGE

Study Title: PREVALENCE OF GENITAL TUBERCULOSIS, ITS RISK FACTORS AND ASSOCIATED CLINICAL FEATURES AMONG THE POPULATION OF NORTHERN PART OF BIHAR

Sample Size-Calculated size323, Total study population:342

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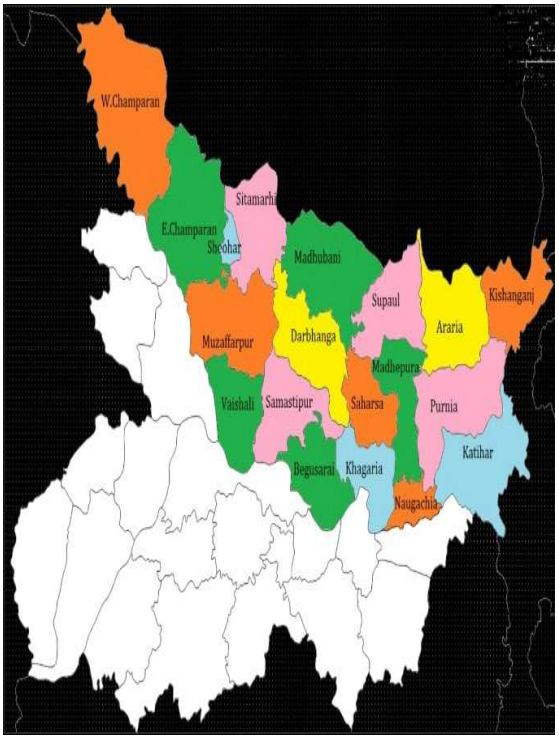

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Aim- To isolate and investigate the person who are either symptomatic or close contact with infected person, to find out the prevalence of genital tuberculosis cases it's early sign and symptoms. Counselling done and all clinical symptoms were noted. After that basic pathological and radiological investigations were done. Advance investigations like gamma interferon or gene xpert were done as per need. Specially then when all investigations are negative but symptoms were strongly suggestive. These pathological findings and symptoms help us to diagnose the disease accurately.

Place of study-Ganga Diagnostic Centre Begusarai, District Hospital Khagaria, Begusarai and Serum Analysis Centre P LTD Kolkata.
Duration of Study- January 22 to April 23
Study Design- cross-sectional study

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Under the Supervision of-
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Area of work-The study was carried out among people either symptomatic or asymptomatic with family history of tuberculosis.

Data Collection- Study population was obtained from Sadar Hospital Khagaria and also by camping and by home visit in Begusarai & Khagaria district.

Choice of specimen- Whole blood in EDTA, 3.8% sodium citrate vial and 3 special tubes for interferon gamma assay, vaginal swab (pap smear) and FNAC.
Method of collection- noninvasive, minimum contamination

Tools of the study- pre-tested, pre-designed questionnaire for subjects with consent form in local language/English.
Physical appearance, clinical symptoms, family and past history. Clinical examination findings. Tests on specimens- Whole blood in EDTA, 3.8% sodium citrate vial and 4 special tubes for interferon gamma assay, vaginal swab (pap smear) FNAC and tissue for HPE.

Result-
Female genital tuberculosis = 07 = **2.04 %**
2 out of 207 males are suffering with genital tuberculosis = **0.96 %**
7 out of 135 females are suffering with genital tuberculosis = **5.18%**
TOTAL INFERTILE PATIENT=**12 (3.5 %),** MALE=**02 (0.58 %),** FEMALE=**10 (2.92%),**
Infertility due to tuberculosis in Male=0
Infertility due to tuberculosis in Female=**4 (1.16%)**

Conclusion-
Overall study tells that there are many hidden cases which are responsible for tuberculosis spread. Some hidden cases are due to public ignorance and also due to absence of accurate diagnostic tools. Main reasons are poverty, ignorance of people and unavailability of advance diagnostic tools which can detect the disease accurately in its early stage and have no inhibition effect of amplification inhibitory drug.

CHAPTER-1
INTRODUCTION

1. INTRODUCTION

Genital tuberculosis (GTB) is a form of extrapulmonary tuberculosis caused by *Mycobacterium tuberculosis s* that affects the reproductive organs of both male and female. It is generally associated with the infection of lungs but it may spread to whole body though lymphatic system. It can affect fallopian tubes, endometrium, ovaries, cervix and vagina in females and epididymis, prostate, seminal vesicles and testis in males. Genital tuberculosis is mild or non-specific with symptoms like infertility, menstrual cycle, pelvic pain, or no symptoms found which makes more challenging issue in diagnostic and treatment plan and generally underreported in regions. Tuberculosis bacilli transmitted from infected to health person through air droplets during coughing, sneezing, or talking. Healthy person inhales these infected air droplets and gets infected[1, 2]. After that they reached to alveoli of lungs and form ghon focus that leads to primary tuberculosis. After the hematogenous spread these tuberculous bacilli reaches to different organ and cause tissue damage by causing morphological changes in it. About 90% of transmission through lymphatic system, whereas 7% transmission occurs through peritoneum bowel and lymphatic mesenteric route, 3% of transmission occur by ascending route like visceral route, using infected sputum as a lubricant during sex, coitus with infected etc.

The major aim of this study was to evaluate the people of Begusari (Bihar) where many articles published in local newspaper related to tubercle infection among the smoker and socially, economically week sections. As we know Tuberculosis (TB) remains one of the most significant infectious diseases affecting global public health, particularly in developing and resource-limited countries. Despite the TB control programmes and advancements in diagnostic and therapeutic measures, TB continues responsible for high morbidity and mortality. India has the highest burden of TB if we see the world level scale and facing challenges in early detection, prevention, and management of tubercle infection. While pulmonary TB is the most common disease among the population, extrapulmonary tuberculosis (EPTB) requires special attention due to its diverse clinical manifestations, diagnostic difficulties, and potential long-term complications.

Clinical sign and symptoms usually same or different in all types of tuberculosis. The common symptoms of genital tuberculosis are infertility[3,4]. In most of the genital tuberculosis at initial stage patient do not show any sign and symptoms. They look healthy but most of the cases eventually found during the diagnosis of infertility screening or they came for treatment of infertility treatment like IVF [5,6]. In case of female genital tuberculosis up to 11% may be asymptomatic[7,8]. So GTB of female is called as silent and chronic disease because bacilli can live without causing any symptoms[9]. In case of pulmonary tuberculosis common symptoms are coughing, weight loss, feeling uneasiness, sweating at night, fever etc. But in case of extra pulmonary tuberculosis like GTB, above symptoms may not be present. Vaginal discharge, PID and fever are the common complication in acute stage of infertility as well as menstrual disturbances, vaginal discharge after sex, loss of pregnancy, abdominal pain and distension, chronic pelvic pain and tumor are other clinical symptoms[10,11]. In Fitz hug Curtis syndrome Symptoms of FGTB post-menopausal are bleeding, leucorrhea, pyometra etc[4,12]. Menorrhagia and intermenstrual bleeding are the typical symptoms of active genital tuberculosis this may even change to fibrosis in uterine tubes [3].

In case of male common involve organ due to hematogenous spread is kidney 80% fallowed by epididymis 22-55%. But other organ like bladder, urethra, penis, lymph nodes, pleura and skeleton system are also affected. Globus minor is the site from where disease usually starts to grow. In male transmission of bacilli to genital organ is responsible for male infertility.

In women's this infection involves the different urogenital organ like ampullary region of fallopian tube (90 to 100%) fallowed by endometrium (50 to 80%) ovaries (20 to 30%) cervix (5 to 15%) and vaginal rarely up to 1%[13-15].

Among all extrapulmonary tuberculosis cases GTB resulting morphological changes in fallopian tube leads infertility, PID and menstrual disturbance etc. Most dangerous thing is that they cause irreversible changes in genital organ. It can also remain in body up to 20 years without causing any abnormalities. But when these latent forms get once activated

disease appeared. Breakdown of host immunity occur due to low vitamin d level leads to reactivation of latent form. Tuberculous bacilli commonly cannot directly infect genital organ without involvement of pulmonary organ. In genital tuberculosis the transmission of disease to the genital organ is almost always secondary to pulmonary infection[16].MTB bacilli present in air reaches to the alveoli during inhalation and causing primary pulmonary tuberculosis[4,7]. From pulmonary sites it is transmitted to the various organ by hematogenous spread[17,18].

According to statistics of Bihar the numbers of TB-related deaths rose to 5,158 deaths in 2023 (as compared to 1,073 deaths in 2018). These trends shows that even after the improvement in the diagnosis and availability of treatment, there is still critical problem in follow up of patient, resistance to drugs and socioeconomic condition.

As per the report of WHO (2014) told that 9.6 million people got ill due to TB and 1.5 million death occur only due to it[22,23].It causes different types of complications like pulmonary tuberculosis, extra pulmonary tuberculosis, genital tuberculosis, tissue involvement results organ damage, lymph node, skeletal system, infertility etc. and death[1,6].Prevalance of tuberculosis is more in economically lower class family due to comparatively lower living standard and due to malnutrition leads to weak immunity[24-26]. Prevalence is less in economically higher class due to strong immunity. The prevalence of TB is 1% in developed countries and 13% or more in developing countries. Lowest prevalence was found in Australia (0.69%) and in India prevalence is very high up to 19%[12]. Incidence of GTB is 192 to 232 per 01 lakh population and 19 to 30 deaths per 01 lakh population in developing countries[27]. In case of male genital urinary tuberculosis most common affected organ is kidney and usually occur in 40-50 years age group[28,29]. In case of female genital tuberculosis commonest effected organ is fallopian tubes and it commonly occur in reproductive age group (20-40 years)[30]. It is very common form of extra pulmonary tuberculosis. Female genital tuberculosis (FGTB) accounts 27% of all cases worldwide and it is 9% of all extra pulmonary cases. In all cases of infertility all over the world 10% are due to genital tuberculosis (GTB). Prevalence of tuberculosis is increased from 19% (2011) to 30% (2015) according to ICMR[31].

It was found that the data regarding genital tuberculosis in areas of Bihar is much in needed Begusarai where cases of tuberculosis are gradually increasing even after availability of modern diagnostic tools and facilities. This lacking of data encourage to work upon genital tuberculosis (GTB). To find out the genital tuberculosis (GTB) it is decided to classify the patient according to their socioeconomic condition, family history clinical symptoms, and on the basis pathological, radiological examination.

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CHAPTER-2
REVIEW OF LITERATURE

REVIEW OF LITERATURE

Research word made up of two words “re” means again and search means “find”. The word research tells that we repeat a search to pick out something new from existing data of earlier researchers. The topic upon which our current research-based tuberculosis and infertility both have been disturbing mankind since times immemorial. The whole world unit against it, even after this disease has been surging and reemerging by improving their strength. This disease affects almost all organs of the body.

Since ancient times almost all disease including TB has caused as misery and suffering upon human being and other animal in all aspects of life such as social, economic and health. History of tuberculosis described here highlights the struggle of mankind against a disease that dates back to ancient times and we can say that it is a story of failure and success and hope

Tuberculosis is the commonest cause of mortality with 9 million new cases and 2 million deaths per year[5]. Tuberculosis is currently the world’s one of the biggest health related problems effecting and causing major health problem in one third of the world’s population[8,10]. GUTB is commonly found in 40-50 years men age group and in female prevalence is twice[13, 14].

TB is still now a very dangerous health related disease. Uncountable research has done and lots of work has done upon it but unfortunately, we are unable to found a gold standard test which can give accurate result in its initial stage.

2.1.1 Present scenario-

India is the largest contributor about 26 % of the total tb cases in the world, (WHO, 2024). India documented the highest reported cases of TB in the country with 2.55 million notified cases in 2023, indicating a better notification system and active transmission (MoHFW, 2024). The trend in TB incidence has decreased over the past ten years, with the figure falling by approximately 17-18% since 2015, with the country experiencing the rate of 237 per 100,000 population in 2015 and 195 per 100,000 population in 2023 (PIB, 2024). Also, TB mortality has steadily declined over time with the help of early diagnosis and expanded

molecular testing as well as the increase in treatment adherence (MoHFW, 2023). According to WHO 7th November 2023, 1.3 million people die due to tuberculosis in 2023 in which 1,67,000 had HIV also. After covid TB is the second most killing disease. In 2022, 10.6 million people diagnose as tuberculosis in which 5.8 million were men (54.7%), 3.5 million were women (33.3%) and 1.3 million (12 %) were children. About 12 % died from TB diagnosed people.

In the financial year 2023, 30 billion Indian rupees was allocated for tuberculosis treatment and elimination purpose. Three countries having highest prevalence of MDR/RR-TB named India (27%), China (14%), and Russia (8%).

2.1.2 History of Tuberculosis and it's diagnosis

Tuberculosis (TB) is about as ancient as humanity itself, although the first documented evidence of the spinal tuberculosis (Pott disease) in a bone marrow dated around 8000 BC (Herzog, 2015). Moreover, abscess smears in the Egyptian mummies maintained in good condition showed that they contained acid-fast bacilli stained using Ziehl-Neelsen stain, which is a confirmation of the ancient presence of Mycobacterium tuberculosis. The Babylonian King Hammurabi (c. 1948-1905 BC) was the author of the oldest known body of law of lung disease in the world. This is written on a stone pillar, which is currently in the Louvre Museum in Paris and shows signs of early understanding of respiratory diseases.

The term phthisis was coined in ancient Greece by the great physician, Hippocrates (460-370 BC) to explain a wasting pulmonary disease that was common and deadly. The Hippocratic School considered phthisis as being an inherited but not infectious disease. The patients were cured in the temple grounds whereby they got nutritious food, milk, and physical activities as part of their treatments. The same Hippocrates noted tuberculosis-like lesions in cattle, sheep and pigs but did not describe autopsy, as no dissections were then done on people. Conversely, Aristotle (384-322 BC) claimed that scrofula (a tuberculous infection of the skin of pigs) was contagious, contrary to the then established theory of heredity. On the same note, the Egyptian Egyptianization of India discouraged marriage

within families whose history of consumption is known to have been the result of earlier social knowledge of the familial or contagiousness of the disease.

Roman physician Caelius Aurelianus gave a detailed clinical description of pulmonary tuberculosis by the fifth century AD. He characterized the patients with an evening fever, which went away by the morning, with chronic cough producing purulent sputum, emaciation, chest wheezing, loss of appetite, and finger clubbing. There were instances of chronic lung disease with purulent sputum because of misdiagnosis at around the same time by Aretacus of Cappadocia. Galen (131-201 AD) subsequently acknowledged the infectious nature of phthisis but still believed that it was hereditary and warned against being in close contact with the consumptives.

During the sixteenth century Girolamo Fracastoro suggested that phthisis was transmitted by seeds of contagion or viruses invisible and able to survive in the garments of infected patients to a period of two years. He related the disease to purulent contents of pulmonary ulcers. In Opera Medica (1679), Sylvius de la Boe (1614-1672) of Amsterdam stated that phthisis was an illness of the lungs and other organs, which caused hollows and ulcers. He also wrote about its complications such as scrofula (tuberculosis of the skin and lymph nodes). This was further developed by Richard Morton (1637-1698) who identified phthisis as a process which progressed via inflammation, the development of tubercles and ulceration. Sylvius as well as Morton, however, continued to regard phthisis as hereditary.

Later in the seventeenth and early eighteenth centuries, the post-mortem examinations were rejected by Giovanni Battista Morgagni (1682-1771), who felt that the illness was contagious and supported growing doubts about contagion. About the same period, an English medical doctor, Benjamin Marten (1704-1722) took an innovative theory in his book A New Theory of Consumption, More Specially of a phthisis or Consumption of the Lungs. He suggested that minute living organisms, maybe protozoa-like, were introduced to the body by inhaling polluted droplets and were the cause of the disease. Marten also advised against the long-term exposure to infected persons and this was the pretext of the germ theory of disease.

Modern tuberculosis the current concept of tuberculosis was influenced by Rene Theophile Hyacinthe Laennec (1781-1826), the pioneer of the stethoscope who coined the term tuberculosis in 1834 to refer to the pathological appearances of the disease. Laennec treated the tuberculosis as a tumor like process, but this was later contested by Rokitansky who believed that the ill was contagious. It was experimentally proven that TB can be transmitted when Phillipp Klencke (1813-1881) injected rabbits with tuberculous material, and they became infected with the disease in a generalized way- another of the first experimental demonstrations of contagion. As a continuation of this, Friedrich Henle (1840) developed three postulates on the cause of diseases that were to be later used by Koch:

(1) The organism should be present in every instance of the disease;

(2) It should be absent in other diseases; and

(3) It should always produce the same disease on transmission.

2.1.2 (a) Historical Milestones in the Discovery of Tuberculosis and the Tubercle Bacillus-

In 1865 French military surgeon Jean Antoine Villemin from 1827 to 1892, successfully managed to prove that phthisis could be transmitted from human as well as cattle to another laboratory animals like rabbits and guinea pigs. Blood, pus, sputum and other tubercular infected material from infected laboratory animals when injected and transfer to another healthy laboratory animals under the supervision caused well developed tuberculosis[42].

Jean Antoine Villemin was the first who told infected organism must be present in air.

Theodor Klebs (1834-1913) was the first who succeed to keep tuberculous bacilli in active live state on artificial culture media made up of protein and used further upon other laboratory animal and produced the disease[43].

In 1882 Robert Koch (1843-1910) gave the irrefutable evidence that tuberculosis occurs due to a particular infectious microorganism[44].

Now most awaiting days came, on 24th march 1881 at the Berlin Charite Hospital Robert Koch with his title "Die Atiologic der Tuberkulose" succeed to demonstrate the bacilli about which Villemin and Kleb was shouting from a long time. Robert Koch used a special staining method to demonstrate thin rods like structure recognized as "Tubercle bacilli". Robert Koch was first to successfully done the culture and from which demonstrate the bacilli and again grow in laboratory animal and prove the presence of bacilli in tissue by histopathological examination and finally successfully proved the postulates of his teacher Henle. Now Koch regarded as a pope of medical science in Europe and Amerika and in Japan a temple had made as a honor and he became famous as a demi god.

But tuberculosis bacilli had already seen by Paud Clemens Von Baumgarten from Tubingen in infected tissue just before the Robert Koch, by using sodium hydroxide but published few weeks later than Robert Koch's lecture in Berlin. Later on, Koch's phenomenon had come in which many experiment carried out upon Guinee pig to describe primary and secondary infection. He also succeeds to utilize dead tuberculous bacilli in to glycerin extract and called it "Lymph" and later renamed as "tuberculin". This tuberculin had used as skin test for identifying patient whether he is infected or not. Due to their enormous important use, for his great contribution, in 1905 Koch was awarded by Novel prize. Although this tuberculin had not any therapeutical use.

Another very important and mile stone invention was discovery of X Ray in 1895 by Willium Conrad Rontgen (1845-1923) which now provide the way to diagnose tuberculosis rapidly.

2.1.2 (b) Improvement of Tuberculosis treatment-

During consider it was found that in 19th century approximately all inquire were based on illness pathology and its determination and exceptionally few works were carried out upon its treatment. About all researchers of that time represented it as an incurable and hopeless disease.

Sanatorium Treatment-

Hermann Brehmer in his proposition “tuberculosis primis in stadiis semper curabilis” clarify that the infected person should be put in a safe place so, that the wounds heal by itself. At that period safe place was defined as an area free from tuberculous infected persons.

Vally in the Sudeten Mountain of Silesia Kirghizstan (since it was free from tuberculosis) had utilized as sanatorium and presently we known that these and most of the portion of central Asia have most elevated tuberculosis dynamic cases worldwide.

Again, Brehmer’s student diminish Dettweler begun an unused sanatorium in Germany at the Taunus Mountain in Falkenstein in 1876. After him in 1881 by St. Blasien, in 1888 by Schomberg in the region of dark timberland and by Terrible Hannet close bonn in 1893. But not at all like all Brehmer and dwindle Dettweiler etc. a previous Prussian specialist adjusted sanatorium but with strict rules. He presented a strict rest remedy, deck chair, take spittoon etc.

Although Dettweiler denied to acknowledge that the tuberculosis was caused by Mycobacterium tuberculosis, he gave the concept that it is caused by parasite indeed after the disclosure of tuberculous bacilli by Robert Koch in 1882. All over sanatorium was making like in Switzerland at tall mountain valley, moreover in the zone of Valtellina as well as at Sondalo in Italy and level range close by ocean in France. All begun the strict medication rule.

Sanatorium surprisingly work on some people but its long-term results were not satisfactory. 60 % of discharge patient die in the time interval of 6 years in which 17% as a cure patient, 51% stable and 72 % as better condition.

Sanatorium union was established in Hanover in 1888. Main aim was to open new people clinic.

In 21th November 1895 “Germany central committee was created. Its main works were to-

- Establishment of TB sanatorium
- Provision to provide economic and hygienic health service to consumptives.
- To promote science and research on tuberculosis.
- Public education.

Collapse therapy- Pneumothorax and thoracoplasty-

After sanatorium treatment it was very disappointed that long term results were unsuccessful but due to collapse therapy this problem of patient had solved. In early 1800 there were many reports which told about its success. From Pavia, Carlo Forlanini was the first carried out artificial pneumothorax by suppling nitrogen in plural cavity. He successfully carried out his work for 18 years on 25 patients and published his article and describe his successive results. John Benjamin Murphy was most likely to the first person who use x-ray in collapse therapy. Later on, for next 25 years this therapy became most popular method. In this therapy lungs collapse sufficiently causing lungs tissue get soften resulting reduced in cavity volume, lungs move towards each other which disturb the supply in bronchus and because of that bacillus found negative in sputum. In 1933 Banyai in USA accidently inject air into peritoneal cavity and it leads to establishment of pneumoperitoneum as an accepted treatment method. Due to this method bacilli disappear from the sputum but impair lung’s function. So, due to many complications of lungs this method had abandoned[46].

Pneumothorax was replaced by thoracoplasty. A surgical procedure to reduce thoracic cavity volume. This method was invented by Max Schede in Germany (1890). This way of extra pulmonary procedure gave good result[47].

Pneumothorax and thoracoplasty were found most popular surgical methods of those era.

2.1.2 (c) Tuberculosis avoidance by vaccine-

Vaccination against tuberculosis had to begin with carried out in children ((1921) in Paris. But in 1930 out of 240, 73 children die and numerous of lively children had developed

contaminated tuberculosis. In 1945 BCG vaccination had started again. It was found that in case of children most dangerous form of tuberculosis had been eliminated but in adult results were unclear[48].

2.1.2 (d) Prevention by combating Bovine Tuberculosis-

Bovine tuberculosis was consideration as nonpathogenic and enough study had not done on it because Robert Koch regarded it as nonpathogenic and no one at that time can stand against him. But in 1908 Chicago established guidelines to make milk pasteurization compulsory[49].

2.1.2 (e) Modern drug treatment-

A scientist named Victor Babes in Rumania found that the product of metabolism of Saprophytic microorganism like staphylococci as well as Bacterium prodigiosum slow down the growth of tuberculous bacilli and just opposite streptococci as well as pneumococci increase the growth of tuberculous bacilli[50]. In 1913 Vaudremer showed that the infectivity of tuberculosis bacilli got lost due to culture filtrate of mold aspergillus fumigate. Salman Waksman systematically work on sail fungi and bacteria since 1914 and around near about the era of 1939 few fungi in which actinomycetes group was successfully found to inhibit the growth of tuberculous bacilli[51].

Waksman and his team got succeeded to develop an antibiotic named actinomycin in 1940, although it had minimal therapeutic use in animal and human. In 1942 another antibiotic had developed by them named streptothricin. At last, finally in 1943 they successfully developed streptomycin. It had relatively low toxicity and side effect on laboratory animals. In 1952 for this work Waksman received the Nobel Prize[52].

Feldman and Hinshaw used the antibiotic on laboratory animal and found that it inhibits the growth of tuberculosis infection and on 20th November 1944 mention in history as victory of man over the deadest enemy by using streptomycin. After 10 years follow up have satisfactory result were found and suffering patient was found healthy and later, she successfully gave birth to three healthy children[53]

After streptomycin many drugs were introduced like in 1949 p-amino salicylic acid, in 1953 isoniazid, in 1954 pyrazinamide, in 1955 cyclomerize, in 1962 ethambutol and in 1963 rifampin.

Two important properties of anti-tuberculosis drugs are-

- Antibacterial activity- isoniazid, rifampin and streptomycin
- Drug having ability to reduce drug resistance- isoniazid, rifampin and ethambutol.

2.1.2 (f) Historical evolution of tuberculosis treatment: from cod-liver oil and gold therapy to streptomycin invention-

According to the literature "The History of **Gold Therapy** for Tuberculosis" by Thomas G. Benedek done the detailed Study between the era of 1912 and 1943. According to this study gold compounds were used during first half of the 20th century[54].

Initially their uses were increase but after arising health issue stopped and finally eliminated from treatment. According to this study many researchers of this era not properly mention about gold compounds advantage and disadvantage. One review article written by PD Hart in 1946, in his article he properly mentions about gold compound treatment.

Tuberculosis was known as most dangerous and death causing disease in mid-19th century, specially in urban area. According to Henry Ancell (1802-1863) in 1847 in England 16.1% death occur due to tuberculosis in which 78.4% due to primary tuberculosis. Where as in London 17.7% of death occur due to tuberculosis in which 71.5 % were pulmonary tuberculosis in 1851.During almost all 19th century **Phthisis** (pulmonary tuberculosis) as well as **Scrofula** (lymph node tuberculosis) remained an unsolved mystery, neither its cause nor its treatment could be found.

Tuberculosis had considered as same as other viral disease in these era.

According to B. Phillips in 1846 there was no any proof against the perception about whether it occur due to virus or it is genetic or both.

In 1849 a report had published by London hospital for consumption and disease of the chest, according to this report cod-liver oil was uses upon 542 cases of phthisis and 63% patient had improved their health,18% had diagnosed and 19% had uncontrolled. Gaining weight of patient was most surprising benefit[55].

Charles T. Williams from 1838 to 1912, in 1883 specified this as a result of current medical treatment.

Phthisis was considered as hereditary disease and also comes from some lower animals.

It was found that the main goal of this period was to improve the immunity or strength of person so that they can fight with the disease.

According to William in 1887 monograph that cod liver oil should be kept on the head of anti -phthisis treatment and prophylactic indication.

A scientist of Montpellier named Jean- Andre Chrestien from 1758 to 1840, in 1810 has known to introduce gold compound as phthisis treatment.

But there are many evidences who shows that gold compound was very less in use.

Robert Bartholow (1831-1904, Philadelphia) told that gold salt cause amenorrhea, impotence and depression.

Robert Koch from 1843 to 1910, after the invention of tuberculous bacilli in year 1882, tried to find its medication and on 4th august 1890, he gave the statement internation medical that he used a verity of substances upon tuberculosis bacilli culture and find out that not a few substances but also small concentration is sufficient to prevent the growth of TB bacilli[56].

They start to work on growth of bacilli having one to two million of dilution.

Robert Koch introduced subcutaneous injection of diluted tuberculosis bacilli in 1890.

Joseph Lister (1827-1912) concluded that although tuberculin injection is beneficial but cannot be cure[57]. Tuberculous tissue is got rid of by this injection but bacilli are not killed

by it. After the unsuccessful result of tuberculosis injection Robert Koch again on 1897 introduced an advance and improved preparation named as “new tuberculin” but many articles had published with doubt of this new tuberculin within a month. In 1913 waxing and waning of tuberculosis was reviewed. Some were refused the tuberculin therapy but some were accepted it as the only one available treatment. In 1912 Koch’s 1890 findings were reevaluated to adept it as medicinal use.

Two scientists from department of dermatology Breslaw named Bruck and Gluck Albert Neisser was the first detail about the toxicity of gold cyanide. 20 patients suffering with lupus vulgaris (cutaneous tuberculosis) of age between 6 to 60 were successfully treated without any harmful effect but lesions become cure unfortunately no further study is available like biopsy or other on these patients about side effect.

Adolf Feldt published his article and described that gold should be antituberculosis agent instead of cyanide.

Antimicrobial effects of drug were explained by Ehrlich in 1920. The theory developed by Ehrlich became the base of gold theory.

Lydia De Witt, a pathologist confirmed Robert Koch’s results that tells the function and uses of gold in vitro as well as in vivo. Many studies had carried out and conclusion given that gold salt have no any role in the of tuberculosis treatment.

In 1925 Sweany and Wasick talked about inhibitory effect of sanocrysin upon growth of tuberculosis bacilli. In 1931 British pathologist agree and support the effect of sanocrysin. Krysolgan a gold compound was used from most early treatment but in case of pregnancy Krysolgan became dangerous due to occurrence of abortion. In 1937 a comparative study had done and published upon the efficiency of gold chloride, sanocrysin, solganal and solganal B. According to above study sanocrysin (1:140000) have most efficiency against growth of tuberculosis bacilli[58].

Holger Mollgaad (1885-1973) a Danish physician requested to protect the experimental animal from sanocrysin shock. First clinical study on sanocrysin done by Kund Faber (1862-1956) on advance pulmonary tuberculosis. Main observation was-

Best result obtains during the 1st year of illness.

- There were many cases having no benefit.
- It was proved dangerous in some cases.

According to the medical research council of Great Britain - gold therapy was frequently used in treatment of tuberculosis without any guideline and without collecting information.

According to FE Koch in 1930, 500 articles had published upon sanocrysin.

P.W. Edwards in 1932 considered sanocrysin as a routine drug in treatment of pulmonary tuberculosis. Although he used sanocrysin indiscriminately but told "a dangerous drug" eight criteria had made by Hawker to define improvement of patient health[59]-

a. global improvement

b. clinical improvement

c. improved hearing

d. improved radiological finding

e. improved erythrocyte sedimentation rate

f. improved hemoglobin

g. disappearance of cough

h. disappearance of M. tuberculous bacilli from sputum.

Hacker didn't include weight gain in its criteria

According to Jensen based on comparative study of microscopic examination and culture methods on patient treated with sanocrysin having no any specific differences.

In 1912 collapse theory was being recommended in which lungs had to compress and asked to keep it in same position for a sufficient time interval. 50 % of advance cases were reported permanently cured by these techniques.

Forestiers in 1932, explain that there are many similarities between tuberculosis and arthritis like anemia, leucocytosis and temperature rise. So, some types of treatment of tuberculosis could be helpful in the treatment of arthritis. In 1944 streptomycin was introduced as anti-tuberculosis treatment at mayoclinic (Rochester MN) despite of its drug resistance effect.

2.1.3 Structural view of the Molecular factors that make it highly dangerous-

According to the article "Emerging advanced in identifying signal transmission molecules involve in the interaction between Mycobacterium tuberculosis and the host" written by Yue Wang, Qiyuan Shi, Qi Chen, Xuebin Zhou, Huiling Yuan, Xiwen Jia, Shuyuan Liu,Qin Li and Lijun Ge[60]-

Mycobacterium is an intracellular obligate aerobic slender and slightly curved having microcapsule, non-spore forming, acid fast bacteria having size of 1-4x 0.4 µm.

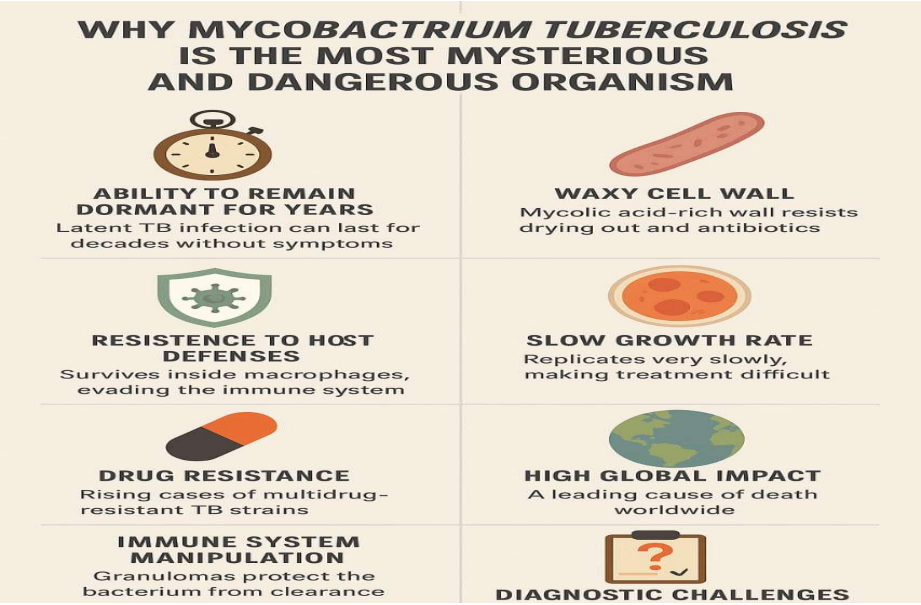

Figure 1: Characteristics of Mycobacterium tuberculosis

Its cell wall is made up of Peptidoglycan (PG), Arabinogalactans (AG), Trehalose-6, 6' dimycolate (TMD), Mycolic acid (MA), Muramyl dipeptide (MDP). About 60% all dry weight of cellular wall is composed of lipid and it is the main cause of slowly growth of tuberculosis bacilli. Due to these components all antituberculosis drug target cell wall.

Peptidoglycan (PG)- similar to all gram-positive bacilli tuberculosis bacilli cell has also made up of peptidoglycan. Main function of peptidoglycan is prominently participated in cell growth, different types of communication and to stimulate immune response of host.

Arabinogalactans (AG)- outer layer named as arabinan chain composed of highly branched arabino galactose (AG) and it's non reducing end of glycan chain is connected with MA. Mycolic acid (MA) - Composed of lipid and carbohydrates which plays an important role in its pathogenesis and its survival.

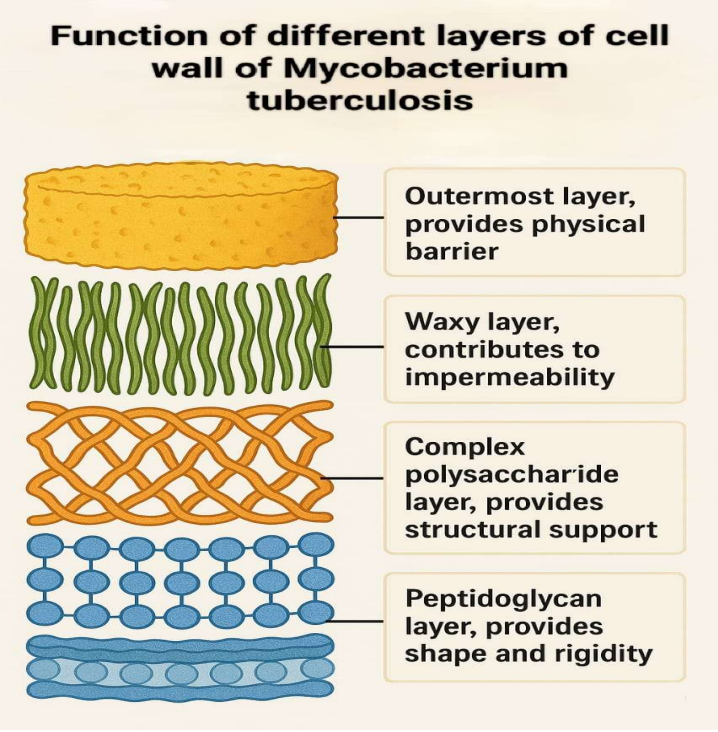

Figure2: Showing Function of different layers of cell wall of Mycobacterium tuberculosis.

It is also found that MTB has efflux pump system by which it reduced the concentration of anti-tuberculosis drug and not only protect itself but also developed immunity against this drug.

There are 5 transporters present in plasma membrane plays an important role in efflux pump system-

- ATP binding cassette (ABC)
- Major facilitator superfamily (MFS)
- Resistance Nodulation Division (RND)
- Small Multidrug Resistance (SMR)
- Multidrug and toxic compound extrusion (MATE)

"Mechanism of interaction between MTB effector proteins and host"

LPS (lipopolysaccharide) and LAM (lipoarabinomannan) are present in MTB cell wall which is tracked by TLR2/TLR1 or TLR2/TLR6 and after that it activate the NF-kß and cytokines resulting host cell injury. Most of the current research describes different types of activities taking place between MTB effector protein [PtpA, MCE family protein (Mce2E and Mce 3E)] and the host cell. Some researcher points out that in host cytoplasm, innate signaling pathways can be regulated by PtpA and inter in the nucleus and regulate the target genes.

Ubiquitin molecule binds with MTB PtpA and get activated. This activated PtpA can regulate many functions. Activated PtpA in the host cell nucleus hamper the transcription of TNFRSF8 in the host nucleus resulting inhibit NF-αß signaling pathway. Now PtpA direct combined with the promoter region of GADD45A or RNF187 gene and hamper transcription. Genome of MTB have four MCE (Mammalian cell entry) operons (Mcel-4). Encoded protein by MCE form large class of MCE family. This MCE family is very important in bacterial entry; it's survival and its pathogenesis. MTB is one of the successful pathogenic microorganisms which can modify their morphology colonies, virulence, immunogenicity and resistance against drug and survive even in unfavorable condition.

MTB and its Molecular mechanism which provide ability to escape from host PPR signal transduction-

MTB modify and change the immune response of host cell by releasing many different types of effector protein at different stage of life period. After entering in to host cell the macrophages recognized by membrane surface pattern receptor, the component of cell of MTB such as PIM (phosphatidylinositol mannose, LAM (lipoarabinomannan) etc.

Membrane surface receptors are-

- Toll like receptors (TLRs)
- Nod like receptors (NLRs)
- C type lectin receptor (CLRs)
- Complement receptor (CRs)
- Mannose receptors (MRs)
- Fe receptors of immunoglobulin (FeRs)

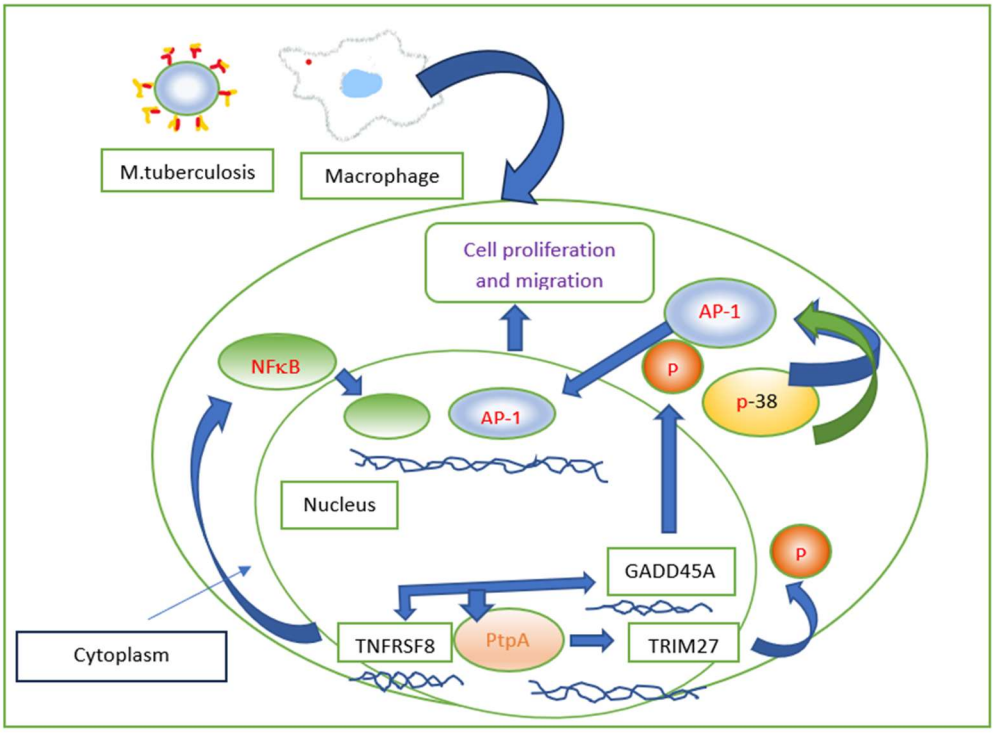

Figure 3: Interaction between MTB effector protein and host

Toll like receptors are single membrane spanning receptor intrinsic in nature and plays very important role in TB infection. PAMPs (pathogen associated molecular patterns) present in the microorganism are identify by TLRs which indued the release and synthesis of cytokines and also antigen presenting cells maturation to acquired immune response.

On the basis of its localization and reorganization properties TLR protein are classified into two groups-

A. Plasma membrane anchored TLRs
B. Endosomal TLRs

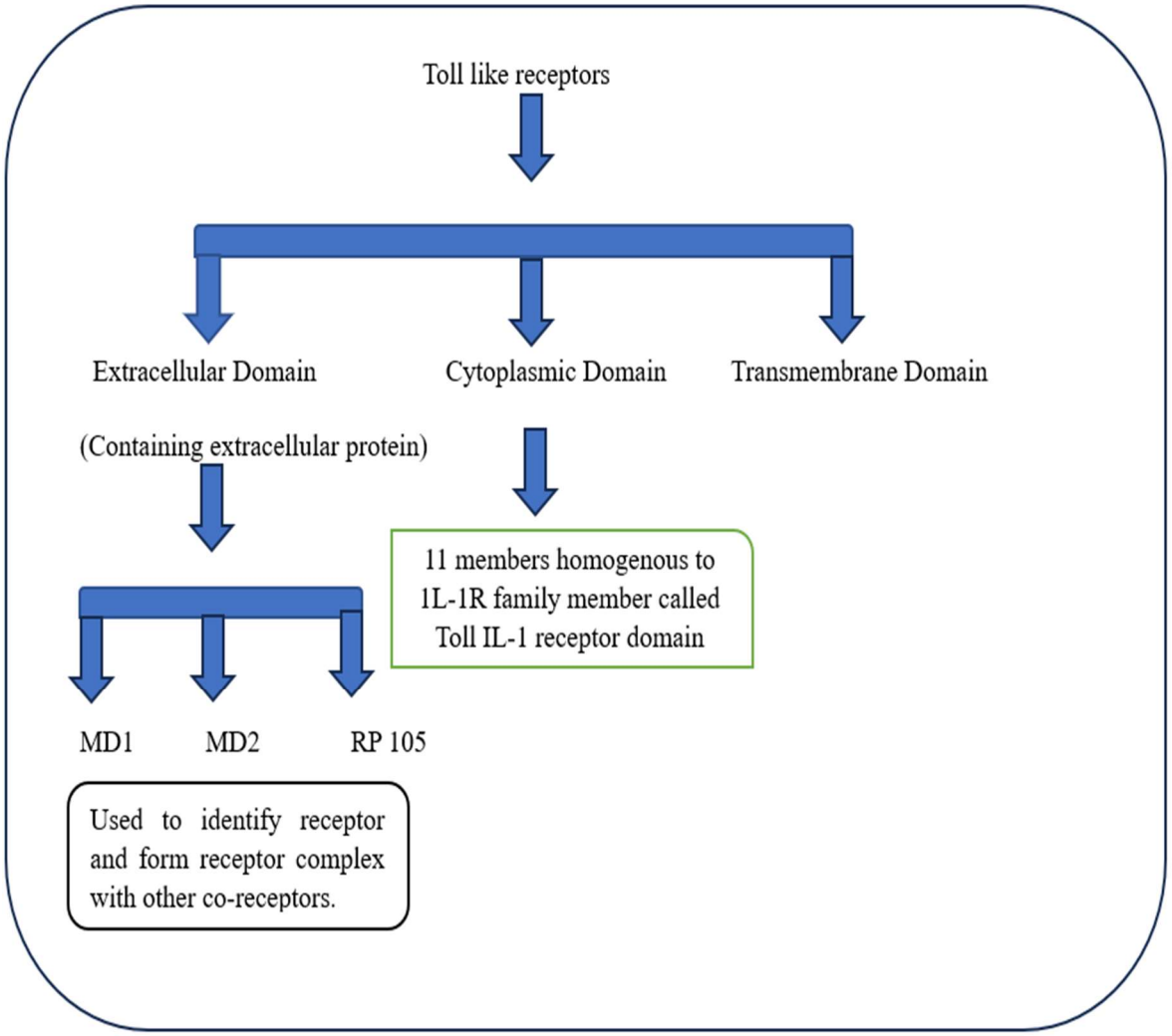

Figure 4: TLRs and its function

TLR3, TLR7, TLR8 and TLR9 have also virus recognition property.

TLRs works through two pathway dependent and independent myeloid differentiation factors (MyD88).

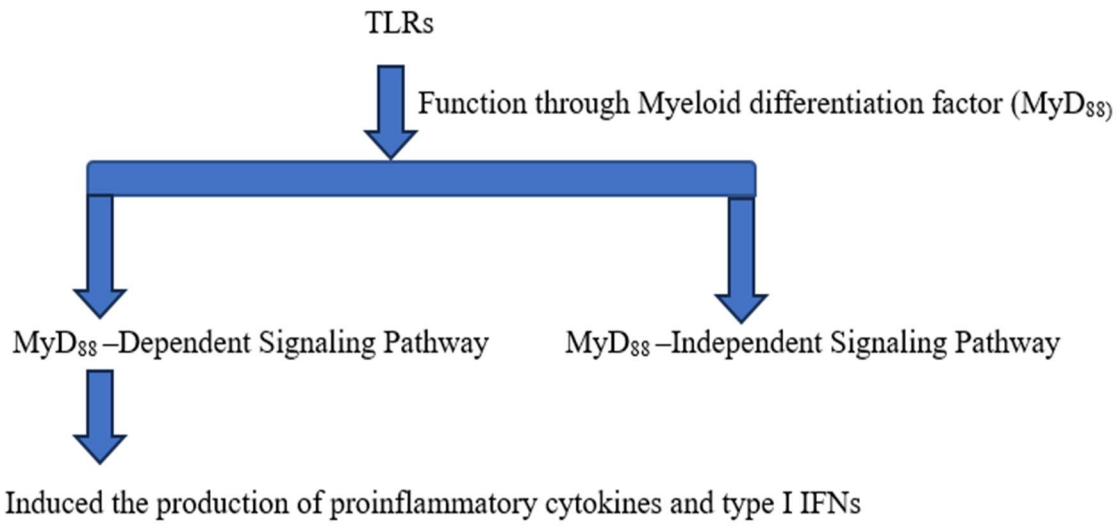

Figure 5: TLRs and its pathway

TLR_2 recognized lipoprotein, LPS and CpGDNA at MTB cell wall also can activate. NF αß signaling pathway which induced the secretion of TNF-α resulting activation of macrophages. It is believed that TLR_2 has crucial role in the activation of innate host defense against MTB.

TLR_4 – It activate both MyD_{88} dependent as well as MyD_{88} independent pathway resulting release of inflammatory cytokines and costimulators and type I interferon (IFN) simultaneously resulting initiation of both innate and acquired immune response.

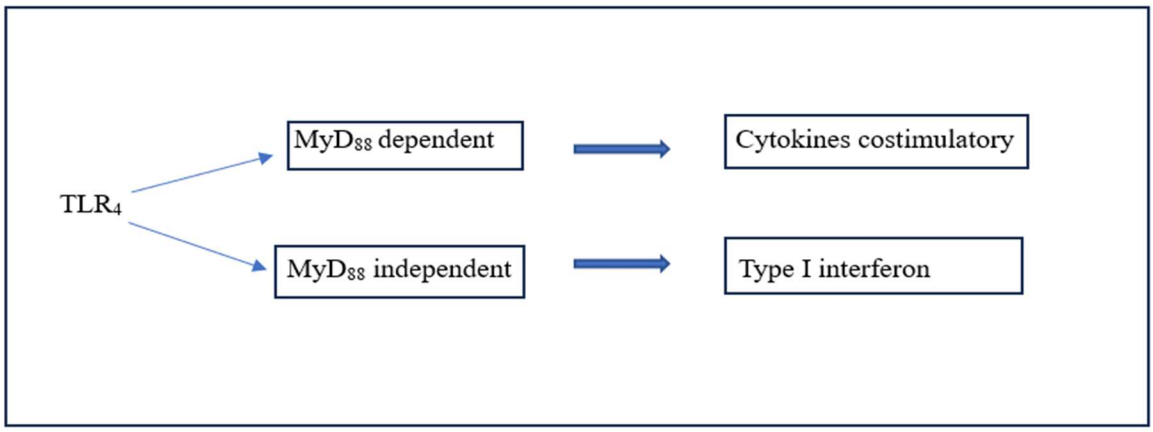

Figure 6: TLR_4 and its pathway

MTB infection is directly related to excessive expression of human genome proteins like TLR_2 and TLR_4.Among all, TLR_9 is the only which is detectable in immune cells.

MTB, its Molecular mechanism which provide ability to escape the host NLR receptor signal[61] –

NOD (nucleotide oligomerization domain) like receptor (NLR)-

These are receptor protein which plays a vital role in expression of innate immunity it is present inside the cell cytoplasm.

Molecular mechanism of MTB which provide the ability to evasion of host CLR r (C-type lectin receptor) signaling-

CLR receptor are from protein super family having carbon hydrate recognition domain (CRDs) CLR plays an important role in innate immune system.

Molecular mechanism of MTB which provide the ability to escape host autophagy-

Autophagy is the mechanism by which cell maintain or indued it's homeostasis to eliminate damage cell or unwanted cellular structure.

Autophagy ⟶ Autophagy Vesicles (Double membrane structure)

Autophagy first form double membrane autophagy vesicles ingulf body are digested when inclusion bodies fused with lysosome. According to research MTB can present in autophagy vesicles of host cells and lysosome collected from MTB containing phagocytes eliminate MTB by acidification.

2.1.4 Global Evolution of MTB Lineages: Virulence, Immune Response, and Resistance Mechanisms-

According to Hansong chae and Sung Jae Shin on 14 February 2018, MTB is a pathogen having the ability to control the immune response of the host and protect itself from anti tuberculosis drug. The following causative agents are responsible for tuberculosis[62]-

1. *Mycobacterium Tuberculosis*
2. *Mycobacterium Africannum*
3. *Mycobacterium Bovis.*

The virulence, symptoms, impact on health and death are not identical in all the cases. Some are in latent, some have mild symptoms, some are moderate, some are strongly symptomatic. Some have developed major health issues whereas some have active TB without symptoms. Patient immunity, patient health strength, surrounding environmental condition, economic condition and strain of tuberculosis bacilli play an important role in different types of impact and clinical presentation. In the article written by Cosolla and Gagneux in 2010 that some clinical isolates have different virulence properties.

According to Merker et al.2015, Beijing family (Lineage-2) and Euro -American strains (Lineage-4) also known as modern lineage are considered as more virulent in comparison with ancient lineage like East African Indian (Linease-1) and *M. Africannum* strains (Lineage-5 and 6)[63]

There are seven lineage –

1. Lineage 1-East African – Indian or Indo-oceanic
2. Lineage 2- Beijing family or East Asian
3. Lineage 3- East- African- Indian or Central Asia lineage strain

4. Lineage 4- American strains
5. Lineage 5-*M. Africannum* Strains or West African type
6. Lineage 6- Africannum strains or West African type 2
7. Lineage 7- Ethiopia

Lineage 2,3 & 4 – known as Modern Lineage- TbD deleted

Lineage 1,5,6 & 7- known as Ancient Lineage- TbD is conserve.

These lineages are particularly known by their geographical area of existence-

Lineage 2 & 4 – these lineages are present and isolated from all over the world.

Lineage 1 & 3 – these lineages are present and isolated from east part, south part and central part of Asia only.

Lineage 5 & 6- these lineages are found and isolated only from west Africa.

Lineage 7- It is newly discovered and isolated from Ethiopia and its immigrants.

Lineage 1 to 4- it consists of 99.2% of total cases of MTB.

Lineage 5,6 & 7- comprise the rest part, reason behind it is, it's restricted area.

Modern lineage-2,3 & 4 found worldwide than another lineage. These modern MTB lineages having these characteristic by which they spread globally and these three lineages are differ by 970 SNPs.

In early phase of infection these modern lineages induce low level of inflammatory cytokines.

Representation of TNF-α, IFN-ϒ, IL-12 and IL-7 is high in modern lineage comparison to ancient lineage (1 &6).

According to many studies modern lineage (2,3&4) has high virulence phenotype resulting delayed inflammatory response.

Modern lineage has high replication rate in macrophages.

Lineage 3 -this lineage has strong anti-inflammatory response mechanism in compared to lineage-4.

Lineage 2 (Beijing family) induce low level immune response in comparison of MTB H37Rv which is associated to lineage 4 also low expression of TNF- α, IL-6, IL-10 and GRP- α.

According to Sarkar et al (2014) shows that modern Beijing strains causes high toxicity resulting worse health issues in C57BL/6 mice.

W-Beijing family become one of the most study subjects due to its dangerous pathogenic phenotype. Endemic area is East Asia region but spreading of this strain to another region also occur like- New York, Russia, Latvia and South Africa according to sreevatsan et al 1997). According to him and many other study – in case of animal W-Beijing family become much more hyper-virulent, high bacilli load in sputum, high dissemination causes early death.

In Texas prisons in 1995 an outbreak occurs due to W-Beijing family the HN 878 strains of Lineage-2.

Barczak et al,2005 Dejong et al told that, its characteristics is more rapidly progression of disease resulting death in very short interval of time in immunocompromised patient and have prevalence of latent relapse cases[64].

In the year 1998 K strain of Beijing family causing outbreak of TB at large scale in South Korea. BCG vaccine is also less or no effective against this Beijing family according to Han et at 2015[65].

Lineage 3 causing out -break of TB in Leicester (UK) shows rapidly growth to an active form within one year which have low IL-12p 40 and high IL-10 expression. Due to deletion of RD750 it is not detect by innate immune response.

Vaccine and its efficacy against MTB strains-

Not a single vaccine in our hand having license for or proved effective against tuberculosis except BCG. But the success and effective rate of BCG are not identical against all the strains of tuberculosis. Sometimes BCG vaccine has proven very effective and sometimes found less effective to no effective. A number of factors are responsible for this type of effective variation like nutritional status of the patient, their surrounding environmental condition, genetic variation of MTB strain and interaction of host immunity according to Abebe and Bjune-2006.It is assumed that the variation in efficiency of BCG vaccine is due to genetic diversity.

It was observed that the Beijing family strains are found in large scale in Russia and East Asia and in neighboring countries of China. It was also reported by several studies that this strain is found in patient immunized by BCG vaccine. So many studies also suggested that the extensive vaccine of BCG is the one of the major causes of emergence of Beijing family.

According to the report of Lanzas et al 2013, the development of MDR and XDR are occurred due to mutation of genome[66].

Drug resistance MTB developed due to mutation in resistance genes (rpo A, K at G AND rrs) resulting defect in life cycle of bacilli.

According to ford et al, 2013 and Coscolla and Gagneux 2014, drug resistance TB have ability to spread[67].

Global discover of strains proved that drug resistance strains can spread between people. From several study it is found that Lineage 2 and KwaZulu-natal (KZN) strain have highest possibility to develop in to drug resistance in comparison of other lineage and stain.

KZN strain of lineage 4 is responsible for outbreak of TB in the KwaZulu natal region of South Africa.

In 1998 whole genome sequencing of MTB had carried out, and Rose et al 2013 published whole genome sequencing of MTB, which enable us to clarify the characteristics of all SNPs.

Publication of whole genome sequencing done by Rose et al (2013). Resulting identification of all SNPs.

The ser 375 thr mutation in Kat G causes resistance to isoniazid.

2.1.5 Mechanisms of *Mycobacterium tuberculosis s* Infection: From Aerosol Entry to Granuloma Formation-

According to the article "The Pathology of Mycobacterium tuberculosis Infection" written by -K. Sakamoto in 2012[68]- Mycobacteria is a non-motile, non-sporing, weakly gram-positive, acid-fast slightly curved rod-shaped bacilli. The cell wall is made up of lipid which make it acid fast, protects form dying, protects from acidic as well as alkalinity, protects from a number of antibiotics and also protect from immune defense mechanism. It is slow, growing pathogenic bacilli having 12 to 24 hr. division rate with a prolonged culture period 6 to 8 weeks. Some believe that the slow growth rate is due to low nutrient intake because of its cell wall having properties of impermeability and a highly low RNA synthesis mechanism.

MTB is facultative intracellular bacilli that grow and multiplies inside the phagocytic cells, specially macrophages and monocytes. The important genetic difference between MTB and BCG is 16 foci present in BCG, designated as RD_1 through RD_{16} (Region of difference).

Cell biology of MTB-

MTB also interacting with PRR (Pattern recognition receptors) like TLR_2 (toll like receptor 2) and initiates a proinflammatory cascade.

Pathogenesis and lesion development –

A healthy person gets infected predominantly by inhalation of contaminated droplets released by the infected person during talking or sneezing. A single aerosol droplet should have at least 1 to 200 bacilli to become infectious. A single aerosol droplet can contain 1 to 400 bacilli; however, the relevant dose is still unclear. The upper lobe of the lungs is favorable for growth of MTB bacilli due to comparatively higher pressure of oxygen and delayed immune response. Inhaled bacilli reach the alveoli where they interact with macrophages, whereby stimulation by TLRs and PRRs they produced cytokines and chemokines to fulfill the requirements of leucocytes at the affected site. The primary affected site is where bacilli implant itself called a ghon focus.

Among leucocytes, neutrophils and monocytes arrive first. Secretion of more cytokines and chemokines causes formation of early granuloma. A well-organized granuloma contains MTB consisting of macrophages surrounded by epithelioid macrophages, a few multinucleated giant cells, lymphocytes, foam cells and a fibrous capsule.

Sometime later necrosis is initiated in the center part of the granuloma because of high lipid and protein contents of dead macrophages forming caseous material. MTB can actively present within the macrophages and extra cellularly in the granuloma. It was believed that the majority of the MTB bacilli were present at the periphery and sparse in the caseous region when ZN stain was used, but after use of Auramine O and Rhodamine B it is found that most of the bacilli are present in the core of caseous granuloma.

MTB adapts the unfavorable environment by increasing cell wall thickness and decreasing replication by using fatty acid. This stage of bacilli is called the “dormant stage”. Bacilli can also disseminate through lymphangitis and regional lymph node causing lymphangitis and lymphadenitis. Lymphangitis and lymphadenitis along with ghon focus it is called as “Ranke’s complex”[69].

In chronic lesion MTB is present at the periphery inside the foamy macrophages. The most typical form of secondary tuberculosis is generally restricted to the lungs.

2.1.6 Types of tuberculosis: -

In 2010, S. Vijaya Kumar et.al address in their article "A systematic review of different type of tuberculosis[70]-

(A) Tuberculosis lymphadenitis-

Clinical features –

Asymptomatic non tender swelling in immunocompetent patient. Cervical lymph node is the characteristic of TB adenitis and present in one third of the patients.

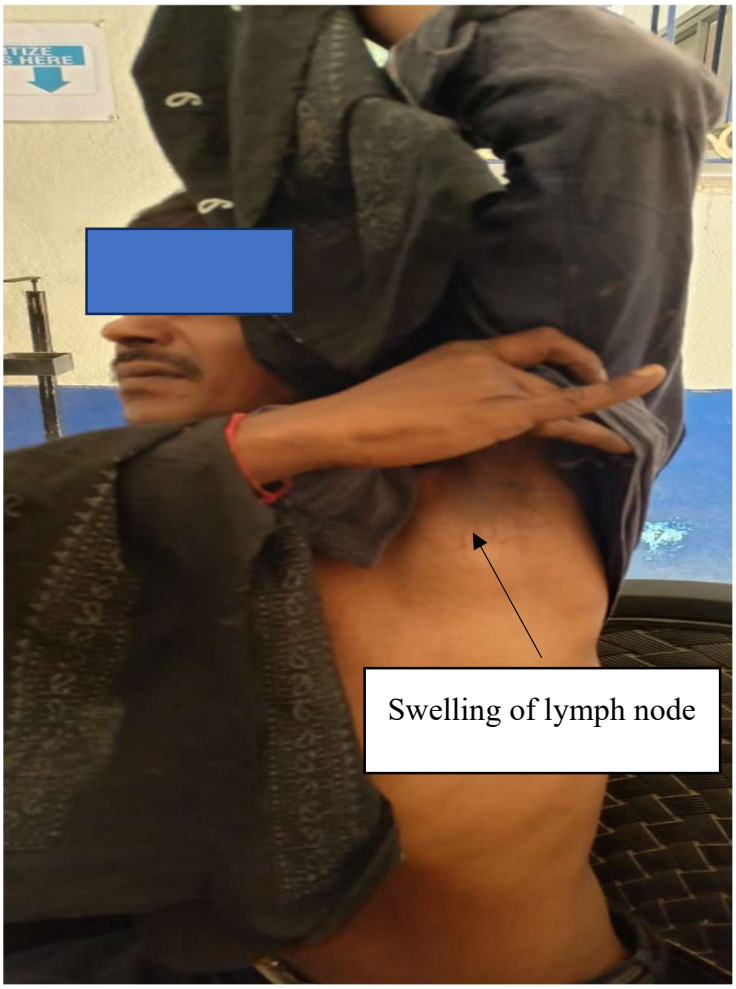

Figure 7: Lymphadenitis at arm pit.

Figure 8: Cervical lymph-node swelling.

Diagnosis-

FNAC has 46% sensitivity for TB adenitis and it has 53% sensitivity in case of granulomatous inflammation.

Histopathological examination- It shows reactive hyperplasia and granulomatous with or without caseation.

X- Ray- in 18% of TB adenitis radiological changes are noted.

Tuberculosis lymphadenitis is more common in 20 to 40 years person and it is predominantly found in female. Dissemination of MTB bacilli from pulmonary site to lymph node occur through hematogenous spread. In case of HIV patient when CD_4 count is below 300 cells per μL then tuberculous lymphadenitis occurs.

(B) Pleural tuberculosis-

Pleural effusion occurs in 30-60 % of cases. Pleural tuberculosis occurs due to reactivation of pulmonary tuberculosis and miliary tuberculosis.

Diagnosis- TST found positive in 80% and 61% of primary TPE (tuberculous pleural effusion) and reactivated TPE respectively. No pathological changes found in pleural tissue.

Figure-9- an 8 years gild having pulmonary tuberculosis with pleural effusion.

(C)Tuberculosis Emphysema-

Advance degree of tuberculosis and long duration is a cause of pulmonary emphysema. It occurs due to rapture of cavitary pulmonary TB along with pus in pleural area. In case of emphysema there are presence of a large number of tuberculosis bacilli. Untreated or poorly treated tuberculous emphysema resulting emphysema necessitates. Which is often bronchopleural cutaneous fistula. Emphysema may drain in to retroperitoneal space and spinal abscess may also drain in to pleural space.

Clinical Symptoms-

Chest pain, cough and dyspnea often with fever, weight loss and anorexia.

Pathological Examination-

Pleural fluid having low P^H cell count 500 to 2500 with predominantly lymphocytes (80% or high) in TPE cases. ZN stain shows positive up to 5 to 20 % in case of immunocompetent hosts and up to 50% in case of AIDS ($CD_4 < 100$). PCR and interferon γ also very useful in diagnosis. Confirmation diagnosis can be achieved by culture of pleural fluid.

(D) Miliary, central nervous system and genitourinary tuberculosis: -

Miliary tuberculosis is a potentially life-threatening form of tuberculosis in which tuberculous bacilli spread to every part of the body through blood stream (hematogenous spread). It is fetal approximate 30 % of cases. It has a lesion of size less than 2 cm not diagnose on contrast computed tomographic (ct). According to a review from 545 patients of MTB, 64.6% having HIV and 28.2% of non-HIV patient had died.

CSF fluid analysis shows lymphocytosis with high protein level. Spread of caseous focus of TB to the cortex of brain in miliary tuberculosis. From here it reaches to subarachnoid space and cause diffuse meningitis.

Tuberculosis bacilli spread through hematogenous spread and form focus in different region of brain like cortex, meninges choroid plexus or ventricles wall at the time of infection, at the time of primary infection or after later during infection. The site where infection established is called “Rich Focus”.

Clinical symptoms are-

Its typical symptoms are evening fever, weight loss, headache, vomiting or behavior change. Neurologic changes loss of consciousness or convulsion may also develop due to delay treatment.

Pathological Examination: -

Cerebrospinal fluid (CSF) examination having low cell count < 300 m^3 predominantly lymphocytosis, low glucose level, high protein level, PCR sensitivity is 56% whereas specificity is 98%.

(E) Genitourinary tuberculosis –

Spread of MTB to genitourinary organ by hematogenous spread and complications arise like burning on micturition, increase frequency (29%) and renal colic (13%).

Sterile pyuria and antibiotics not respond on urinary symptoms indicates renal TB. Many other further complications can arise due to delay treatment- parenchymal necrosis and later calcification calyceal distortion urethral strictures and bladder fibrosis which may lead to renal failure. In case of female fallopian tube and in case of male epididymis is the commonest involve organ.

Pathological examination-

PCR technique used to detect mp^{+64} gene of MTB to ruled out genitourinary tuberculosis endometrial aspiration, endometrium biopsy and fluid extract from POD (Pouch of Douglas) can be used in pathological examination.

(F) Abdominal tuberculosis-

There are two known modes of MTB dissemination from a primary focus through hematogenous spread or miliary tuberculosis which spread through lymphatic channel causes abdominal lymph node involvement resulting abdominal tuberculosis. Ileocecal formation, mucosal ulceration, caseation fibrosis and scarring can also occur due to delay treatment.

Symptoms-

Fever (40-70%), weight loss (40-90%), abdominal pain (80-95%), diarrhea (11-20%) and constipation. Some patient can also complain for fatigue, malaise and anorexia. Dysphagia and odynophagia can be seen in case of esophageal TB. Dyspepsia or duodenal obstruction found in duodenal tuberculosis.

(G) Ocular tuberculosis: -

It is most commonly found in children affecting eyelid. Lupus vulgaris on eyelid is characterized by reddish brown nodule having “apple jelly” colour secretion by applying

pressure on it. Conjunctive is most common involve organ in children. Conjunctiva tuberculosis can cause scarring of involve tissue. Mucopurulent discharge and edema of eyelid is typical symptoms. By ZN staining MTB can be detected in conjunctival smear.

(H) Laryngeal tuberculosis-

It is a complication of primary tuberculosis (12 to 45% cases) depends upon severity of pulmonary lesion. Tuberculosis lesions can be healed by calcium salts. Healing can also done encourage by increased blood supply. In case of tuberculosis ulcers electric cautery is used.

(I) Musculoskeletal tuberculosis-

It is very rarely occurred approximately 1 to 3 % of cases. Involvement of osteoarticular regions is due to hematogenous spread of TB bacilli from pulmonary area causing lesions. Any bone, bone joint, or bursa (fluid fill sac) can be infected but prominently spine, hip and knee are very preferred site of infection from 70-80 % of infection. Due to indolent nature diagnosis is often delay.

(J) Spinal tuberculosis-

It is also called “pott’s disease”. About 50% and more cases of Musculo skeletal tuberculosis. In endemic area young adults are more affected but in developed countries older person are affected. In spinal tuberculosis involvement of thoracic spine is 50% lumber and cervical involvement is 25% each. Due to the disease herniation of intervertebral disk into vertebral bodies occur, resulting loss of vertebral height causing “step-off” kyphosis. In 10 to 47% of spinal tuberculosis, most dangerous neurological complication develops due to spinal deformity or due to formation of epidural abscess.

(K) Extra axial musculoskeletal tuberculosis-

Tuberculosis arthritis can affect any joint but weight bearing joint like hips and knee are commonly affected. Single joint is involved usually but multiple joints can involve.

Osteomyelitis as well as bursitis have been also reported. Necrotic cartilage and classic rice bodies found in synovial fluid.

(L) Pericardial tuberculosis-

Mycobacterium tuberculosis also affects the heart pericardium it can lead to a condition called pericardial tuberculosis. It occurs due to hematogenous spread or spread from mediastinal lymph node or pulmonary tuberculosis or rarely spread from infected spine and tracheobronchial region.

(M) Pericardium become thickened due to fibrosis-

Clinical symptoms- dyspnea (shortness of breath) orthopnea (shortness of breath during lying flat and is relieve by sitting or standing) chest pain, pleuritic pain during breath, cough, night sweat, weight loss etc.

Diagnosis- ECG, histopathological examination of pericardial tissue, culture, PCR, or combination of all used in the diagnosis.

2.1.7 Impact of Tuberculosis on Health-Related Quality of Life-

Muhammad Atif et.al in 2014 in their article[71] "Impact of tuberculosis treatment on health-related quality of life of pulmonary tuberculosis patients: a follow-up study" detailed about impact of tuberculosis on health. According to this article-

Impact on health due to tuberculosis is very important in management and treatment aspects. Health related quality of life (HRQoL) has substantial and encompassing impact occur due to tuberculosis. TB patient is found with deficit in physical and mental wellbeing.

Common symptoms are loss of weight, loss of appetite, fever, fatigue and body pain etc. Some patients were also reported anxiety and fear.

There is only one study from Asia are available and acceptable as a HRQoL assessment tool. HRQoL is a very important tool to access different types of impact caused by tuberculosis on health. To measuring it a study was conduct on health care facility at Malaysia having 1,107 bed capacity named as Penang General Hospital (PGH) during

march 2010 and February 2011. 400 new smear positive subject are taken in study. Short form 36(SF-36) even now widely accepted and uses despite the avaibility of a number of questionnaires. Translation of SF-36v2 is controlled and managed by IQOLA (International quality of life assessment.) researchers. There are eight scale of SF-36v2 to measuring different eight conditions of HRQoL :-

1. PF- Physical Functioning – 10 items
2. RP-Role physical --- 04 items
3. RE-Role Emotion ----- 03 items
4. BP- Bodily pain ------02 items
5. VT- Vitality -------04 items
6. SF- Social functioning -----02 items
7. GH-General health --------05 items
8. MH-Mental health --------05 items
9. 1 item is used to record the change in health status compared to previous year.

These eight scales are further classified into two groups

PCS-Physical component summary

MCS- Mental component summary

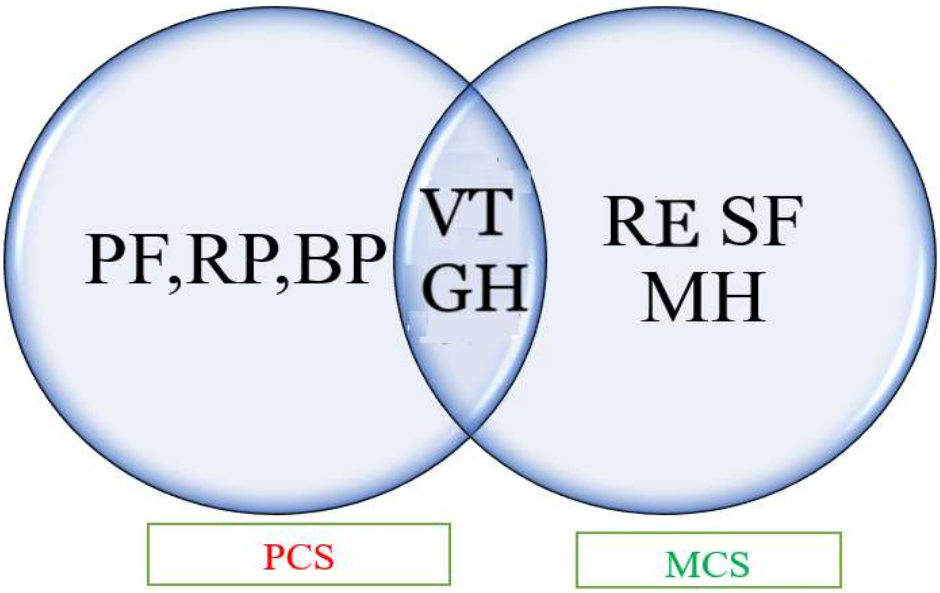

Figure 10: Scales of HRQoL

According to Muhammad Atif article[71] NBS (Norm based scoring) of above describe 8 HRQoL and by scoring algorithms (United States weights) components were summarized. Patient how were educated and age more than 18 years had given and asked to complete the SF-36V^2 at before starts the treatment, after the intensive stage and after the final treatment. HIV patient, drug abusers, malignant, arthritis patient not included in study.

NBS from 47 to 53 indicate---------- Normal

NBS less than 47 ---------- impaired function

NBS less than 42 ---------- risk of depression

Result of this experiment according to Muhammad Atif et.al **2014-**

It was found that 67% of patient before start of treatment 35% at intensive phase and 23.5% after the end of treatment had at the risk of depression. It was also found MCS have low score in comparison of PCS resulting more psychological distress. After the treatment improvement in HRQoL score occur (by SF-36V^2).

2.1.8 Risk factors of genital tuberculosis

1. Socioeconomic and Environmental Factors –

Low socioeconomic status is among the greatest risk factors of GTB. High-crowding, malnutrition, lack of sanitation and access to healthcare services predispose to Mycobacterium tuberculosis infection. In some developing countries, the late diagnosis and partial treatment of pulmonary TB often lead to the transmission of extrapulmonary ones, such as genital involvement (Jana et al., 2019). The poor living standards and malnutrition inhibit the immune mechanisms leading to the development of the active form of TB in the genital tract.

2. Pulmonary or Extrapulmonary Tuberculosis History–

Previous history of tuberculosis, especially pulmonary TB is a well-reported risk factor. In its most frequent manifestation, genital organs are not the main place of infection; instead, GTB develops due to the hepatogenic or lymphatic dissemination of bacilli in a primary focus in the lungs (Sharma, Sharma, 2020). Severe TB is also a disease that patients who have suffered in the past with inadequate medical attention are at risk of getting genital involvement in future life.

3. Immunosuppression and Co-morbid Conditions-

Co-morbid conditions such as HIV/AIDS, diabetes mellitus, and chronic corticosteroid use are associated with a high probability of extrapulmonary and genital tuberculosis (World Health Organization, 2022). The patients are especially susceptible to HIV-positive people, whose immunity is weakened, which promotes the re-activation of dormant TB bacilli and their spread in the genital organs. There is also further immune compromise by malnutrition and chronic ailments.

4. Reproductive Age and Hormonal Factors-

GTB predominantly affects women between 20 and 40 years of age, the most active reproductive period (Thangappah et al., 2017). The high vascularity of the genital organs

and cyclic hormonal changes during this phase may favor localization of the infection. Postmenopausal GTB is uncommon but may occur due to reactivation of latent infection.

5. Infertility and Gynecological Procedures –

GTB can cause infertility and infertility can also increase the risk of GTB infection. Women who have undergone repeated uterine instrumentation, dilatation and individually, curettage (D&C;), or intrauterine device (IUD) insertions are marginally increased in the risk of infection because of potential reactivation/introduction of dormant bacilli (Tripathy & Tripathy, 2021). Moreover, GTB may are asymptomatic over years and tend to emerge only in the process of infertility research.

6. The Exposure to TB Cases and Family History-

The close contact with TB patients, especially in the family. One of the key risk factors is households. Exposure to a family puts the risk of primary infection higher, which could subsequently spread to the genital tract. It has been revealed that women who have family history of TB are more at high risk of emerging with genital involvement (Varma, 2020).

7. Socio-cultural and Behavioral Factors –

There are some socio-cultural factors that might contribute to poor performance including the practice of early marriage. Menstrual hygiene, and poor knowledge about TB symptoms, are the reasons that lead to late diagnosis, persistence of infection. The rural women might postpone medical assistance because of stigma or sex. Barriers, making the disease chronic and spreading (Indian Council of Medical Research, 2021).

Risk Factors Female-Specific.

a. Reproductive and Menstrual Factors - early menarche, repeat pregnancy or abortion.

b. Postpartum or Post abortal Sepsis - destruction of endometrium is a source of infection.

c. Uses of Intrauterine Device (IUD) - enhancing the ascending infection.

d. Hormonal and Nutritional Disproportions - associated with endometrial TB.

e. Pelvic Inflammatory Disease (PID) History.

Male-Specific Risk Factors

a. Urinary tract tuberculosis (UTTB) - the commonest site of infection.

b. Unprotected Sexual Practices - infrequent, but possible.

c. Local Trauma or Surgery - is capable of facilitating the spread of infection.

d. Infertility or Chronic Epididymo-orchitis - could be a cover to TB.

2.1.9 Associated clinical features of Genital Tuberculosis-

Genital tuberculosis (GTB) is a persistent granulomatous disease that is caused by Mycobacterium tuberculosis and it mostly attacks the reproductive organs in both males and females. It represents a major percentage of cases of extrapulmonary tuberculosis (EPTB) in India and is a major cause of infertility and reproductive morbidity (Sharma et al., 2019). The disease has a mixed clinical presentation and it is commonly linked with systemic, gynecological and reproductive manifestations making it difficult to diagnose and manage.

1. **General Associated Features** - Genital tuberculosis is in most cases the secondary TB of pulmonary TB or abdominal TB, which disseminates either through the hematogenous route, lymphatic route, or by direct extension. Low-grade fever, night sweats, loss of appetite, fatigue, and weight loss can be observed as systemic symptoms, but most of the patients do not show any symptoms (Varma, 2018). The infection can be chronic and anemia and general malaise may be the result of this being confused with other infectious or systemic disorders of the pelvis.

2. **In females Gynecological and Reproductive Gynecological In females**-GTB most commonly affects the fallopian tubes (90-100%), then the endometrium (50-60), the ovaries (20-30), cervix (5-15), and vagina/vulva (<1) (Arora et al., 2020). The infection causes a large spectrum of reproductive and menstrual malfunctions such as infertility, menstrual malfunctions, chronic pelvic pain, abnormal uterine bleeding, pelvic masses, and vaginal discharge. Beaded fallopian tubes and adhesions are some of the most suggestive laparoscopic findings (Sharma et al., 2019).

3. **Companied Features in Males**- Male genital tuberculosis tends to impact the epididymis, testes, prostate and seminal vesicles. The typical characteristics are scrotal edema, infertility and obstructive azoospermia, dysuria, hematuria, and sinus formations (Sinha et al., 2021).

4. **Imaging and Histopathological** - Tubal occlusion or hydrosalpinx is observable on ultrasonography and hysterosalpingography (HSG). Caseating granulomas and Langhans giant cells are also visible in endometrial biopsy (Arora et al., 2020). Laparoscopy is used to detect tubo-ovarian masses and dense pelvic adhesions characteristic of an advanced disease (Sharma et al., 2019).

5. **Immunological and Systemic Associations**- Women patients who have genital tuberculosis tend to have a high ESR and CRP. HIV-positive persons and immunocompromised ones might have more aggressive disease (WHO, 2024).

2.1.10 Persistence of Mycobacterium tuberculosis and Its Implications for Treatment Failure-

Unlike other disease the TB patient did not develop immunity to protect themselves from reinfection.

According to the article “Emerging strategies for the treatment of pulmonary tuberculosis: promise and limitation” by Wing Wai Yew and Won-Jung Koh[72]-

There are different kinds of MTB bacilli population present in the lesion of the host. In this population of bacilli, numerous of bacilli maintain and increase their population regularly by rapidly replicating and growing, where-as others are either grow rapidly or present in hibernation. These bacilli are known as persisters and can only be diagnosed by agents which have sterilizing activity.

Table 1: Drug and its activity

TB Drug	Activity
Isoniazid	Bacterial
Aminoglycosides	Bacterial
Rifamycin	Sterilization
Fluoroquinolones	Predominantly Sterilizing + some Bacterial
Pyrazinamide	Only Sterilizing

It is believed that dormant bacilli are present in highly calcified and fibrotic areas of the lungs and so safe from antituberculosis drugs.

Directly observe and treatment short course (DOTs) need a number of workers to decrease the relapse incident to prevent MDR-TB, because due to long treatment period, some patients leave their course within the treatment period resulting in development of MDR.

2.1.11 PREVENTION-

2.1.11(a) Preventive Strategies That Make TB Less Dangerous in HIV-Affected Populations-

The article "Prevention of Tuberculosis in People Living with HIV" written by Reuben Granich et.al 2010 details about prevention[73]. According to this Prevention is better than cure. Prevention became very important because-

- 2.2 billion people are infected and suffering with TB and 33 million due to HIV. So, there are much more chances to be infected with pulmonary tuberculosis and also infected with MDR TB, XDR TB or TDR TB directly and develop the respective disease directly. In HIV patient the recovery rate is slow and possibility of present of MTB bacilli in sputum cannot be easily disappear and make the sputum comparatively highly infectious.
- In HIV patient prevention become much more essential, because it has 20 to 37 times more at risk.

- TB is pivotal cause of death in HIV infected person
- 30% of HIV suffering patient will develop TB.
- Prevention is lifesaving activity not only for TB but also for MDR, XDR and TDR cases.
- Health care workers are always at greatest risk of TB infection
- Health care HIV positive workers are always comparatively at very highest risk of getting TB infection.

World Health Organization (WHO) gave 12 activities for HIV/TB (Isoniazid prevention treatment [IPT], intensified case tracing and infection control for TB) for prevention, care and treatment for HIV patient[74].

HIV prevention is prevention of TB. Patient tracing, diagnosis and successful treatment are done under DOTs (Directly observing therapy short course).

ART- Anti retroviral therapy not only prevent HIV infection but also prevent TB spread. 98% transmission of HIV in Africa reduces due to ART, counselling and treatment.

Infection control for TB- In 2007 many countries around 127 make infection control policies for health care facilities.

IPT (Isoniazid preventive treatment)- in 1998 WHO and United Nations Joint Programmed make a new IPT policy which have 6 concepts to prevent TB infection in HIV patients-

1. Encourage the HIV patient to participate in counselling and early diagnosis and treatment.
2. All patient should be diagnosed for active TB before starting IPT and avoid monotherapy.
3. IPT benefit persons specially ones whose TST is positive should be targeted first.
4. IPT must be given for at least for 6 months of daily dose be self-administered.
5. & 6. To monitor the toxicity adherence and outcomes.

In 1998 WHO and UNIDS (United Nations joint programme on HIV/AIDS) issue guidelines for management of children infected with TB and in 2004 WHO issue interim

policy on TB/HIV control. In 2008 again WHO held a meeting on HIV/TB coinfection and reemphasized the important of IPT for HIV/TB. 42 countries used IPT on HIV positive patient to control TB infection.

Rationale and Regimens-

Isoniazid treatment for 6 months to 12 months reduces the risk of TB. Isoniazid treatment result is found equivalent to rifampicin and pyrazinamide combined result. IPT reduces up to 33% the risk of active TB in high burden area of HIV and in case of TST positive case reduction occur up to 64%. In about all study, it was found that isoniazid was safe and tolerable and study proved that 9-month treatment gave optimal benefit[75].

IPT and Children-

In 2007, 2.5 million children are infected with HIV in which 3,30,000 deaths occur. The outcome of children are worst in comparison of adults and death in children 6 times more than adults. Death and pulmonary disease in children infected with HIV occur due to TB. By using IPT, TB infection rate reduces to 72% and mortality rate reduces to 54%. WHO advice to give 10 mg/kg of isoniazid per day to children age equal to or more than 3 months but no any advice available for children age less than 3 months.

2.1.11(b) Structural Insights in to IPT and ART: Duration, Safety, and Impact on TB Control

Studies carried out before ART shows that IPT protection covered up to 18 months and some studies shows that duration of IPT effect present up to 2.5 yrs. Studies shows that ART can reduces TB infection up to 80%.

IPT and ART-

Cohort studies done in Brazil and south Africa shows a 76 to 89 % reduction in TB infection when used IPT AND ART.

Screening for TB-

A result of inadvertent treatment of IPT, isoniazid and polydrug resistant cases arrived. So, in 1998 WHO/UNADS issue guidelines to carry out chest x ray before IPT.

Drug resistance-

A systematic review of data since 1952 summarized and published in may15 2010, according to this report the drug-resistant risk with IPT and placebo in same viral strains are not statistically different[75]. Patient resistant with isoniazid and resistant without isoniazid both respond identically. Possibilities of development of isoniazid resistant TB cannot be ignored.

Toxicity-

Isoniazid had more toxic side effect than placebo. Side effect occur due to isoniazid are vomiting, fever, hepatitis, nausea and peripheral neuropathy. Hepatoxicity sometimes became fetal. The risk to develop Hepatitis is high in alcoholic patient with isoniazid treatment. Although seeking advice, careful counselling, careful clinical monitoring and patient education regarding TB help to reduce the toxicity. Many studies suggested IPT is safe and effective up to 2.5 years period by reducing risk of TB by 33 to 64%[76,77].

According to the article “Control and prevention of tuberculosis: a code of practice” published by Joint tuberculosis committee of the British thoracic society- The Mycobacterium tuberculosis load reduces by 99% after 2-week adequate anti tuberculosis treatment and now patient become non- infectious from infectious.

2.1.11(c) A Structural Overview of Household Infection Control, Childhood TB Prevention, and Advances in Vaccines-

The treatment result of pulmonary tuberculosis at hospital is not better than home. In hospital there are many admit patients of different disease and due to illness, their immunity is comparatively weaker and have high risk of infection but at home the risk of infection to family member is negligible according to several controlled studies.

Segregation of smear positive patient are necessary until to become smear negative. Bearing mask and gown is fail to protect so smear positive patient should cover the mouth and nose during coughing, sneezing and talking. Separate wash room etc. are unnecessary. Infectious material like sputum or sinus discharge should be heat sterilization before disposable. Fumigation of rooms or ward is useless.

"Prevention of tuberculosis in household members, estimates of children eligible for treatment"-article written by Yohhei Hamada et. Al 2019[78]. According to this article-

In young children conversion of latent tuberculosis to active tuberculosis is comparatively high compare to other age group. So, tracing of such individuals is essential to start preventive treatment.

Number of child household contacts eligible for preventive treatment is calculating as

N= n/c hp. (1-T) L **[L** is included in high TB burden country only]

N= number of eligible children house hold

n= number of notified cases of tuberculosis

c= average number of active tuberculosis case per household with an index case

h= average house hold size

p= population of the nation younger than 5 yrs

T= proportion of child household contact had active TB

L= prevalence of a confirmed latent TB among child house hold

In 2017 there were only 23% of children eligible for preventive treatment.

C. Fordham von Reyn and Jenni M. Vuola in his journal, 2002 "New vaccine for prevention of tuberculosis" explain that[79]**-**

BCG (bacille Calmette Guerin) vaccination at childhood prevents children from tuberculosis but fails to prevent from reactivation of tuberculosis and HIV related tuberculosis.

Due to a number of disadvantage development of a new advance vaccine become world's one of the top priorities. Work on development of candidate vaccine being initiated by consideration following points-

- Natural history of Mycobacterium tuberculosis immunology of MTB infection.
- Reanalysis of non-tuberculosis Mycobacterium.
- Draw fall of BCG.
- Identification of immune dormant antigen by molecular method.
- Mode of antigen delivery.

2.1.11(d) From Natural Immunity to Next-Generation Vaccines: A Structural Perspective on TB Prevention

It was found that among 10% of infected person, 5% develop active tuberculosis within 1-2 years and rest 5% develop after reactivation[79].

Immune factor responsible for latent infection which are unable to identify.

Tuberculosis bacilli reach to the alveoli of lungs with small droplets here MTB bacilli engulfed by macrophage.

Macrophage activation occurs due to INF-ϒ release by TB infection.

According to study previous tuberculosis infection provide natural protection against upcoming identical infection. Animal and human study shows that previous infection of non- tuberculosis bacilli generates natural protection immunity against upcoming Mycobacterium tuberculosis infection. Mycobacterium avium is the commonest non-tuberculosis bacilli infecting human and 40% of adults are infected with it in united state[80].

Three types of vaccines found to be effective on tuberculosis prevention-

BCG vaccine-

Protection method-

BCG is the exceptional vaccine in our hand having licensed for preventing the tuberculosis infection.

BCG is live weakened strain of Mycobacterium bovis, identically similar to M. tuberculosis. It provides immunity against M. Tuberculosis.

Efficacy-

BCG is frequently administered in newborn in countries where TB is endemic and countries where TB administrated in high-risk infants. It has been found that BCG can only prevent the disease not the infection.

Table 2: Showing negative and positive aspects of BCG

NEGATIVE ASPECTES	POSITIVE ASPECTS
Limited efficacy in mycobacteria exposed children	Protection of new born from death due to childhood tuberculosis
Effect on skin test result.	Protection of new born from Miliary meningeal TB
Potential for disseminated BCG in HIV patient.	Protection of new born from Tuberculosis infection
Duration of protection is less.	Protection of new born from Non-tuberculosis Lymphadenitis, leprosy Buruli ulcer
Not protected from reactivated tuberculosis	
Booster dose of BCG is not effective, because strain of vaccine unable replicate due to previous immunization.	

Previous exposure to tuberculosis infection retards the efficacy of BCG by reducing it's replication. Also protect from non-tuberculosis infection and mycobacterial adenitis, due to non- tuberculous bacilli (M. Avium complex).

Immunogenicity-

BCG induced both cellular and humoral response to MTB. BCG vaccination at birth develops th_1- type immunity against MTB antigen. It was also found that Intradermal immunization is more immunogenic in comparison to subcutaneous immunization. Low dose of BCG is less immunogenic then normal or high dose. So normal or high dose of BCG had preferred.

Mycobacterium vaccine-

In 1950 live strain of Mycobacterium microti was used to carry out a trial by medical research council, upon 54239 tuberculin negative subjects and found 5-year efficacy rate of 84%, similar to BCG. This trial proven that other strain of Mycobacterium like M. bovis (BCG) can protect from tuberculosis.

Inactivated Whole Cell Mycobacterial Vaccines and its application-

Inactivated whole cell mycobacterial vaccine were being used before BCG acceptance and it also prevent tuberculosis infection. Control trial done on 1930. It is a multiple dose series of heat killed M. tuberculosis carried out by Dr Jles Freund he demonstrated that It's efficacy had 42%.

Characteristics of an improved tuberculosis vaccine-

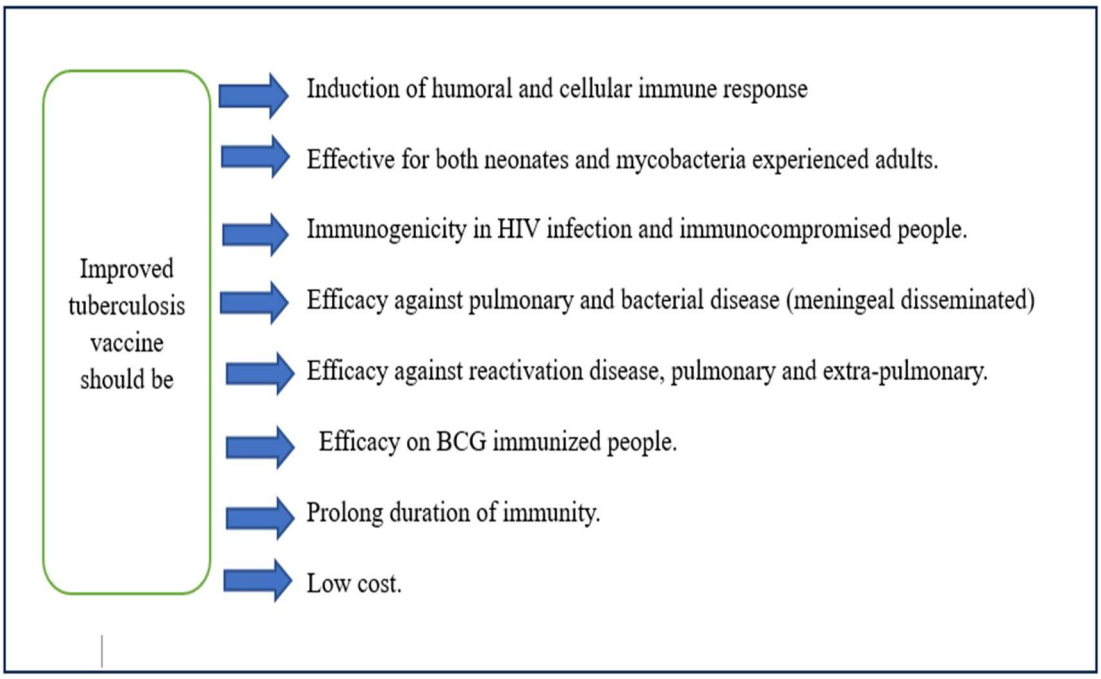

Figure 11: Improve tuberculosis vaccine characteristics

BCG vaccine is utilized as a "gold standard"

Design of human trial: -

Trail on human being basically undergoes in three phase-

A. Initial phase (phase I)-
   First carried out in adult then children and then immunocompromised people. Safety trial carried out in mycobacteria naïve and mycobacteria experienced patient with previously BCG vaccinated or without BCG vaccinated from endemic area.
B. Immunogenicity testing (phase –II) –
   It is essential for safe testing.
C. Controlled efficacy trials (phase-III)
   It is for ethical and logistic issues.

Vaccine under Development-

A. Whole cell live vaccines-

⇒ Attenuated BCG or M. tuberculosis- continuously research in the field of bioinformatics and molecular microbiology enable us to know about the genome sequence of tuberculosis and explore the field of vaccine development by targeting desirable mutant. In a study it was found that when an immunodeficient mice immunized by advance BCG, mice die but with auxotrophic vaccine mice not die, supporting that auxotrophic vaccine would be safe for HIV patient[81].

⇒ **Enhance BCG-**

A more advance form of BCG had made by adding plasmid coding for the 30-kDa major secretory or extracellular protein, also called as "Antigen 85B".

In a guinea pig model, this recombinant BCG vaccine lowers the CFU in liver and lungs and found long survival compared with parent BCG.

⇒ **Whole cell inactivated vaccines**-

Mycobacterium vaccae is found safe and preventive to tuberculosis in animal model. M. vaccae is also found safe in human subject and stimulate Mycobacterium specific antibody lymphocyte proliferative and IFNϒ response in healthy, as well as in HIV infected and BCG primed person

⇒ **Subunit vaccine**-

Subunit vaccines are prepared to deliver mycobacterial antigen known for immunogenic and protective in animal models.

⇒ **DNA Vaccine-**

In DNA vaccine, vaccination carried out with DNA plasmid having vaccine antigen encoding a gene sequence. It is efficient to induced cellular and humoral response in rodent.

⇒ **Prime boost vaccine**-

It is successive administration of the identical mycobacterial antigen expressed by two non-identical vaccine vectors.

2.1.12 Prevalence of Genital tuberculosis in different geographical areas of India-

According to the literature "Prevalence of Genital tuberculosis among infertile women: A systematic Review and meta-analysis"[82] published in April 25 – the prevalence of genital tuberculosis in infertile population were as follows-

Table No-3: showing prevalence of GTB among infertile population in India according to different authors-

SN	Name of first author	Published in	Sample size	Prevalence of GTB (%)
1	Punyashetty[83]	2012	230	3.9
2	Jindal[84]	2006	150	7.2
3	Nadgouda[85]	2010	170	10
4	Shanti Sri[86]	2013	100	12
5	Marii[87]	2003	110	13.6
6	Chhbra[88]	1986	150	14
7	Patted[89]	2009	96	21
8	Khanna[90]	2011	100	26
9	Gupta[91]	2007	150	26.7
10	Jindal[92]	2012	443	38.1
11	Parikh[93]	1997	300	39
12	Neelam[94]	2005	186	44.1
13	Singh[95]	2008	70	48.5
14	Rozati[96]	2006	65	49.2
15	Kulshrestha[97]	2011	196	60.2

According to the article "Prevalence of genital tuberculosis in infertile women, a study from a tertiary care center in north India, that out of 193 infertile patient 13 (6.73%) were found suffering with Mycobacterium tuberculosis[98].

Based on the study of an article “Genital tuberculosis in female “published in April 2017 prevalence of genital tuberculosis is 9% among extrapulmonary tuberculosis cases in India[99].

According to different studies the prevalence of Genital tuberculosis varies from 3 to 16 % among infertile women in India[100,101].

Table 4 -Prevalence of Genital tuberculosis in different geographical areas of India-

State / Region	Study Population & Setting	Diagnostic Methods	Sample Size	Prevalence (%)	Reference
India (Pooled)	Infertile women across many studies	Mixed diagnostics	30,846	20%	Ahmed et al.
North India (Multiple clinics)	Tertiary infertility clinics	Clinical + radiological	Variable	3–16%	ICMR Review
Delhi (AIIMS)	Unexplained infertility	Laparoscopy + HPE + PCR	212	26%	Sharma et al.
Uttar Pradesh (Lucknow)	Infertile women (SGPGI)	PCR + histopathology	327	18.3%	Tripathi et al.
Bihar (Patna)	Gynecology OPD infertility cases	HPE + AFB + GeneXpert	241	12.4%	Kumari et al.
Haryana (Faridabad)	Clinically suspected	Xpert Ultra + culture	623	2.73%	Gunasekaran et al., 2024
Punjab (Ludhiana)	Chronic pelvic pain + infertility	Laparoscopy + biopsy	176	14.2%	Gill et al.
Rajasthan (Jaipur)	Infertility evaluation	Laparoscopy + HSG + PCR	264	10.8%	Maheshwari et al.
Maharashtra (Mumbai)	Infertility clinic attendees	TB-PCR + laparoscopy	301	19.2%	Mehta et al.
Gujarat (Ahmedabad)	Laparoscopy-based infertility study	Laparoscopy + biopsy	187	11.6%	Patel et al.
Tamil Nadu (Chennai)	Infertility & menstrual disorders	HPE + PCR	205	9.1%	Rao et al.
Karnataka (Bengaluru)	Tubal-factor infertility	Laparoscopy + PCR	174	16.7%	Nayak et al.

Kerala (Thiruvananthapuram)	Suspected GTB cases	TB-PCR + biopsy	263	6.8%	Nair et al.
West Bengal (Kolkata)	Primary infertility patients	PCR + laparoscopy	198	8.4%	Banerjee et al.
Jharkhand (Ranchi)	Infertility OPD	HSG + PCR	156	7.9%	Singh et al.
Madhya Pradesh (Bhopal)	Menstrual dysfunction + infertility	Histopathology	142	9.8%	Verma et al.
Assam (Guwahati)	Chronic pelvic pain	PCR + biopsy	118	6.4%	Deka et al.
Jammu & Kashmir (Srinagar)	Infertile women	Laparoscopy	126	13.5%	Qureshi et al.
Telangana (Hyderabad)	Reproductive complaints	TVS + laparoscopy + PCR	233	11.2%	Reddy et al.
Andaman & Nicobar Islands	Community screening	Survey + clinical	-	45.1 per 100,000 women	Parvez et al., 2017

- The highest prevalence is always reported in the population of tubal-factor infertility, which may range up to 20-40, particularly in North India.
- At the level of the community (e.g., Andaman Islands), prevalence is significantly lower due to the fact that genital TB is mostly asymptomatic and not detected.

- States that have high TB burden (Bihar, UP, Delhi, Maharashtra) tend to exhibit higher clinical prevalence of FGTB in infertility clinics.

- Strong influence on prevalence has diagnostic method:

Laparoscopy + PCR	→	highest detection.
Histopathology	→	Lower detection.
GeneXpert	→	Less sensitive with FGTB since bacillary load is low

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CHAPTER-3

HYPOTHESIS FOR RESEARCH

As the cases of infertility increasing in Bihar particular post pulmonary Tubercle infection in the family as per the news agency. So, researcher thought it may be due the breach of tubercle infection in other organs. To the best of our knowledge, there has not yet been any research on prevalence and risk factor of genital tuberculosis in northern part of Bihar.

India is known as the top TB prevalence country of the world because of the major barrier to socio-economic development of the country. The alarming situation in the country is the increasing number of tuberculosis patients which indicates the higher need to raise the control measures for the disease. Control of this disease largely depends on early detection of disease and treatment of patients. The present study provides an insight for current knowledge in root cause & pathological investigation for patients with tuberculosis.

Despite advances in medical science technology, the diagnosis of Tuberculosis is still primarily relied on clinical symptoms of the tuberculosis patient. For many researchers all over the world, prevalence of genital tuberculosis, its risk factor and associated clinical features among the population of northern part of Bihar is must be a new topic for further research.

CHAPTER-4
OBJECTIVES OF THESIS

Objectives: -

1. Analysis of the socio economic back ground reveals the genital tuberculosis
2. To investigate the early sign and symptoms in suspected cases of genital tuberculosis.
3. Isolation and identification genital tuberculosis infection by using routine and advance diagnostic techniques.
4. To find out the relationship between infertility and genital tuberculosis.

CHAPTER-5

MATERIALS AND METHODS

5 (A) STUDY DESIGN;

Our study is a cross-sectional study which design to find out the disease in different types of symptomatic and asymptomatic (having family history) patients carried out at Ganga Diagnostic Centre and Department of pathology Sadar hospital Khagaria and Begusarai.

The proposed work was carried out between January 22 to August 24 under the guidance of my supervisor and with the inputs from well-known pathologist at Ganga Diagnostic Centre as well as Department of Pathology, Sadar Hospital Begusarai and Khagaria

PURPOSE OF THE STUDY-

During reviewing literature, it was found that literature on prevalence of genital tuberculosis in Bihar is very negligible. Also available data on genital tuberculosis is also much in need. Infertility is the most common cause of genital tuberculosis. In these areas reproductive ability is very valuable. Infertile male and female both feel guilty and inadequacy. In society even in family they are treated as a social stigma. Due to this stigma and misconceptions, they face isolate from family, friends and society.

5. (B) SAMPLE SIZE:

342 subjects were selected instead of 323, in accordance with the inclusion-exclusion criteria to compensate for drop-outs, and sample size calculated by sample calculator formula given below-

$$S = Z^2 \times P \times \frac{(1-P)}{M^2}$$

- S = sample size for infinite population
- Z = Z score
- P = population proportion (Assumed as 50% or 0.5)
- M = Margin of error

Note: Z score is determined based on the confidence level.

Confidence Level	z-score (±)
0.70	1.04
0.75	1.15
0.80	1.28
0.85	1.44
0.92	1.75
0.95	1.96
0.96	2.05
0.98	2.33
0.99	2.58
0.999	3.29
0.9999	3.89
0.99999	4.42

$$S = (1.96)^2 \times 0.30 \times \frac{(1- 0.30)}{(0.05)^2}$$

$$S = 3.84 \times 0.30 \times \frac{(0.70)}{(0.0025)}$$

$S = 3.84 \times 0.30 \times 280$

$S =$ **322.56**

Ethical Clearance-

Ethical clearance from Independent Ethics Committee (Clinical Research) India Address 119, Rajdanga Gold Park, RB Connector, Kolkata – 700107 also from Chief Medical Officer Sadar Hospital Khagaria

5. (C) INCLUSION AND EXCLUSION CRITERIA OF PATIENT AND METHODS:

Exclusion Criteria:

1. Age – below 18 years and above 50 years.

Inclusion Criteria:

- Person having symptoms of infertility, mild fever in evening, chronic coughing, weight loss, loss of appetite.
- Primary and secondary infertile female
- Post menopause women with abnormal uterine bleeding (AUB)
- Irregular menstrual cycle
- Presence of lymph node or gland.
- Past history of tuberculosis either subject or family member.

Proforma and Consent Form

DATE(दिनांक)		PATIENT ID(रोगी पंजीकरण संख्या)	
PATIENT NAME(रोगी का नाम)		FATHER / HUSBAND NAME (पिता/पति का नाम)	
AGE(उम्र)		SEX(लिंग)	
ADDRESS,(पता)		OCCUPATION (पेशा)	
ABDOMINAL PAIN (पेट में दर्द)		DOCTOR NAME(डॉक्टरका नाम)	
PREVIOUS HISTORY OF TB (टीबी . का पिछला इतिहास)		PRESENCE OFLYMPH NODE OR GLAND(लिम्फ नोड की उपस्थिति)	
WEIGHT(वजन)		HEIGHT (ऊंचाई)	
FEVER HISTORY(बुखार इतिहास)		WEIGHT LOSS HISTORY(वजन घटने का इतिहास)	
MARITALSTATUS(वैवाहिकस्थिति)		EDUCATION (शिक्षा)	
NO OF CHILD(बच्चे की संख्या)		MISCARRIAGE HISTORY(गर्भपात इतिहास)	
INFERTILITY TYPE(बांझपन प्रकार)		MENSTRUATION STATUS (मासिक धर्म की स्थिति)	

I have provided true and reliable information and fully understand the indication, intended purpose of study.मैंने सही और वि
जानकारी प्रदान की है और संकेत, अध्ययन के इच्छित उद्देश्य को पूरी तरह से समझता हूं।

Signature of Patient(रोगी के हस्ताक्षर)		Contact Number (संपर्क संख्या)	

Figure 12-Consent form.

Roles and responsibilities: -

Multi Task Worker-02- (Mr. Rishi Kumar and Guddu Kumar)-arrangement of camp, provide pre-information to all the concerned people and help in maintaining records.

Laboratory Technician- 02- (Chandan Kumar and Ramu Kumar)- perform counselling, documentation, marking unique id and collection of different types of samples like blood, sputum, urine etc**.** Performing investigation under the supervision of Dr Vijay Kumar and Dr Manish Kumar.

Dr. Umesh Kumar MBBS-performs counselling on a priority basis.

Late Dr Amrita MD(Gynaecologist-01)- perform counselling on priority basis, tissue isolation from different sites and vaginal smear preparation.

5(D) Materials and Methodology:

Data collection, clinical examination and other general investigation-

1. Identification-

   Name-

   Age/Sex-

   Patient ID-

   Address-

2. History Taken-
   Occupation-
   Abdominal pain-
   Previous history of TB-
   Presence of Lymph node or gland-
   Weight
   Hight
   Fever history-
   Weight loss history-

Marital status-

Education-

Number of children-

Miscarriage history-

Infertility type-

Menstruation status-

3. Investigation-

Total leucocyte counts and differential leucocyte count

Hb

Erythrocyte sedimentation rate (ESR)

Mantoux test

X ray

AFB (Urine/Sputum)

Pap Smear

TB PCR

Gene-Xpert

FNAC

Histopathological Examination-

Culture

5(E) SPECIMEN AND STORAGE-

1. BLOOD- **342** blood samples were collected
2. Sputum-47 samples were collected.
3. Urine samples -**252** samples were collected for the microscopic examination of AFB in urine sediments.
4. Cervical smear- from a patient having symptoms of vaginal discharge, primary and secondary infertile patient samples were collected-**29** samples.
5. Tissue-**05** samples
6. FNAC-**12** FNAC samples were collected.
7. Pus -**01** sample was collected for culture.

8. Menstrual blood- **17** samples were collected.
9. Semen-**01** sample was collected for culture.

Table No-5- **Sample and its storage guidelines-**

Sample Type	**Container used**	**Preservatives used**	**Storage Temperature**	**Maximum Storage Duration**	**Remark**
Blood	EDTA/Heparin tube	EDTA / Heparin	2–8°C	24–48 hrs. (culture), 7 days (molecular)	Avoid freeze–thaw cycles
Sputum	Screw-capped container	None	2–8°C	Up to 7 days	Process quickly for TB tests
Urine	Sterile container	None / Boric acid	2–8°C	24 hrs.	Fresh sample preferred
Endometrial / Cervical Swab	Transport medium	PBS or TB transport medium	2–8°C	24–48 hrs.	For culture or PCR
Tissue Biopsy	Sterile vial	Normal saline / RNA solution	2–8°C	24 hrs. / long-term −80°C	Avoid drying
Serum / Plasma	Cryovial	None	−20°C	6–12 months	For ELISA or serological tests
Pus	Sterile screw-capped container	None	2–8°C	Up to 72 hours (ideal <24 hrs.)	Do not freeze before culture
Menstrual Blood (day 1 to day 3)	Sterile wide-mouthed container	None	2–8°C	24–48 hours	Best sample for female GTB
Semen (3-5 days abstinence)	Sterile wide-mouthed container	None	2–8°C	Up to 24 hours (ideal 6–12 hrs.)	Allow liquefaction before processing

5.(F) Selection and Rejection Criteria of Sample-

Different types of clinical specimens were collected based on the site of involvement, clinical presentation, and feasibility of sample collection. All specimens were collected under aseptic precautions and transported immediately to the laboratory for further analysis.

1. Blood Sample

Selection Criteria:

a) Collected from all asymptomatic and symptomatic cases for hematological (e.g., ESR, Hb, WBC count) and for molecular testing according to need.
b) 2–3 mL of venous blood collected in EDTA, Citrate and Heparin tubes under aseptic precautions as per need.

Rejection Criteria:

a) Hemolyzed, clot or insufficient blood samples.
b) Patients on recent immunosuppressive or anti-TB therapy.

2. Sputum Sample

Selection Criteria:

a) Collected from patients presenting with chronic cough, fever, or pulmonary symptoms along with suspected genital TB (possible dual infection).
b) Early morning, deep cough sputum collected on three consecutive days in sterile containers.

Rejection Criteria:

a) Saliva or inadequate sputum samples.
b) Contaminated or mislabeled containers

3. Urine Sample

Selection Criteria:

a) Early morning, midstream urine samples. Three consecutive days sample collected in suspected cases of genital TB.
b) Urine sample collected in sterile container.

Rejection Criteria:

a) Contaminated or delayed samples (>2 hours unprocessed).
b) Patients with urinary tract infection unrelated to TB**.**

4. Endometrial Tissue / Curettage Sample

Selection Criteria:

a) Women presenting with infertility, irregular menstrual cycles, or chronic pelvic pain.
b) Endometrial biopsy obtained during the premenstrual phase (Day 21–23 of cycle) or by diagnostic curettage**.** Sample should be sufficient in quantity and without any contamination by vaginal secretions.

Rejection Criteria:

a) Patients having active uterine bleeding or in case of suspected malignancy.
b) Inadequate or necrotic tissue samples.

5. Cervical and Vaginal Swabs

Selection Criteria:

a) Patients with chronic cervicitis, abnormal vaginal discharge, or pelvic pain.
b) Samples collected before pelvic examination or use of antiseptic solutions.

Rejection Criteria:

a) Samples collected post-coitus or after vaginal medication within 48 hours.
b) Inadequate or improperly labeled samples.

6. Pus

Selection Criteria:

a) Sample collected by aseptic needle by aspiration in sterile, leak-proof container.
b) Received in laboratory within 1 to 2 hours.
c) ATT (anti tuberculosis treatment) more than 7 days.

Rejection Criteria:

a) Sample collected in cotton, without labelling and cracked container.
b) Delayed transported sample.
c) Patient on ATT.
d) Non-representative tissue or samples compromised during collection or transport.

7. Menstrual Blood Sample

Selection Criteria:

a) Women of reproductive age group or suspected case of genital tuberculosis.
b) Collection during the first or second day of menstrual flow using sterile containers.

Rejection Criteria:

a) Contaminated sample with urine or vaginal discharge.
b) Women on anti-TB therapy or under hormonal treatment.

8. Semen Sample-

Selection Criteria:

a) Abstinence period should be 3 to 5 days.
b) Sample should be collected by masturbation in sterile, leak proof container with proper labelling.
c) Should be received in laboratory within 1 hour of collection.
d) Should not be under treatment with ATT.

Rejection Criteria:

a) Sample collected in condoms.
b) Delayed sample, insufficient sample and leak sample.
c) Under treatment of ATT.

5.(G) SAMPLE COLLECTION PROCEDURE-

Blood-

Sample A- 2 ml blood collected in EDTA vial to process the tests of TLC, DLC, Hb, ESR

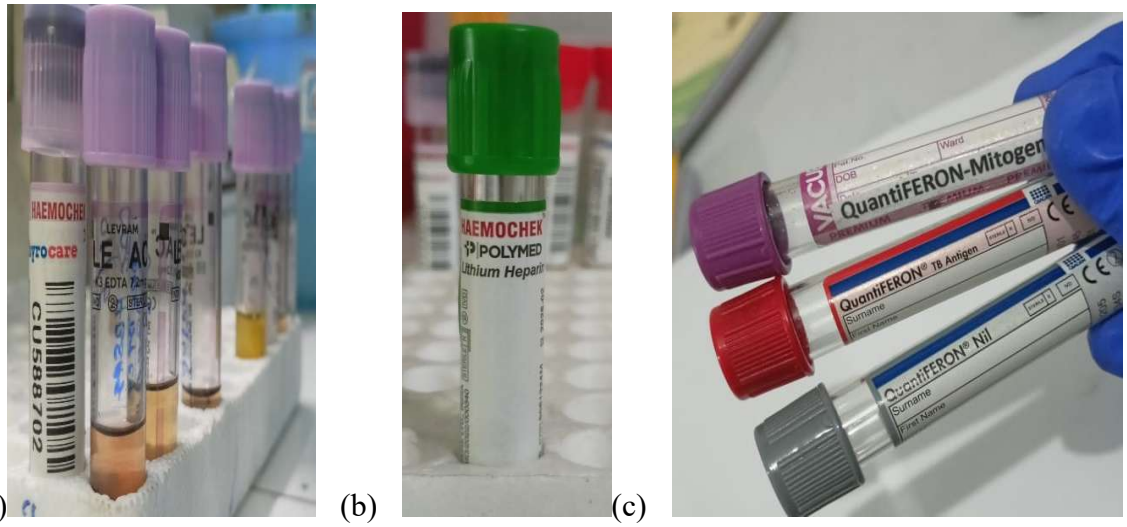


Figure 13- Blood collection tubes (a) EDTA (b) Heparin (c) Nil, Antigen & Mitogen tube

Sample B- 4.0 ml blood collected in lithium heparin vial for INF-γ assay.

Blood sample should be stored at 2-8 ºc except for the heparin sample, it should be store at room temperature but not more than 16 hrs., within 16 hrs,1 ml of blood should be transferred in to each vial nil, antigen and mitogen vial.

Sputum (Expectorated & Induced)

Accurate sample collection is very important for all the investigations. So, it is very necessary to conduct the collection according to the protocol. Well-ventilated open space for sputum collection is used and provided wide mouth, screw capped, and sterile container to the patient. Before given marked the patient's name, age, ID, sample type and date on the container. Patient has trained to first wash the mouth with water and collect the sputum after deep cough. Induced specimen is produced from patients who were not able to give sputum (nebulization with normal saline). For tuberculosis diagnosis purpose collect and conduct 2 sputum examination (SPOT and MORNING).

Sputum – specimen collection: container and PP(Falcon) tube-

Sputum Cups

Figure 14: sputum cap

- 30-50 ml volume capacity wide mouthed with crew capping sputum cap is used. Which was translucent, clear visible, proper and adequate space for marking details and leak proof, disposable, single use container.

PP (Falcon) Tube-

Figure 15: Falcon Tube

50 ml volume capacity of centrifuge tubes (pp tube) made with polypropylene materials is used. Which was translucent, clear visible having proper and adequate space for marking details. This tube is leaking proof, wide mouthed with screw capping, disposable and single use container.

Cervical smear/ pap smear-

Clean glass slides were taken. An Ayer's spatula was taken to collect the material and make a round smear on slide. Stain the slide with Papanicolaou stain for microscopic examination.

Figure-16 pap smear kit.

Tissue-

Different types of tissue send for histopathological examination and bacilli detection in leak proof jar containing 10% formalin with proper labelling of patient details.

Pus-

Sterile syringe was used to collect pus. Then transfer the pus in to sterile, leak proof container. All procedure done aseptically to avoid any contamination. Proper patient information mention on the container before sample transfer. Proper packing done to prevent any leakages.

Menstrual blood-

Day 1 to day 3 was collected aseptically in wide mouthed container. 5 to 10 ml of menstrual blood was collected by gynecologist. Sterile syringe /aspiration cannula was used.

Semen-

Sterile, leak proof, wide-mouthed container was used. Sample was collected after 3-5 days abstinence. If collected outside the laboratory, sample send to the laboratory with in 1 hour.

Direct and Indirect Evidence of Tuberculosis

According to World Health Organization. (2024) and Global Tuberculosis Report 2024. There are two types of methods for the diagnosis of *Mycobacterium tuberculosis s-*

1. Direct Evidence

Direct evidence of tuberculosis is the method by which we detect Mycobacterium tuberculosis bacilli or its components from clinical samples, confirming the disease. This is taken as the confirmatory test in this study.

2. Indirect Evidence

Indirect evidence is the immunological or pathological response of the host to infection by M. tuberculosis without the direct identification of the bacillus.

Table No-6- List of investigation under direct and indirect evidence.

Type of Evidence	Basis of Detection	Common Diagnostic Methods	Interpretation / Diagnostic Significance
Direct Evidence	Demonstration or isolation of Mycobacterium tuberculosis bacilli or its genetic material from patient specimens.	Ziehl–Neelsen (ZN) / Fluorescent Auramine O staining for AFB; Culture (Löwenstein–Jensen) Molecular tests (CBNAAT / GeneXpert, PCR); Histopathological demonstration of AFB in tissue sections.	Confirms the presence of M. tuberculosis infection; considered definitive (confirmatory) diagnosis.
Indirect Evidence	Detection of the host immune response or tissue reaction suggestive of tuberculosis infection.	Tuberculin Skin Test (Mantoux); Interferon-Gamma Release Assay (IGRA); Radiological findings (X-ray); Histopathology (granulomatous inflammation with caseous necrosis);	Indicates exposure or probable infection; supports diagnosis when bacilli are not demonstrated; used as supportive / presumptive evidence.

This study had completed by performing following evaluation:

- Detail clinical history of subject and their family members.
- Routine and Advance investigations.

5(H) ROUTINE INVESTIGATIONS:

Table No 7: **Routine Investigations its method and reagent**

S. No.	PARAMETER	METHOD /REAGENT KIT
1	Hemoglobin	Sahli's method/ Drabkin method
2	TLC DLC (Total leucocyte count and differential count)	Leishman stain- Microscopy
3	ESR (Erythrocyte sedimentation rate)	Western green method
4	MT (Mantoux test)	Tuberculin skin test
5	AFB	Ziehl Neelsen Stain/ Auramine stain
6	X RAY	Digital

5(I) ADVANCE INVESTIGATIONS:

Table No 8: **Special investigations it's method and reagent**

S. No.	PARAMETER	METHOD /REAGENT KIT
1	TB GOLD	Gamma interferon detection
2	Gene Xpert	Nucleic acid amplification
3	FNAC	Microscopic
4	PAP STAIN	Microscopic
5	TB PCR	Polymerase chain reaction
6	Histopathological Examination	Microscopic
7	Culture for MTB	Lowenstein Jensen medium

5(J) Quality Control-

The guidelines of internal as well as external quality control of respective investigations are strictly followed-

Frequently used internal quality controls were-

Table 9: **Investigation and used IQC**

S. No.	PARAMETER	INTERNAL QUALITY CONTROL USED
1	TRUE NAAT	Inbuilt IQC – SAMPLE PROCESSING CONTROL (SPC) AND PROBE CHECK CONTROL (PCC)
2	ZIEHL NEELSEN STAIN/AURAMIN STAIN	PANEL SLIDE (1+,2+,3+) NEGATIVE SLIDE
3	ZIEHL NEELSEN STAIN/AURAMIN STAIN	E. coli ATCC 25922

Following external quality controls were used in study-

Table No 10: Investigation and used EQAS

S. No.	EQAS TEST NAME	CENTER ACCREDITED FOR TESTING
1	MYCOBACTRIUM TUBERCULOSIS	Tuberculosis (NEPT) Nation Tuberculosis Institute Bengaluru, Karnataka
2	MOLECULAR MICROBIOLOGY	IAMM EQAS NEW DELHI (Sir Ganga Ram Hospital New Delhi)

5(K) TEST METHODOLOGY-

HEMOGLOBIN-

Principle

Hemoglobin is converted to acid hematin when blood sample is mixed with N/10 HCl. It produced brown colour. Distilled water is added drop by drop until it visually matches with the comparator; the tube scale then gives hemoglobin in g/dL (gm%).

Materials & reagents

- Sahli's haemoglobinometer (comparator box with brown glass standard, graduated hemoglobin tube).
- Hb pipette (marks to contain 0.02 mL = 20 µL.
- Dropper/Pasteur pipette, glass stirring rod.
- 0.1 N (N/10) HCl
- Distilled water.
- EDTA anti-coagulated blood (well mixed).

Procedure-

1. Clean and dry the haemoglobinometer tube and pipette. Comparator and brown glass standard should be free of dust.

2. Fill the hemoglobin tube with N/10 HCl up to the lower mark of 2 gm% by using a dropper.

3. Properly mix the EDTA blood. Draw blood up to the 20 µL mark in the Hb pipette. Wipe out the excess blood from the tip of the pipette.

4. Add 20 µL blood into the HCl in the hemoglobin tube. Mix properly with the glass rod.

5. Leave it to stand 10 minutes, required to convert hemoglobin to acid haematin. (About 95% conversion occur at 10 min but in some literature waiting time up to 20 min is required for completeness.)

6. After that add distilled water drop by drop and mix until the brown colour of the solution matches with the comparator.

7. When colour matches, read the hemoglobin value directly from the graduated scale on the tube (read the lower meniscus). Report in g/dL (gm%).

Reference Range-

In male; 13.0 g/dl to 17.0 g/dl

In female: 12.0 g/dl to 15.0 g/dl

TOTAL LEUCOCYTE COUNT AND DIFFERENTIAL LEUCOCYTE COUNT-

Total Leucocyte Count and Differential Leucocyte Count done by the help of fully automated CBC analyzer. But mild and moderate case of leucocytosis microscopic examination was also done.

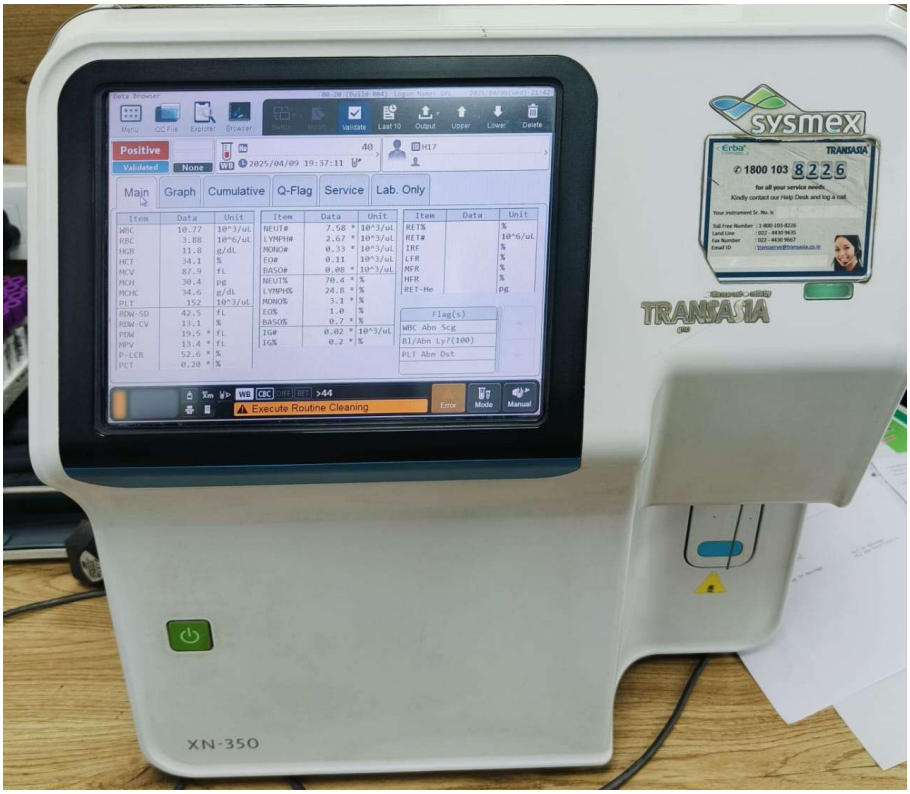


Figure 17- CBC analyzer- 5 part

Differential Leucocyte Count-

Principle-

Different types of leucocytes were counted microscopically based on their size, nucleus and cytoplasmic granules. 100 cells were counted and number of cells denotes their percentage.

Material required-

EDTA Blood

Clean glass slide

Leishman stain

Distilled water

Microscope

Oil immersion

Test procedure-

A well-mixed small drop of blood was taken on one end of the slide.

Using a spreader slide at 30^0 to 45^0angle spread this drop over the slide to make a well spread tongue shaped thin smear.

Air dry the slide.

After drying slide flood, the Leishman stain over it and leave for 2 minutes.

Add double the volume of distilled water.

Wait for 8 to 10 minutes.

Wash the slide with tap water.

Air dry and examined under oil immersion (100x).

ESR (ERYTHORCYTE SEDIMENTATION RATE)-

It is a prognostic test but used widely for treatment and diagnostic purpose[103,111].

Westergren method was used to determined ESR value.

Material required-

1) Westergren ESR tube.
2) 3.8% Sodium citrate
3) Pasteur pipette
4) Stop watch

Test procedure-

1.6 ml whole blood was taken and 0.4 ml 3.8 % sodium citrate mixed in it.

By the help of Pasteur pipette, Westergren tube was filled up to 0 mark. Now Place this tube vertically on ESR stand. Leave it for 1 hour at RT with disturbing it.

Stop watch was started and after 1 hour reading was taken. Level of clear plasma above the RBC column gave the ESR value.

Figure 18- Western green ESR pipette with ESR stand.

Reference Range:

In male: 0 to 15 mm in 1 hour. In female: 0 to 20 mm in 1 hour

Very high ESR > 50 mm in 1 hour are strongly suggestive of tuberculosis infection.

MANTOUX TEST (MT)-

The word Mantoux comes from the name of “Charles Mantoux” a French physician who discovered the Mantoux test in 1907. It is the simple, traditional and worldwide accepted tool for the diagnosis of Mycobacterium tuberculosis. It is also called as Tuberculin skin test (TST). The first tuberculin was developed by Robert Koch in 1890 called as “Old tuberculin”. It was prepared by killing the tubercle bacilli at 100ºc in a steam cabinet. Again in 1941, Seibert and Glenn developed a purified product and named purified protein derivatives (PPD). This PPD was prepared by using sterilizer and by repeating precipitation with ammonium sulphate, protein was purified.

This PPD was accepted by WHO in 1952[114-116]. In 1957 PPD was prepared for international use, it was known as Tuberculin PPD RT23[117].PPD RT 23-Purified protein reference tuberculin. 23 was the particular batch. Over 500 grams of this batch were prepared to fulfil the global requirement.

There is no any accepted conversion factor between new PPD RT 23 and PPD-S. But generally, 2 TU of PPD RT 23 are assume to equivalent to 5 TU of PPD-S by WHO and International Union Against Tuberculosis and Lung Disease (IUATLD)[118]. At present there were only two accepted tuberculin one is PPD-S and PPD RT 23 by WHO.

In developing countries having high prevalence of tuberculosis, it is recommended dose suggested by WHO is 1TU of PPD RT23. Because in our country it was found that non-specific reactions to atypical Mycobacteria are high so 1TU of PPD RT23 is found to distinguish between infected individuals with disease from infected individuals without disease[119]. International union against tuberculosis & lung disease (IUATLD) has also recommended 2TU of PPDRT23 for tuberculosis survey purpose[117]. Diluted form of Tuberculin PPD (1 TU/2 TU/5 TU and 10 TU) is available commercially conducting Mantoux test. Its source material is calibrated with RT23 batch prepared by Staten serum institute [120] and diluted by specific buffer which containing tween-80 as a stabilizer[121].

Principle-

Individuals with tuberculosis infection, BCG vaccination or other infection with other Mycobacteria causes proliferation and sensitization of T cells in lymph nodes. When PPD administrated intradermally these sensitized T cells recognized the antigen and produce cytokines including IFN-γ leads to cause delay type of hypersensitivity reaction. This hypersensitivity reaction release INF-γ and other cytokines plays a central role in the formation of induration. This hypersensitivity reaction starts from 5 to 6 hrs. After administration and comes at maximum level at 48 to 72 hrs.

The first response develops as vasodilation, oedema fibrin accumulation and collection of inflammatory cells at administration site resulting formation of induration of variable size

depending upon the dose of tuberculin PPD or sensitivity of the individuals. Diameter of this induration is directly proportion to the intensity of the degree of sensitization.

Procedure-

PPD is administered intradermally at the middle third of the forearm, as the reaction may be weaker near the elbow joint or at the wrist. The preferred site for injection is either the flexor or dorsal surface of the forearm, about 4 inches below the elbow joint, avoiding any areas that are swollen, red, or have visible veins. Then clean the injection site with 70% alcohol and allow it to dry. Then also clean the stopper of the PPD vial with 70% alcohol before drawing 0.1 ml of tuberculin into a syringe using a sterile needle.

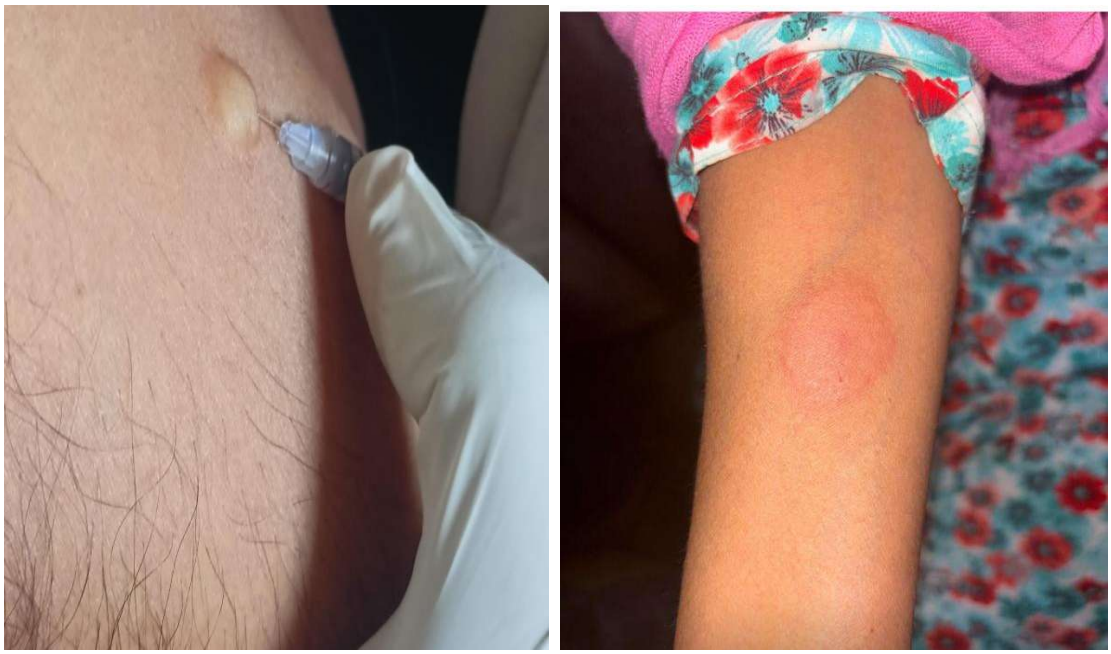


Figure 19- (a) showing PPD administration with formation of small papule & (b) MT-positive

Next, stretch the skin and hold the syringe parallel to the skin, inserting the needle into the superficial layer so it is visible in the epidermis. Then slowly inject the tuberculin solution, which results in the formation of a small papule. After injection, remove any blood from the site using cotton. If a papule does not appear, it means the injection not done correctly

or placed too deeply. In such a case, repeat the test on the other arm. If same arm is used then choose a site at least 5 cm away from the previous injection site and mark it with a circle to help identify the correct location during result interpretation[122].

Interpretation of results

- Result should be taken between 48 to 72 hours[115], from the injection time. Only induration is taken for result not the erythema. Transparent ruler used and record the result in mm[123,124].

  Negative: 0 to 5 mm

  Doubtful- 5 to 9 mm

  Positive: 6 to 14 mm

  Strongly positive: more than 15 mm.

According to the standard guidelines of Tuberculin Skin Test (TST), a positive result is ≥5mm induration in people who fall into the high-risk groups, including patients with HIV, people that used to be in close contact with an active case of tuberculosis, organ transplant recipients, immunosuppressed individuals, and individuals with the results of chest radiography with old healed TB lesions.

The induration of ≥ 10 mm is a positive TB infection in individuals with moderate risk, such as recent immigrants to countries with high rates of TB, injection-drug users, children under the age of four years, or those exposed to high-risk adults, people who live or work in high-risk environments, like hospitals, prisons, and shelters, and people with underlying conditions such as diabetes, renal failure, or malnutrition.

In case of a TB infection of individuals with no known risk factors, a TB case is defined as an induration of ≥15 mm in diameter, which indicates a large threshold in patients at low-risk groups.

Conversely, a TB infection is considered negative when the induration is less than 5 mm, that is, when there is no significant response to the tuberculin antigen, and a 0 mm induration is considered a total lack of the measured immune response, thus being negative.

Positive result may be occurred due to latent, current or early infection of Mycobacterium tuberculosis and also due to Mycobacterium Complex *(M. bovis, M. africanum, M. micrtii*)[125]. BCG vaccination and some non-tuberculous mycobacterium also cause positive reaction[126,127].

It has limited significance due to false negative or positive result. Previous BCG vaccine or presence of NTB (non- tuberculous bacilli) can give false positive result and steroid medication can give false negative result[128]. According to Abderlrub et al[15] 42.6% GTB patient are TST positive. According to Raut et al[129] sensitivity and specificity was 55% and 80% respectively. Strong the reaction indicates more infection of MTB. Tuberculin antigen is injected intradermal and leucocytosis, leucocyturia, lymphopenia and raised body temperature. Occur after 24 hr. and 48 hr. suggestive of positive result.

ZN STAIN AND AURAMINE STAIN –

Lower Respiratory Specimen: for tuberculosis

Ziehl Neelsen Stain –

This is very popular and authentic diagnostic tools used widely worldwide because it is easy to perform at very low cost. Its sensitivity is 37.1% to 52.1% [14,103,104]. Any type of sample can be used like sputum, urine, tissue, pus, body fluid, semen, prostatic secretion etc. To be ZN positive sample should contain minimum 10^4 to 10^6 bacilli per ml of sample[33].

QUALITY CONTROL OF Ziehl Neelsen / AFB Staining-

- On a slide two smears are prepared one for AFB positive control/clinical specimen and the other for E. coli ATCC 25922.
- These slides are prepared and store in Double packing with biohazard label for further use as a control.
- These controls are used on every new stock of reagent before conducting a test.

Properties of Ziehl Neelsen Stain-

- Mycolic acid present in cell wall of the TB bacilli provide the ability to become acid fast.
- Fuchsin used as primary stain binds with mycolic acid of cell wall.
- Even after the decolourization by strong acid/alcohol primary stains remain inside the cell which provide the red colour to the bacteria known as acid-fast bacilli.
- In case of non-acid fast bacilli primary colour also wash out due to lack of lipid content.
- Methylene blue is applied as a counter stain.
- In case of non-acid-fast cells and the background appear blue after the counterstain stain.

Ziehl Neelsen Staining Reagents-

Reagent Stability & Store- at room temperature

Primary Stain:

- It contains 0.3% carbol-fuchsin.
- 50 gm of phenol should have dissolve in 100 ml of ethanol having strength of 95% or methanol (95%)
- 3 gm of basic fuchsin dissolve in the mixture and add distilled water in to it to make 1.0 L volume.

Decolorization Solution:

- Slowly add 250 mL sulfuric acid (at least 95%) to 750 mL of deionized water. Leave it at room temperature for cooling and proper mix before using.

Counterstain:

- 3 gm of methylene blue is mixed in 1 L of deionized water to make 0.3% of methylene blue solution.

- In case of colour-blind workers, picric acid solution (7 gm/l water) can be use which gives yellow background.

Ziehl Neelsen Staining Procedure-

First, gently heat the slide to fix the smear. Then, place the slide on a staining rack with the smear facing upward. Using filter paper, cover the smear with strong carbol fuchsin solution. Then heat the slide intermittently for 8 to 10 minutes by moving the flame under the slide until steam appears, reheating every 3 to 5 minutes. First make sure the smear doesn't dry out by adding more stain if needed. After staining, pour off the excess stain and gently rinse the slide with distilled water. Next, apply 20% sulfuric acid (H_2SO_4) over the smear and wait for 1 to 3 minutes, then rinse again with distilled water. If any red stain remains, repeat the acid wash. Once the decolorization is complete, apply methylene blue for one minute and rinse it off using distilled or tap water. Finally, place the slide on a rack to air dry. Once it's dry, it is ready for microscopic examination.

Ziehl Neelsen Staining Results:

- AFB are approximately 1-10 um long, typically Red/Pink slender rods (0.2-0.6 μm) which may present in the shape of curved or bent structure.
- Individual tuberculous bacilli can be seen in heavy stain areas or alternating stain area display a beaded appearance.
- Non-tubercular mycobacteria can also be seen as a long filament structure or coccus like form.
- The background material appears blue

Ziehl Neelsen Staining (Grading) Results:

Table 11- Guidelines (WHO and RNTCP) for marking results of ZN stain-

Number of AFB seen (by using 100x oil immersion objective and 10x eye piece)	**Grading**
More than 10 AFB per field in 20 fields.	Positive, 3+
1-10 AFB per field in 50 fields	Positive, 2+
10-99 AFB per field in 100 field	Positive, 1+
1-9 AFB per field in100 field	Scanty- number of bacilli
No AFB per 100 fields	Negative

SPUTUM- SELECTION AND REJECTION CRITERIA (MICROSCOPIC):

Specimen quality is back bone of the investigation. A good quality sample give desirable result whereas a bad quality sample not only gave wrong result but also hamper the diagnosis and treatment of patient. Sputum having large number of pus cell considered as good or ideal specimen where as large number of epithelial cells considered as a bad specimen and should be rejected.

Quality of Sputum for ZN Stain and Culture $\propto$ number of pus cells.

Quality of Sputum for ZN Stain and Culture $1/\propto$ number of epithelial cells.

In this study sample having Q Score > 1 is used.

Q Score (QUALITY SCORE)

Q Score provide us the ability to distinguish between true and wrong specimen. For this purpose, sputum smear on slide is prepared and stain it by using gram stain and examine under 10x objective. Now according to the provided table Q score is calculated on

Table No-12: Q Score

			Squamous cells			
Neutrophils	Cells per field		0	1-9	10-24	>25
	Report		None	Few	Moderate	Many
		Q value	0	-1	-2	-3
0	None	0	3	0	0	0
1-9	Few	+1	3	0	0	0
10-24	Moderate	+2	3	1	0	0
>25	Many	+3	3	2	1	0
			Composite Q score			

AURAMINE STAINING-

Also called as Truant auramine-rhodamine stain, having high sensitivity used to ruled out acid fast bacilli by using fluorescence microscope. Acid fast bacilli emit reddish yellow fluorescence.

Principle of Auramine staining-

The dye binds with the mycolic acids and give fluorescent bright yellow colour of auramine.

Sputum (Expectorated & Induced)

Reagents use-

Liquid ready to use reagents:

- TB Auramine M solution
  Auramine O
  Phenol,
  Glycerine
  Isopropanol
  Distilled water
- TB Decolourizer TM
  Hydrochloric Acid:
  Isopropanol
  Distilled water
- TB Potassium permanganate ($KMnO_4$)
  Potassium permanganate
  Distilled water

Reagent Stability & Storage-

- Must be protected from humidity
- Storage temperature should be maintained between 15º and 30ºC
- Avoid to store oxidizing and acidic reagent together.
- Shelf life of storage reagents is up to 2 months

Procedure of Auramine staining-

This procedure is begun by preparing the sputum slide and fixing it through gentle heating. Next, we fill the entire slide with auramine-rhodamine stain and allow it to sit for 20 minutes; heating is not required in this method. After staining, wash the slide with distilled water, taking care to peel and rinse thoroughly as the auramine-rhodamine stain is very sticky in nature. Then apply 0.5% acid alcohol over the entire slide and let it act for 5 minutes to decolorize the stain—if necessary, add more acid alcohol to ensure complete removal of the excess stain. Following this, rinse off the acid alcohol with distilled water. Then pour potassium permanganate (MSDS) over the entire slide and let it sit for 1 minute, making sure not to exceed 2 minutes to avoid over-quenching the fluorescence. Finally, wash the slide again and examine it under a fluorescent microscope.

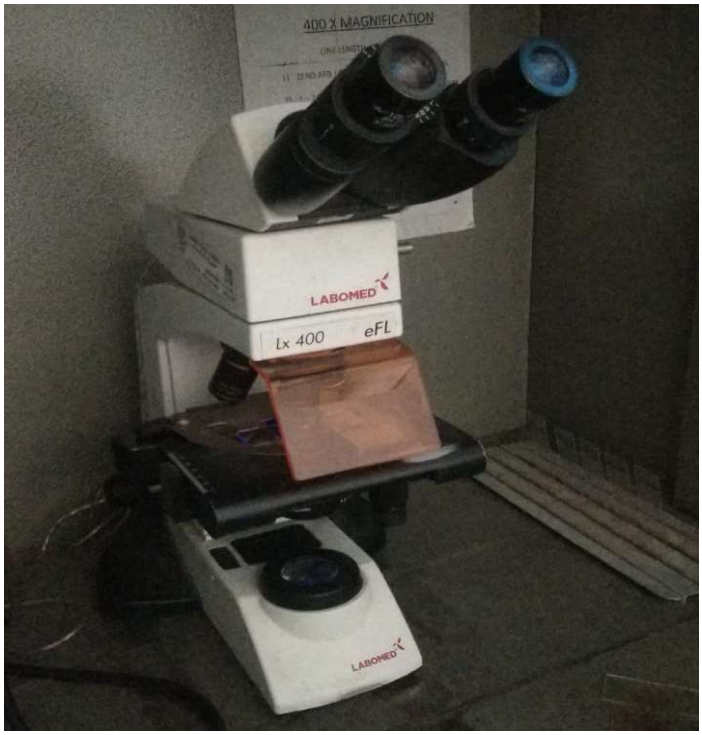


Figure 20- Fluorescent microscope

Table No-13: Guidelines (WHO) for marking results of Auramine staining-

Number of AFB seen (Fluorescence stain) 400 x magnification	**Reporting**
0 AFB /70 Field	No AFB seen
1-2 AFB/70 Field	Doubtful
1-18 AFB/ 50 field	1+
4-36 AFB/10 field	2+
4-36 AFB/ field	3+
>36 AFB/ Field	4+

X Ray-

In all radiological techniques x-ray is widely used all over the world. This technique is used in all suspected patient for MTB diagnosis. By the help of this technique and with combination of routine investigations and clinical symptoms clinician usually starts anti tuberculosis treatment (ATT). This is easily available in all health care facilities. From different study it was found that about 10 to 75% of genital tuberculosis patient's x-ray reports were abnormal[113].

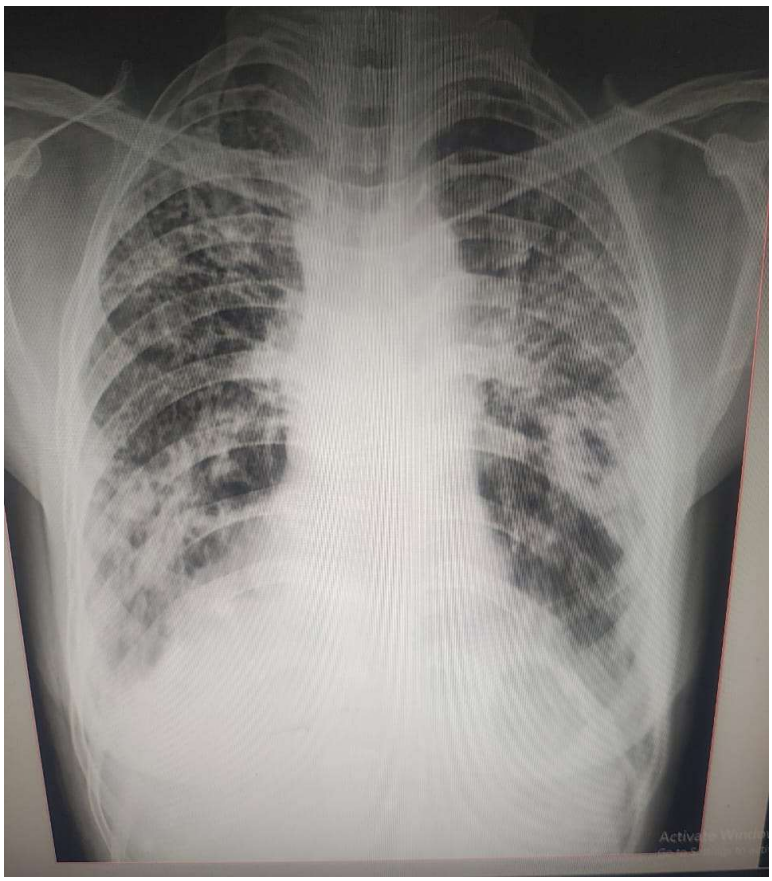


Figure 21- showing pulmonary tuberculosis.

Quanti-FERON-TB Gold (QFN Gold)-

Researchers stared to study around 1980 the role of γ-interferon. In 1990 first Quanti Feron -TB test (QFT-1) was invented by a Australian company named Cellestis limited. PPD was used to activate antigen similar to TST (tuberculin skin test). But it had many limitations due to false (+) results specially in BCG vaccinated patient. Now BCG vaccinated patient becomes very big obstacle for development of γ -interferon.

The second-generation test was developed in 2001 named as Quanti FERON-TB Gold (QFT-G). TB antigen like ESAT-6, CFP-10 were used because it is not present in BCG strain and also most of the mycobacteria. This ability makes it more specific and sensitive. In 2007 tube-based analysis was mixed in blood collection tubes. Three tubes used named as –

Nil-for negative control

TB antigen and

Mitogen- for positive control.

Nil tube- used as negative control to fix base line level of IFN-γ. This tube not contain any stimulatory antigen.

TB antigen tube- This tube contains ESAT-6, CFP-10 and TB 7.7 antigen specific to MTB but absent in BCG strain and most of other non-tuberculous mycobacteria.

Function- when T cell exposed to these ESAT-6, CFP-10 and TB 7.7 it releases IFN-ϒ. High level of INF-ϒ indicate TB infection.

Mitogen tube- used as positive control. This tube contains mitogen to stimulate T-cells.

Function- mitogen is present to confirm that whether patient immune system is capable to produce IFN-ϒ or not.

Theory- INF-ϒ present in blood is measured by the help of this method. Blood in antigen sensitised tube nil, antigen tube, mitogen incubate for 16-24 hrs at 37°c. T-cell interact with these antigens and get reactivated to produce INF-ϒ. Reactivation of T-cell due to antigen stimulation and accurate measurement of released INF-ϒ by reactivated T-cell is the basis of TB-gold technology.

This test is done in 2 steps, in first step blood is collected in antigen sensitised special tube known as nil, antigen and mitogen. Tube must be incubated just after the blood collection and within 16 hours. After incubation tubes were centrifuge and plasma is separated to measure INF-ϒ. If value of TB antigen is above the nil value test is said to be positive. Low value of mitogen said to be indeterminate. Possible reason may be insufficient lymphocytes, retard lymphocyte activity and also due to blood collection error and wrong handling. The value of INF-ϒ of nil tube is subtracted from value of antigen and mitogen tube.

Procedure-

Step 1 – Incubation of blood sample and plasma separation:

This procedure is started by injecting 1 ml of collected blood into each of the three tubes: Nil, TB antigen, and mitogen tubes. After filling, gently shake each tube 10 times or place them on a roller mixer to properly mix the antigens with the blood. Since the process depends on live lymphocytes, avoid vigorous shaking to prevent damage to the blood cells. Next, incubate the tubes at 37°C for 16 to 24 hours. If immediate incubation isn't possible, keep the tubes standing upright at room temperature, but make sure to start incubation within 16 hours of collection. Alternatively, blood can be collected in a heparin tube (at least 3.5 ml), mixed well, and stored at room temperature for up to 16 hours. Before this time limit, must dispense the blood into the Nil, TB antigen, and mitogen tubes for incubation. After incubation, centrifuge the tubes at 2200 to 2300 RCF for 15 minutes.

Step 2 – Measurement of IFN-γ in human blood:

To begin standard solution preparation by labelling four sterile tubes as S1, S2, S3, and S4. Add 300 µl of ELISA diluent to each tube. Then, add 100 µl of reconstituted standard to the S1 tube and mix it well to make a 4 IU/ml solution. From S1, transfer 100 µl to the S2 tube and mix to get a 1 IU/ml solution. Similarly, transfer 100 µl from S2 to S3 to get 0.25 IU/ml. The S4 tube is left as it is and used as the zero standard.

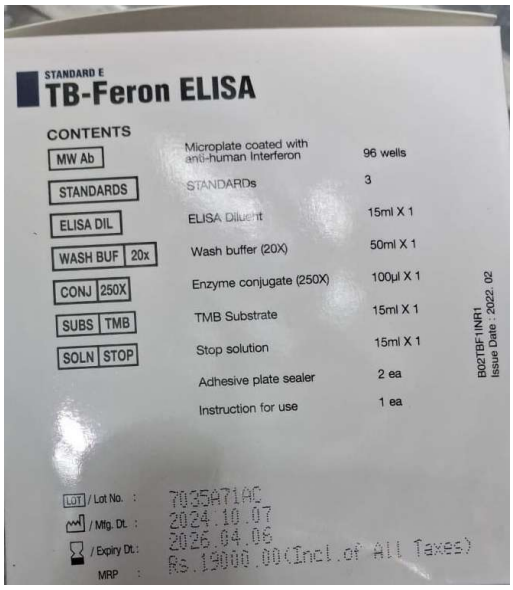


Figure 22- Interferon gamma reagent.

Working detector solution and sample incubation:

Add 50 µl of the working detector solution to each well (both for standards and test samples). Then add 50 µl of standard solutions from S1 to S4 into wells 1 to 4, and add patient samples into the rest of the wells according to their IDs. Shake the plate gently to mix, seal the wells with the adhesive cover provided in the kit, and incubate at 36 to 38°C for 1 hour.

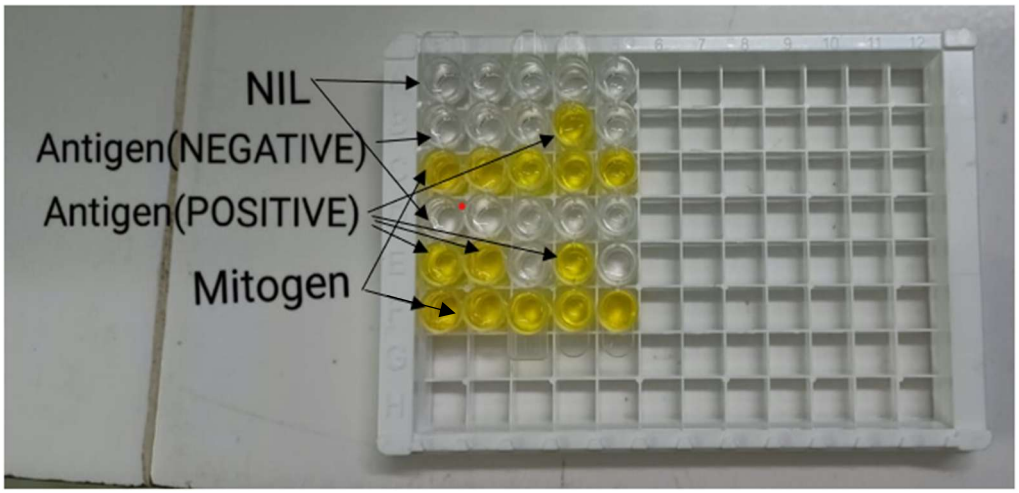


Figure 23- microwells after adding stop solution.

Washing procedure:

Wash each well with 350 µl of diluted wash buffer using an automatic washer. After washing, tap the wells on tissue paper to remove any leftover liquid. Then add 100 µl of TMB substrate to each well and incubate at 15 to 25°C for 30 minutes. After incubation, add 100 µl of stop solution to each well and gently shake the plate to mix.

Measurement:

Finally, read the absorbance of all wells at 450 nm using an ELISA reader, making sure to take the reading within 30 minutes of adding the stop solution.

Interpretation of result-

NIL TUBE IN IU/ml	ANTIGEN TUBE MINUS NIL TUBE (IU/ml)	FINAL RESULT	INTERPRETATION
≤ 10.00	< 0.438	Negative	M. Tuberculosis Infection Unlikely
	≥ 0.438 & < 25% of Nil tube	Negative	M. Tuberculosis Infection Unlikely
	≥ 0.438 & ≥ 25% of Nil Tube	Positive	M. Tuberculosis Infection likely
> 10.00	Any Result	Indeterminate	This may be due to excessive levels of circulating gamma interferon or presence of heterophile antibodies

- Elisa wells are coated with captured antibody specific for INF-γ. This antibody is monoclonal antibody specific to bind INF-γ. Plate is incubated and wash to remove unbounded antibodies. Blocking buffer are added to block unwanted bindings.
- Samples are added to the wells and if INF-γ is present in sample binds with capture antibody.
- A biotinylated detection antibody is added which is specific for different epitope of INF-γ.
- This biotinylated detection antibody binds with capture INF-γ.
- Streptavidin -HRP (Horseradish peroxidase) or other enzyme (alkaline phosphatase) is added. This binds with biotinylated detection antibody.
- A chromogenic substance (TMB-3,3',5,5' tetramethyl benzidine) added in presence of hydrogen peroxide it develop a blue colour. After it stop solution (sulphuric acid) is added it changes the colour blue to yellow
- Absorbance is measure at 450 nm.

Its sensitivity was 84 to 95% and specificity was 85 to 99% respectively. It works on principle of TB specific antigen called RD1 based interferon assay. By the help of this technique, we can diagnose immune reactivity to MTB. It can provide result within 24 hrs. MTB antigen like ESAT-6, CFP sensitized T cell. This T cell stimulate interferon gamma. Presence of this active form is suggestive of tuberculosis.

Gene Xpert-

Sputum sample preparing method for gene-Xpert: -

This procedure is begun by opening the cap of the container and placing it upside down to prevent sputum from leaking onto the surface. Then, add Gene Xpert reagent in an equal amount to the sputum in the container. While adding the reagent, take care not to let the reagent bottle touch the sputum container. If accidental contact occurs, discard the sputum container. Next, recap the container and shake it clockwise for 10 to 20 minutes. After shaking, leave the container at room temperature for 15 minutes to allow liquefaction.

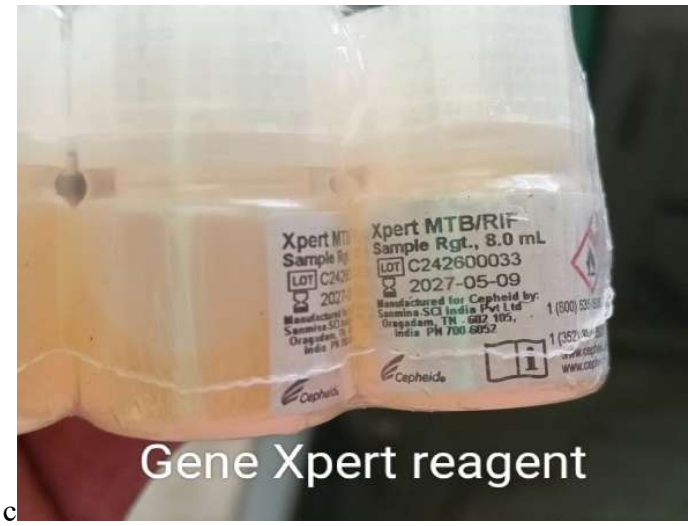
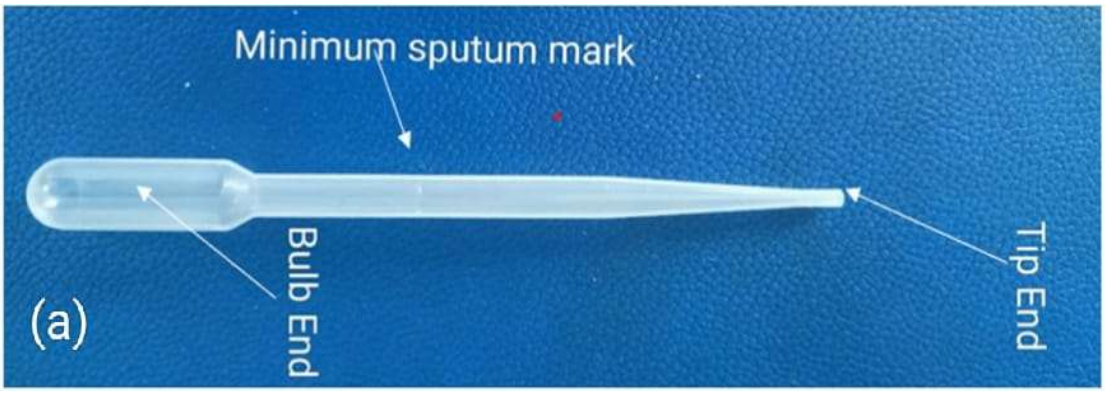
c 

Figure 24- Gene Xpert reagent

During this waiting time, open the foil packet of the cartridge, place it upright on the table, and mark the patient ID beside the barcode. After 15 minutes, shake the specimen again 10 to 20 times and leave it for another 5 minutes. The sputum should become completely liquefied, with a consistency similar to water, as only then is it ideal for Xpert testing. It must be ensured that the test begins within 30 minutes of liquification.



For Gene Xpert cartridge preparation, use a sterile pipette provided with the cartridge to transfer the liquefied sputum sample. Then carefully draw the sample up to the marked level on the pipette and transfer it into the large opening of the cartridge, ensuring no air bubbles form. Then, close the lid of the cartridge, scan it, and place it inside the module to start the test. The results are displayed after approximately 2 hours.

Figure 25- (a) disposable sterile pipette and (b) cartridge showing large opening for sample

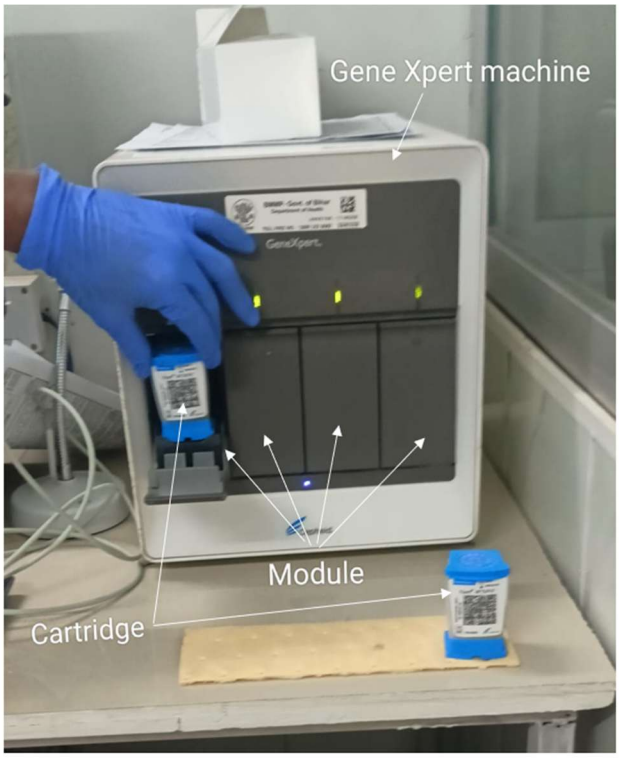


Figure 26- Gene Xpert analyzer with cartridges.

FDA approved it since 2013. It detects Mycobacterium tuberculosis as well as rifampicin resistance conferring mutation directly from sputum, fluids, tissue (except blood and urine).

Table No 14: Advantages and disadvantages of Gene Xpert-

Advantages	**Disadvantages**
Rapid turnaround time	False results due to presence of amplification inhibitors in samples.
Detection of Tuberculous bacilli and drug sensitivity can be done by sputum sample	Costing per test is high
Complete automation possible eliminating of possible human errors and cross contamination	Nucleic acid contamination gives false results. Amplification also from dead bacilli DNA so monitoring of treatment is not possible.
	Requirement of QC and QA.
	Machine and cartridge are temperature sensitive. Blood and blood mixed sputum cannot be used because these samples contain PCR inhibitors, DNA recovery is poor, and the bacterial load in blood is highly insignificant, which causes invalid or false-negative results. GeneXpert can be adapted to tissue, pus or body fluid samples that contain Mycobacterium tuberculosis DNA more centrally and are less inhibited.

It is very recent and advance diagnostic tools. It can provide reports just within 2 hrs. It is fully automated equipment and has very negligible contamination risk. It is FDA approved technology and used widely all over the world. It has one special feature rifampin resistance which distinguished it from other. It is nucleic acid amplification-based technique said **gold standard test**. It is also called CB-NAAT (cartridge based nucleic acid amplification test). Its sensitivity is 80% and specificity is 98-99% on sputum sample but in urine sample it is 94.3% to 95.6% and 85.7 to 94.3% respectively[14,104]. But can't differentiate active and latent case. According to different study from 80.9% of GUTB cases were diagnose by it[112].

FINE NEEDLE ASPIRATION CYTOLOGY (FNAC)-

It is minimally invasive diagnostic procedure used to collect the cellular sample from a lump or gland for microscopic examination.

A. Pre-procedural preparation:

It is begun by taking informed consent from the patient. Then, identify the target site either by palpation or with the help of USG/CT guidance if needed. After identifying the site, clean the area thoroughly with alcohol.

B. FNAC procedure:

In this procedure a needle (22 to 25 gauge) attached to a 10-20 ml syringe is used. Carefully insert the needle into the gland and apply negative pressure to aspirate cellular material. To collect an adequate sample, move the needle slightly within the gland. Before withdrawing the needle, release the suction to minimize the risk of contamination.

C. Post-aspiration handling:

Smear preparation:

Press the syringe holder to place the collected sample onto a clean glass slide. Using another clean slide, thin smear has made. Then either air dry the slide or fix it in 95% alcohol.

Staining procedure:

For staining, use ZN stain and Papanicolaou stain.

Papanicolaou stain has used to rule out cytological changes to identify whether it is inflammation, infection, or malignancy. Whereas AFB stain has used to detect the presence of Mycobacterium tuberculosis. These stains provide both morphological and microbiological information. Help us to achieve accurate diagnosis genital tuberculosis.

Steps of Papanicolaou stain:

1. Fixation (wet fixation)

Done immediately after preparation of the smear, place the slide in 95% ethanol (or 95% ethanol: ether 3:1 if used) for 15–30 minutes or fix by flooding the slide with 95% ethanol for 15–20 minutes. Do not allow air-drying before fixation — air-dried cervical smears give poor nuclear detail.

2. Hydration / rinse

Remove slide from fixative and wash in distilled water quick to remove alcohol film before nuclear staining.

3. Nuclear staining — Hematoxylin

Place slides in Hematoxylin (Harris or Gill's) for 3–5 minutes avoid overstaining.

4. Rinse

Rinse slides gently in running tap water or distilled water for 2 minutes to remove excess hematoxylin.

5. Differentiate (acid alcohol)

Differentiate briefly in 0.5% acid alcohol (0.5% HCl in 70% ethanol) — a few second. It washes out excess background nuclear stain; avoid over-differentiation which makes nuclei pale.

6. Rinse and blueing

Rinse in tap water to stop differentiation.

After that put the slide in Scott's tap water substitute or 0.2% lithium carbonate for 1–2 minutes (or until nuclei turn crisp blue).

Rinse briefly in distilled water.

7. Dehydrate briefly before cytoplasmic stains

Dip slides 10 times in 95% ethanol (or immerse 30–60 seconds) to remove water before Orange G step.

8. Orange G-6 (OG-6) — keratin stain

Stain in Orange G-6 for 30 seconds – 1 minute (time depends on OG strength. This stains keratin and superficial squamous cell cytoplasm orange.

9. Dehydrate / quick rinse

Rinse in 95% ethanol (10 dips) to remove excess OG-6.

10. EA (Eosin Azure) — counterstain for cytoplasm

Stain in **EA mixture (EA-36 / EA-50 / EA-65)** for **2–3 minutes**.

EA contains eosin Y, light green SF and Bismarck brown (in some formulas) — it gives multi-hued cytoplasm (pink, green, blue).

11. Dehydration (graded alcohols)

Immerse slides in 95% ethanol (10 dips), then 100% ethanol (2 changes, 10 dips each or 2 × 1 minute) to complete dehydration.

12. Clearing

Clear slides in xylene (2 changes, 2–5 minutes each) until slides appear transparent.

13. Mounting

Mount with a permanent mounting medium (e.g., DPX) and cover slip. Allow to dry and harden.

14. Label and examine

Examination done under microscope.

PAP STAIN (Papanicolaou Stain)-

Principle-

It is a multichromatic staining procedure used to detect morphological details specially nuclear and cytoplasmic detail in exfoliated cells.

Material Required-

Clean glass slides.

95% ethyl alcohol used as fixatives.

Hematoxylin

Acid alcohol

Scott's tap water

EA stain

Absolute alcohol

Xylene

DPX mounting medium

Procedure-

Fix the smear in 95% alcohol quickly and wait for 20-30 minutes.

Hydration done- 95% alcohol then in 70% alcohol then in distilled water. 2 to 3 minutes in each step.

Hematoxylin used for 3 to 5 minutes for nuclear staining.

Wash with distilled water

Dip for few second in 1% acid alcohol.

Wash with distilled water.

Place in scott's tap water for 1 to 2 minutes.

Wash with distilled water.

Put in 70% alcohol then 95% alcohol.

Stain in OG-6 and wait for 2 minutes.

Rinse in 95% alcohol. Stain in EA stain for 3 to 4 minutes.

Put in absolute alcohol 2 changes, then xylene (2 changes) after that mount the slide with DPX mount and put the coverslip.

Done the examination microscopically.

MTB chip based Real Time PCR for detection of mycobacterium tuberculosis: -

Based on polymerase chain reaction in which the segment of 16s r RNA gene is amplify. It contains sequence that hybridizes with an oligonucleotide probe specific for MTB complex bacteria.

Mycobacterium tuberculosis (MTB) complex diagnose by PCR (Polymerase Chain Reaction) technique is based on amplification the selective region of the MTB genome. Nucleic acid amplification test applied for the quantitation of M. tuberculosis DNA in various human samples. In Real Time PCR the amplified product (AMPLICON) is detected via fluorescent dyes. These are usually connected to oligonucleotide probes which bind specifically to each amplified region and dissociate in each PCR cycle. Monitor of the

fluorescence intensities during each PCR cycle (Real Time run, Graph), allows the detection and quantitation of AMPLICONs. Thus, no more prediction of results with Gel Electrophoresis and through densitometers (like end point (PCR) after the PCR run is necessary. Thus, detection and quantitation of MTB are much faster with far greater sensitivity and specificity.

Method- Real Time PCR

Probe used- Dual labeled oligonucleotide with fluorescence probe (TaqMan) used.

Instrument Used- Rotor- Gene Q from Corbett Research, Australia

Subtypes used- All common MTB Complex (M. tuberculosis, M. bovis, M.bovis BCG, M. H37Rv, M. pinnipedi)

Measuring Range: 70-2.5 X 10^7 copies/ml.

Molecular techniques like polymerase chain reaction (PCR) or Real time PCR are more sensitive than sputum microscopic examination and culture. These instruments were used-

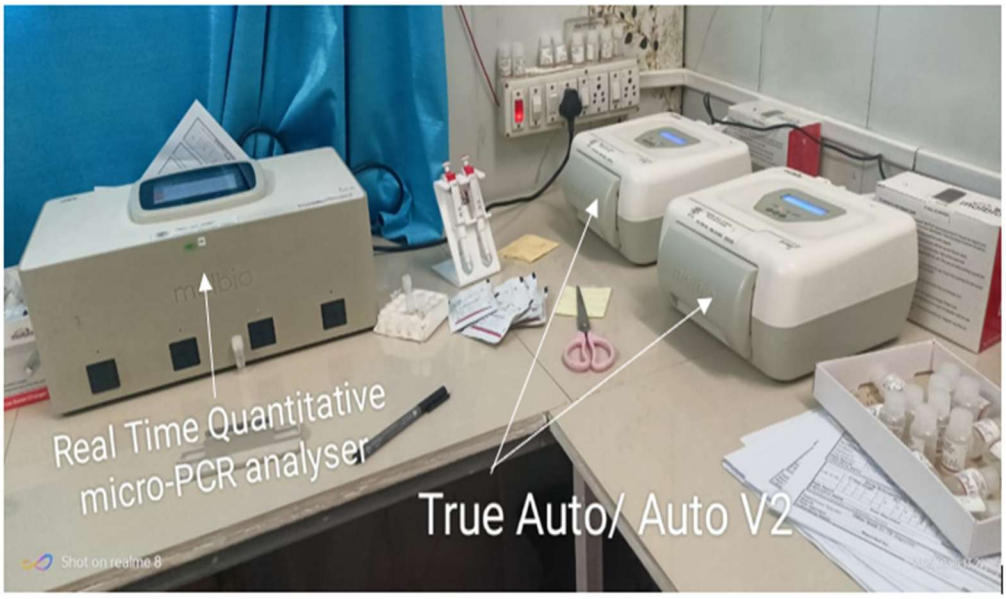


Figure 27- PCR Analyser with true auto sample prep. device, micro pipette

1. True Auto/Auto V2: - It is a universal cartridge-based sample prep. device can work at room temperature to extract purified DNA for RT PCR study.
2. Battery operated Real Time Quantitative micro-PCR analyser.

Working principle of Real time polymerase chain reaction based on TaqMan chemistry. Purified DNA from patient sample is extracted by the help of True prep Auto Universal Cartridge Based Sample Prep Device. Purified DNA extracted in form of elute. Now 6µl of purified DNA is dispense in to microtube containing freeze dried PCR reagent and leave it stand for 30 to 60 seconds to get clear solution. Now 6µl of this clear solution is put in the reaction well of the MTB chip and insert the chip in to the analyser and start the test. A positive amplification results the dual labelled fluorescent probe in the RT PCR test to release fluorophores in exponential manner which is captured by in built sensor and display Real time amplification curve on the screen of analyser. The cycle threshold (CT) is the number of amplification cycles required for fluorescent signal to cross the threshold. CT levels are inversely proportional to the amount of target nucleic acid. In case of negative sample amplification not occur and a horizontal line draw on screen. Based on the presence or absent of the bacilli. MTB detected and MTB not detected display on the screen. Target sequence for MTB RT PCR is nrd B gene which codes for ribonucleoside diphosphate reductase large subunit.

Test procedure-

The whole test procedure takes place in two steps. First done extraction of purified DNA. Since samples of all types may contain PCR inhibitors, have uneven distribution of bacilli, or be paucibacillary in the case of non-sputum samples, it is important to remove such complexes. The combination of reagents in the MTB sample pre-treatment pack helps digest these complexes, and obtain purified DNA in the elute chamber.

Figure 28- lysis buffer and cartridges used for purified DNA extraction.

In the second step, amplify the target sequence using the RT PCR analyzer. After entering the patient details, we press the start button and then the eject button to open the chip tray. Open the pouch and take out the micro-PCR chip, microtube, and DNase- and RNase-free pipette tips. Stand the microtube with the freeze-dried PCR reagent vertically and dispense 6 µl of the purified DNA into it using the special tips. Let it stand for 30 to 60 seconds until get a clear solution. Then take 6 µl of this clear solution and dispense it into the reaction well of the chip, which is placed in the tray slot. After loading the tray into the analyzer, start the reaction and view the result on the screen. Once the measurement is complete, we press the eject option to remove the chip and discard it according to BMW (Biomedical Waste) guidelines

Figure 29- Microchip for MTB PCR

If the MTB result is positive and we need to check for rifampicin resistance, follow another procedure. Open the pouch of the MTB RIF DX kit and take out the chip-based Real-Time PCR test and the microtube. Place the chip on the tray slot, stand the microtube containing the dried PCR reagent vertically, and add 6 µl of purified DNA. After letting it stand for 30 to 60 seconds to get a clear solution, we dispense 6 µl of the solution into the reaction well of the chip. Then start the reaction and read the result on the screen, which will show whether rifampicin resistance is detected or not. After the test, we discard the microchip as per BMW guidelines.

Figure 30- Microchip for MTB PCR to detect rifampicin resistance

Principle- presence of mutations (SNPS) the RRDR region of rpo B is detected by prove melt.

Target selection-

Rifampicin resistance determining region (RRDR) region of rpo B (RNA polymerase Beta subunit) gene and the codon numbering (H37RV). Codon 428-435 and codon 445-452(E. coli codon numbering codon 509-516 and codon 526-533. Representing mutation hot sports known as rifampicin resistance.

It is rapid and advance technique. It has high sensitivity and it can diagnose both alive and dead bacilli. But sample should have minimum 10 or more bacterial load per ml is required for a positive result. But false negative and false positive results are its biggest draw back and because of that treatment of tuberculosis cannot be start based on its report even after positive result[108, 109, 110]. But some article favors it, like Jindal et al[108]. According to him if infertility treatment has done according to PCR result, we can get high pregnancy rate

Limitation of TB PCR-

False Negative-

- In case of low bacterial low
- Error in sample collection or during handling

False Positive-

- It cannot differentiate between live and dead bacteria. So, in case of previously treated patient, it can give false positive result. PCR is not able to distinguish between living and dead bacteria as it identifies DNA, which does not change even after the bacteria die and is still available and can be detected. It does not determine bacterial viability and metabolic activity.
- Contamination during processing can give positive result. So, processing done in DNA and RNA free environment is crucial.

HISTOPATHOLOGY-

Different types of tissue like endometrium, cervix, ovaries, fallopian tube, lymph node, breast lump, can be sent for histopathological examination as well as for bacilli detection. It is recommended to take multiple tissue from different site to get maximum yield. Presence of granulomatous caseous lesion along with giant epithelioid cell on histopathological examination are suggestive of tuberculous infection[75]. Endometrial tissue is the most popular and widely accepted sample for culture but have very low sensitivity[31,105]. Late secretory phase of menstrual cycle is ideal for tissue collection[106,107].

Procedure-

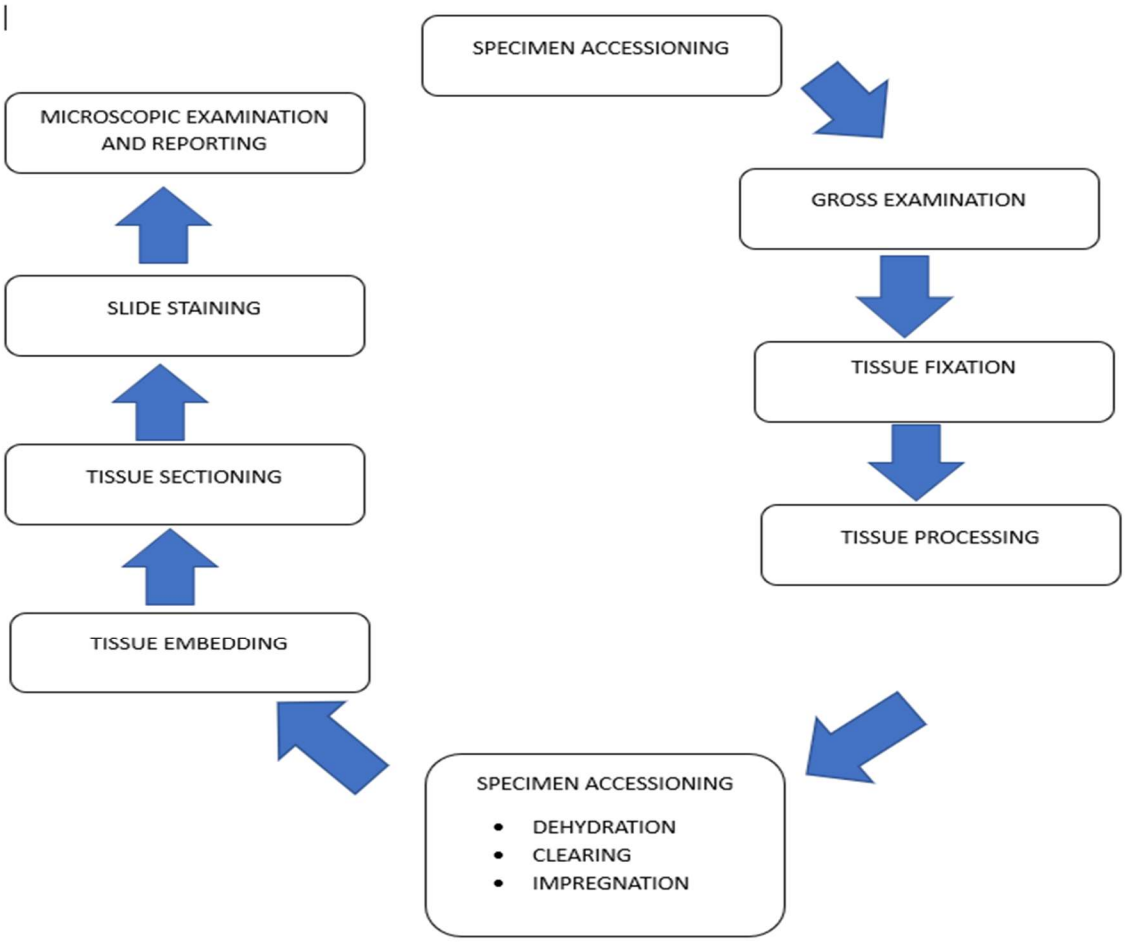


Figure 31: Histopathology processing

Table No-15: list of reagents used in histopathology

Embedding Material	Paraffin Wax (MP 50-56 ºC)
Fixative	Formaldehyde (Formalin) Ethyl alcohol Xylene Ether
Mounting Medium	Paramount and Canada Balsom, Egg Albumin, Glacial acetic acid, Nitric acid, HCL
Stain	Eosin, Hematoxylin

Fixation-

After receiving the tissue fixation is done to prevent autolysis.

This process of fixing is carried out twice-

1. At the operation theatre
2. At the histological laboratory after cutting bits or blocks.

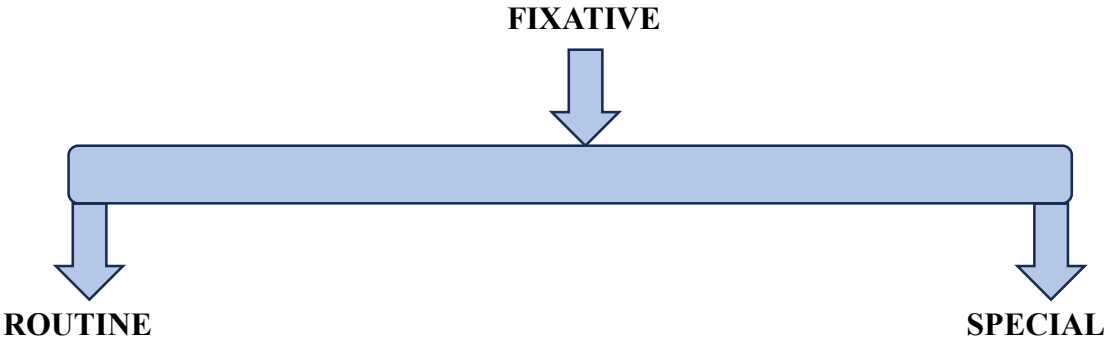
The volume of the fixative = 10 to 20 x volume of tissue block.

Specimen must be securely held in a holder along with the identification number.

In this study 10% formalin used as fixatives due to-

- Relatively inexpensive
- Easy to prepare
- Compatible with most of stains
- Penetrate the tissue wall.
- Interferes in staining procedures

Classification of fixative-



Routine fixative-

Formalin-

Removal of formalin from formalin fixed tissue-

- By water followed by placing 70% alcohol
- Formalin is extracted more rapidly in 70% alcohol than in unchanged water.

Problem with formalin fixative-

- It produces dark-brown or black precipitate (due to lacked haemoglobin) creates disturbance in microscopic examination.
- To remove precipitate first deparaffinise the section to water (xylene-alcohol- water) and place in following solution

Ammonium hydroxide (58%) ---------- 2 ml

Alcohol (70%) ---------- 100 ml

Special fixative-

- Staining for specific cellular details or for carbohydrates requires special fixatives.
- If the biopsies are previously fixed by formalin, then de-formalization become necessarily followed by secondary fixation with Zenker's fluid (used in our study), Helly's fluid, Blouin's fluid or other.
- Deformalinization and secondary fixation-

- Deparaffinise and take the section to water. Then treat it with ammonia water (30 drops of 58% ammonium hydroxide in 100 ml of H_2O) for 1 hr, followed by washing in running water.

Processing of tissues-

Paraffin embedding gives the tissue the necessary hardness for microtomy.

It is known that paraffin wax is not miscible or soluble in water so water should be removed from the specimen to allow the paraffin to infiltrate in to the tissue.

There are four major steps for processing-

a) Dehydration
b) Clearing
c) Infiltration
d) Embedding

(a). Dehydration-

Dehydration is the process by which water is removed from fixed tissues. The most commonly used dehydrant is-

Ethyl alcohol (Ethanol) – It is miscible with both water and xylene. Xylene is a solvent for the embedding material, paraffin wax. So, ethanol stands intermediate between these two dissimilar solvents. The use of ethanol is highly satisfactory except that is requires detailed record keeping for the excise department.

Isopropyl alcohol (99%) is the best substitute for ethyl alcohol

Tissue is kept in cassettes or capsule which is perforated stainless steel or plastic. Small tissue is wrapped with lens paper. We change the concentration of alcohol in every 1 hr. strength of starting alcohol is about 80% but in case of soft tissue strength may be 30%,50% or 70%. After that change the absolute alcohol three times.

(b) CLEARING –

Alcohol must be removed (dealcoholization) before the infiltration of the paraffin because absolute alcohol is not miscible with paraffin.

Routine staining procedures-

Haematoxylin –

- It is a natural staining reagent and one of the most important part of histopathological work.
- It has very low affinity towards tissue but with the combination of mordent like Al, I, Cr or W salt it becomes powerful "nuclear stain" and "chromatin stain". Hematein is formed by the oxidation of haematoxylin. Hematein is active colouring agent.

Ripening-

- The process of conversion of Haematoxylin to hematein by oxidation hastened by adding oxidizing agent (mercuric oxide, hydrogen peroxide, potassium permanganate, sodium perborate or NaI)
- In histopathological examination haematoxylin is used with combination of Al to form ALAM.
- The Section stained by haematoxylin can be counter stain by eosin, cargo red, eosin or safranin.
- In case of haematoxylin there are two types of staining method is used-

1. Progressive- Haematoxylin contain an large amount of Al salts or acid, thus enhancing the selectivity for nuclei. After staining process with haematoxylin washing of slide must be carried out before counter stain.
2. Regressive- in this method staining is done by over staining in a relative neutral solution of haematoxylin, after it remove the stain from other constituents by using acid alcohol or differentiating agent.

Frequently used Haematoxylin are -

1. Harris's alum Haematoxylin (used in our study),

Can also be use-

2. Mayer's Haematoxylin
3. Delafield
4. Ehrlich
5. Bullard
6. Bohmer

STAINING OF SECTION-

1.) Haematoxylin and eosin (H.E)-

Haematoxylin obtain from free named Haematoxylon campechianum (Mexico)

oxidation Haematoxylin ⟶ Haematin	Essential staining element Cell nuclei take up haematin

Preparation-

Harries's Alum Haematoxylin-

Haematoxylin	1 gm
Absolute alcohol	10 ml

Aluminium potassium-

Sulphate (potassium alum)	20 gm
Distilled water	200 ml
Mercuric oxide	0.5 gm

Dissolve the haematoxylin in alcohol and alum in water by adding heat and these two solutions are then mixed. Bring rapidly to boil. Remove from heat and add mercuric oxide reheat until it assumes a dark purple colour. As soon as the colour forms, flame is removed and container is plunged into bowl of water. It is ready to use.

2.) Eosin aqueous-

Stock solution- 5% in water add a crystal of thymol or, 1% formalin.

Working Solution- dilute to 1% with water.

Procedure –

This procedure is begun by treating the section with xylene in two changes to deparaffinize it. Then, wash off the xylene using graded alcohols in the order of 100%, 90%, 70%, and finally water. After that, wash the section in tap water and drain it well. Then stain the section with Harris's haematoxylin for 10 to 20 minutes. Once stained, tip off the excess stain and wash the section thoroughly with tap water for 15 to 30 seconds. Then differentiate the section in 1% acid alcohol (prepared by mixing 1 ml of HCl with 99 ml of 70% alcohol) for 10 to 20 seconds. After that wash off the acid alcohol with tap water. Next, perform blueing by placing the section in cold tap water, which is slightly alkaline, for 10 to 20 minutes. Alternatively, 2% ammonia water can be used for 5 to 15 seconds. Then counterstain the section with 1% aqueous eosin for 1 to 5 minutes. After staining, rinse off the excess stain using tap water. We then apply 95% alcohol for a few seconds, followed by absolute alcohol for a few seconds to dehydrate the section. After dehydration, we clear the section by treating it with xylene in two changes. Finally, mount the section using Canada balsam or DPX**.**

Mounting-

Media of mounting-

1.) Canada balsam
2.) DPX mount

Technique-

This procedure is begun by placing a drop of mountant on a clean 22x22 mm^2 coverslip. Then, carefully place the slide with the section onto the coverslip so that the section comes into contact with the mountant. As the mountant begins to spread, quickly turn the slide over. If the coverslip is not perfectly aligned on the slide, gently adjust it using a fine glass rod. Finally, keep the mounted slide in paraffin wax for 12 to 18 hours or place it in an incubator at 37°C for 24 hours to allow proper setting**.**

Microscopic examination of the slide-

Microscopic examination of mounted slide –

Cyst filled by granulomas with central caseous necrosis, Langhans giant cells, epithelioid cells, surrounded by lymphocytes and a peripheral fibrotic edge suggestive of tuberculosis.

CULTURE BY LOWEN STEIN JANSEN MEDIUM (L J. medium)-

L.J medium is a gold standard technique for isolation of Mycobacterium Tuberculosis. It provides viable bacilli which confirms the active form of the disease. A variety of samples can be processed by this method like-pus, menstrual blood, semen, tissue and fluid etc. It also provides the colony characteristics which provide the presumptive identification of the Mycobacterium Tuberculosis. These colony can be used in ZN staining and drug sensitivity testing.

Two culture methods were used Mac-Conkey and L.J medium (Lowenstein Jensen's medium). Mac-Conkey medium was used to detect non mycobacterium species and L.J medium is selective medium for detection and isolation of mycobacterium tuberculosis.

1. Mac-Conkey Medium-

Table 16- Constituents of Mac-Conkey agar medium

(a) Peptone	20 gms	To differentiate the lactose fermenters from non-lactose fermenters, produce pink-coloured colonies.
(b) Sodium taurocholate	5 gm	
(c) Agar	20 – 25 gms	
(d) 2% Neutral red in 50% ethanol	3 to 4 ml.	
(e) Water	Up to 1000 ml	
(f) PH at 7.4 to 7.6		

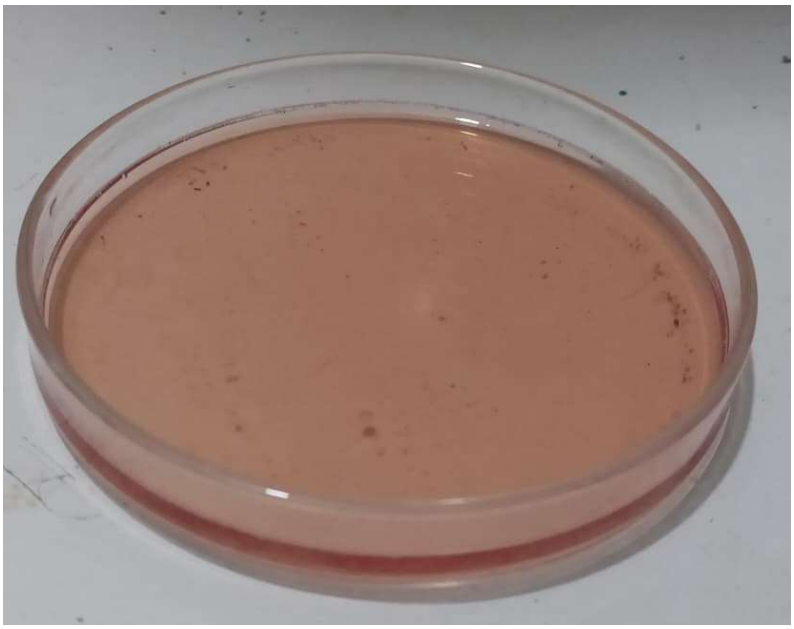


Figure 32- Mac-Conkey agar medium

The constituents of this medium inhibit the growth of mycobacterium tuberculosis.

2. Lowenstein Jensen Medium-

Table 17- Constituents of Mac-Conkey agar medium

(a) Anhydrous KH_2PO_4	2.4 gm	Eggs were washed with soap and water or spirit dried. It is cracked with sterile knife and collected in a sterile beaker. Mix well by egg beater.
(b) MgSO4	0.24 gm	
(c) Hydrated Magnesium Citrate	0,6 gm	
(d) Asparagine (N2 source)	3.6 gm	
(e) Glycerol (enhances the growth of human type)	12 ml	
(f) Water to add	600 ml	
Dissolve the ingredients hy heating		
(g) Malachite green solution- 2%		Mineral salt and malachite green solution is mixed with beaten egg. Now pour 5 ml of the whole mixture into Mac-Cortney tube and screw the cap tightly.
Mineral salt- 600 ml		
Malachite salt-20 ml		
(h) Beaten whole egg (hens)	20 to 22 pics (up to 1 litre)	

Figure 33- L.J. Medium

Culture Techniques-

By the help of loop specimen was transferred on to the slanted surface of the LJ medium by "**Stroke culture method**".

After that incubation of the inoculated media carried out at 37^0c on slanted position for up to 8 weeks.

Growth Observation-

- After 4 days of incubation. It was observed to find whether growth of any organism occur or not like mycobacterium, fungus or other non-MTB bacilli.
- We continued this observation every week till 8^{th} weeks.
- Growth of colonies found which having unique characteristics- rough, dry, wrinkled, creamy to buff coloured appearance.
- In case of no evidence of any growth occur, it was considered as culture negative.

- The growth which occurred during 3 to 8 weeks and having characteristics of mycobacterium were confirmed by ZN stain method.

In this study symptomatic patient and family members either symptomatic or asymptomatic of both treated or under treatment MTB patients were included. Patients were primarily classified on the basis of their clinical history and physical appearance in to two groups-

A. **Asymptomatic**-252 patients-Further classify in to 3 subgroups on the basis of pathological findings-
   A1. Asymptomatic clinically healthy -192 patients
   A2. Asymptomatic clinically mild suspected - 49 patients
   A3. Asymptomatic clinically strong suspected- 11 patients

B. **Symptomatic**-90 patients- Further classify in to 2 subgroups on the basis of pathological finding-
   B1. Symptomatic clinically mild suspected -70 patients
   B2. Symptomatic clinically strong suspected -20 patients

CHAPTER-6

RESULT

After the experimental work was completed, the results were analyzed based on the selection and rejection criteria. The population under study was broadly divided into two groups—those who were asymptomatic with family history and those who were symptomatic—along with an explanation of additional laboratory parameters.

A1. Asymptomatic clinically healthy-

192 patients were classified as asymptomatic clinically healthy patients. They were close relatives of MTB patients. These 192 patients (130 male and 62 female) were further classified on the basis of pathological findings.

Table no-18: Classification of asymptomatic clinically healthy patient on the basis of diagnostic findings-

Pathologically Normal (Total=156)	**Pathologically mild Suspected (Total=28)**	**Confirmed Cases (Total=8)** 8 asymptomatic patients are POSITIVE
Pathological finding of 125 patients- HB- 12 to 14 gm% TLC=5000 TO 10000/mm^3 DLC= all cells are within normal limit ESR= 10 mm to 20 mm 1^{st} hr. MT=Negative Urine AFB= Negative	**Pathological findings of 18 patients-** HB- 12 to 14 gm% TLC=5000 TO 10000/mm^3 DLC= Lymphocytes (50-65 %) ESR= 10 mm to 20 mm 1^{st} hr. MT=**POSITIVE (18)** Urine AFB= Negative	**Pathological findings of 5 patients-** HB- 12.0 to 14.0 gm% TLC=5000-10000/mm^3 DLC= within normal limit ESR= 10 mm to 20 mm 1^{st} hr. MT=**Strong positive-05** Urine AFB= Negative X Ray-**POSITIVE (3)** TB GOLD- **POSITIVE (2)**
Pathological findings of 19 patients- HB- 10 to 12 gm% TLC=10000 TO 12000/mm^3	**Pathological findings of 4 patients-** HB- 10 to 12 gm% TLC=10000 TO 12000/mm^3	**Pathological findings of 1 patient-** HB- 12.0 gm% TLC=12000/mm^3

DLC= Neutrophils (70-80%) ESR= 10 mm to 30 mm 1st hr. MT=Negative Urine AFB= Negative	DLC= Neutrophils (70-80 %) ESR= 10 mm to 30 mm 1st hr. MT=**POSITIVE (4)** Urine AFB= Negative	DLC= Neutrophilia (75%) ESR= 30 mm 1st hr. MT=**POSITVE** (22 mm) Urine AFB= Negative TB GOLD- **POSITIVE**
Pathological findings of 5 patients- HB- 8 to 9 gm% TLC=12500 TO 15400/mm^3 DLC= Neutrophils (70 to 80%) ESR= 20 mm to 50 mm 1st hr MT=Negative Urine AFB= Negative TB Gold- Negative	**Pathological findings of 4 patients** HB- 8.0 to 9.0 gm% TLC=15400 TO 17400/mm^3 DLC= Lymphocytes (50-65 %) ESR= 20 mm to 60 mm 1st hr X Ray= Normal TB Gold=Negative MT=**POSITIVE**-04 Urine AFB= Negative	**Pathological findings of 1 patient-** HB- 9.0 gm% TLC=14500/mm^3 DLC= Neutrophilia (80%) ESR= 48 mm 1st hr. MT=**POSITIVE** (20 mm) Urine AFB= Negative TB GOLD- **POSITIVE**
Pathological findings of 7 patients HB- 7.5 to 9.8 gm% TLC=15400 TO 18000/mm^3 DLC= Lymphocytes (50-65 %) ESR= 20 mm to 60 mm 1st hr. MT=Negative Urine AFB= Negative	**Pathological findings of 2 patients** HB- 7.0 & 9.0 gm% TLC=14500 & 15400/mm^3 DLC=Neutrophils (75&80%) ESR= 20 mm to 50 mm 1st hr. MT=**POSITIVE-02** Urine AFB= Negative X-Ray=Negative, TB Gold=Negative	**Pathological findings of 1 patient-** HB- 8.4 gm% TLC=15400/mm^3 DLC= Lymphocytosis (65%) ESR= 60 mm 1st hr. MT=**POSITIVE** (20 mm) Urine AFB= Negative TB GOLD- **POSITIVE**

A2. Asymptomatic clinically mild suspected - 49 patients-

Patients of this group are usually weak and underweight. Their physical appearance seems that they were suspected. Based on their physical appearance and economical status patients were classified in this group. Further several required investigations were done. On the basis of diagnostic results (described in table 19) patients were further classify into these subgroups-

Table No-19: Classification of asymptomatic clinically mild suspected patients on the basis of diagnostic findings-

Pathologically Normal (Total=32)	**Pathologically Suspected (Total=11)-MT- (+)**	**Confirmed Cases (Total=6)**
Pathological findings of 15 patients HB- 10.0 to 14.0 gm% TLC=5000 TO 10000/mm^3 DLC= within normal limit ESR= 10 mm to 20 mm 1^{st} hr. MT=Negative Urine AFB= Negative	**Pathological findings of 4 patients** HB- 10.0 to 14.0 gm% TLC=5000 TO 10000/mm^3 DLC= within normal limit ESR= 10 mm to 20 mm 1^{st} hr. MT=**POSITIVE** Urine AFB= Negative X Ray= Negative	**Pathological findings of 1 patient** HB- 10.0 to 14.0 gm% TLC=5000 TO 10000/mm^3 DLC= within normal limit ESR= 10 mm to 20 mm 1^{st} hr. MT=**POSITIVE** Urine AFB= Negative X Ray= Negative TB Gold= **POSITIVE**
Pathological findings of 10 patients HB- 10.0 to 12.0 gm% TLC=7500 TO 10000/mm^3 DLC= within normal limit ESR= 30 mm to 40 mm 1^{st} hr.	**Pathological findings of 3 patients** HB- 10.0 to 12.0 gm% TLC=7500 TO 10000/mm^3 DLC= within normal limit ESR= 30 mm to 40 mm 1^{st} hr.	**Pathological findings of 2 patients** HB- 10.0 to 14.0 gm% TLC=5000 TO 10000/mm^3 DLC= within normal limit ESR= 10 mm to 20 mm 1^{st} hr., MT=**POSITIVE**

MT=Negative Urine AFB= Negative	MT=**POSITIVE** Urine AFB= Negative	Urine AFB= Negative X Ray= **POSITIVE (1)** TB Gold= **POSITIVE (1)**
Pathological findings of 5 patients HB- 7.0 to 8.0 gm% TLC=5000 TO 10000/ mm^3 DLC= within normal limit ESR= 10 mm to 30 mm 1^{st} hr MT=Negative Urine AFB= Negative TB Gold=Negative	**Pathological findings of 3 patients** HB- 7.0 to 8.0 gm% TLC=7500 TO 10000/ mm^3 DLC= within normal limit ESR= 20 mm to 30 mm 1^{st} hr MT=**POSITIVE** Urine AFB= Negative X Ray- Negative TB Gold-Negative	**Pathological findings of 1 patient** HB- 7.0 to 8.0 gm% TLC=8700/ mm^3 DLC= within normal limit ESR= 30 mm 1^{st} hr MT=**POSITIVE** Urine AFB= Negative TB Gold= **POSITIVE (1)**
Pathological findings of 2 patients HB- 7.8 to 8.5 gm% TLC=15800 TO 17200/ mm^3 DLC= Neutrophilia (80-90%) ESR= 10 mm to 30 mm 1^{st} hr. MT=Negative Urine AFB= Negative TB Gold=Negative	**Pathological findings of 1 patient** HB- 8.0 gm% TLC=16400/ mm^3 DLC= Eosinophilia (38%) ESR= 20 mm to 30 mm 1^{st} hr. MT=**POSITIVE** Urine AFB= Negative X Ray- Negative TB Gold-Negative	**Pathological findings of 2 patients** HB- 7.8 to 8.5 gm% TLC=15800 TO 17200/ mm^3 DLC= Neutrophilia (80-90%) ESR= 70 mm to 110 mm 1^{st} hr.MT=**POSITIVE** Urine AFB= Negative X Ray= Negative. TB Gold= **POSITIVE (2)**

A3. Asymptomatic clinically strong suspected- 11 patients- These 11 peoples were close contact to MTB patients under treatment. They belong to lower economic class and all were very weak and underweight. All were asymptomatic.

Table No-20: Classification of asymptomatic clinically strong suspected patients on the basis of diagnostic findings

Pathologically Normal (Total=06)	**Pathologically Suspected (Total=01)**	**Confirmed Cases (Total=04)**
HB- 7.0 to 8.5 gm% TLC=13500 TO 16400/ mm^3 DLC= Neutrophilia (88-94%) ESR= 80 mm to 94 mm 1^{st} hr. MT=Negative Urine AFB= Negative TB Gold=Negative	HB- 8.2 gm% TLC=15700/ mm^3 DLC= Neutrophilia (90%) ESR= 92 mm 1^{st} hr. MT=**POSITIVE** Urine AFB= Negative TB Gold=Negative **Undefine case**	HB- 7.0 to 8.5 gm% TLC=13500 TO 16400/ mm^3 DLC= Neutrophilia (88-94%) ESR= 80 mm to 120 mm 1^{st} hr. MT=**POSITIVE (3) without fever** MT=**POSITIVE (1) with fever** Urine AFB= Negative X Ray= **POSITIVE (1)** TB Gold= **POSITIVE (3)**

B. Symptomatic -90 patients (39 Male and 51 female)- Further classify in to 2 subgroups on the basis of pathological and radiological findings-

B1. Symptomatic clinically mild suspected -70 (30 male and 40 female) patients
B2. Symptomatic clinically strong suspected -20 (9 male and 11 female) patients

B1. Symptomatic clinically mild suspected – Clinical symptoms and diagnostic findings of these 30 male symptomatic clinically mild suspected patients-

Table No-21: Symptomatic clinically mild suspected 30 male patients and their diagnostic findings-

Clinical Symptoms	**Number of Patients**	**Diagnostic findings**
Fever, Coughing, Weight Loss	20	TLC=8500 TO 11500/ mm^3 Neutrophilia (75-85%)-10 Lymphocytosis (50-65%)-04 Eosinophilia 77%-01 DLC-Normal-05 Hb=11 to 14 gm% ESR=20 to 60 mm MT=**POSITIVE-08** Sputum AFB=**2 POSITIVE** X Ray=**1 POSITIVE** TB PCR**-POSITIVE-01 and** also TB Gold=**POSITIVE-01**
Lymph node (Total = 7 Patient) a. Cervical Lymph node	4 Patients had cervical lymph node without fever	TLC=8800 TO 15600/ mm^3 1=Neutrophilia (82%) 3=Lymphocytosis (55 to 62 %) Hb=9.6 to 14 gm% ESR=20 to 100 mm MT=**POSITIVE-02** FNAC= ZN Stain in lymph node aspirate – Negative TB Gold=**POSITIVE-01**

	01 have cervical lymph node with history of fever	TLC=12800/ mm^3 DLC=Lymphocytes=66% MT= **Positive** FNAC= showing ZN stain in lymph node aspirate shows AFB caseous, necrosis, epithelioid granuloma, suggestive of tuberculosis and his TB Gold= **POSITIVE**
b. Inguinal Lymph node with fever	02 Patients	TLC=8800 & 12400/ mm^3 DLC= Lymphocyte (58%) & Neutrophil (78%) Hb=10.8 & 12.0 gm% ESR=40 mm & 60 mm MT= Both **Positive** (16 mm & 20 mm) FNAC=1^{st} Negative,2^{nd} ZN Stain in lymph node aspirate shows **AFB** and TB Gold=1^{st} Negative
Azoospermia	02	TLC DLC= within normal Limit Hb > 12 gm%, MT= Negative TB Gold= Negative Seminal fluid send for culture -Negative Patient had no other sign and symptoms. Patient was looking fit and fine.
Breast Lump With family history No fever, weight loss	01	TLC &DLC-Normal Hb=12.8 gm%, MT=Negative FNAC=Gynecomastia TB Gold = Negative

B1. Symptomatic clinically mild suspected –

Clinical symptoms and diagnostic findings of 40 female symptomatic clinically mild suspected patients-

Table No-22: Symptomatic clinically mild suspected 40 female patients and their diagnostic findings

Symptoms	**Total Patients**	**Diagnostic Findings**
Irregular Menstruation	15 10-without history of fever	TLC DLC-Normal, Hb-9.0 to 11.0 gm% ESR-20 to 40 mm, MT- 6 **POSITIVE** PAP SMEAR- ALL NEGATIVE TB GOLD-ALL NEGATIVE X Ray- ALL NEGATIVE
	5-with history of fever in which 01 had coughing.	TLC-9000 to 11000/mm^3-02(DLC-WNL) TLC – 11500 to 13500/mm^3-03 Neutrophilia-02, Lymphocytosis-01 ESR-30 to 68 mm 1^{st} hr. Hb- 8.0 to 11.0 gm% AFB-**1 POSITIVE** Pap Smear- Negative (05) X Ray- **1 POSITIVE** **5** menstrual blood sample were sent for culture-all were negative.
Cervical Lymph node	02	TLC -14200 & 19200 DLC-Neutrophilia (76% & 80%) ESR- 60 mm & 120 mm 1^{st} hr MT- Both **Positive** (16 & 18 mm) X Ray- Negative both AFB- Negative both TB Gold- **POSITIVE** 1^{St}

		TB Gold- NEGATIVE 2nd FNAC-NEGATIVE 1st HPE-NEGATIVE 1st FNAC-**Acinic Cell Carcinoma** 2nd
Breast Lump History of Fever and weight loss,	02	TLC-5000 to 10000/mm^3 DLC- within normal limit ESR- 10 to 18 mm in 1st hr. MT- **Positive** both, FNAC-Negative both HPE-Negative both, TB Gold- Negative both
Primary Infertility	Total=07 05= without fever	TLC DLC- Normal, Hb- 9 to 11 gm%, ESR- 10 to 50 mm 1st hr. MT- **POSITIVE**-01 Pap Smear-Negative (05) TB Gold- **POSITIVE -01**
	02- with fever, coughing, weight loss and family history	TLC-11500 & 15000/mm^3 HB- 9 to 11 gm% Lymphocyte- 52 % & 60 % MT- Both **Positive** AFB-Both Negative Pap Smear-Negative-02 TB PCR-Negative both TB Gold- **POSITIVE (01)** 7 menstrual blood samples were sent for culture-**1 POSITIVE**
Coughing and weight loss	14 Patients 05 had history of fever all had family history	TLC=11000 to 15000/mm^3 DLC-09 had neutrophilia (70 to 80%) 04 had lymphocytosis (50-60 %) and 01 had Eosinophilia-23% Hb- 9 to 11 gm%

		MT- **POSITIVE**-06 X Ray -Negative all AFB- **POSITIVE-02** 03 Patient samples were collected for TB PCR and TB Gold TB PCR- **POSITIVE-01** and this sample was TB Gold- **POSITIVE-01** Rest two patient had fever, coughing, weight loss, family history but X Ray, AFB, TB PCR, TB Gold is Negative, only MT was positive and her physical appearance shows that she must be MTB positive. So, **Gene xpert** - test done and got one **POSITIVE** result with rifampicin resistance.

B2. Symptomatic clinically strong suspected -20 (9 male and 11 female) patients-

Physical appearance and symptoms of all patients of this group, shows that they were suffering with MTB. In this group all were from economically lower class, living in house made of mud.(*Kaccha ghar*) even they don't have the proper cloths, all were wearing torn and old cloths, their qualification level was 5th standard pass. All females were also uneducated and not aware about personal hygiene and sanitary pads. All were labor or rickshaw puller.

Table No-23: Symptomatic clinically strong suspected 09 male patients and their diagnostic findings-

Symptoms	**No of Patients**	**Diagnostic Findings**
Coughing mixed with blood, fever and weight loss	02	TLC-14200 & 16400 Neutrophilia (80 & 87%) Hb- 9.2 & 9.0 gm%, ESR- 70 mm & 120 mm MT- Both **Positive** X Ray-**POSITIVE**-02 AFB- **Both Positive**
Coughing, fever, weight loss	06	TLC-6700 to 11400/mm^3 DLC- 4 has neutrophilia > 75 % 2 has lymphocytosis 55 to 65% Hb-9.0 to 11.5 gm% ESR- 60 to 110 mm 1^{st} hr. MT- **POSITIVE** -04 X Ray-**POSITIVE**-01 AFB- **POSITIVE-01-**analysis of rest 5 samples- TB PCR-**POSITIVE-01** Rest four patients were strongly suspected. So, their blood samples were collected for TB Gold, out of 4 ,2 patient were TB Gold- **POSITIVE-02,** Rest 2 patients were also very suspected but TB PCR and TB Gold Negative. So, their sputum samples were collected for Gene Xpert. We got Gene Xpert- **POSITIVE-01**(rifampicin resistance not detected) rest one patient was pathologically negative.

Penis sore	01	One patient had sore in his penis. He had family history of tuberculosis. History of fever and weight loss. Pus discharge from penis. He had five children. His wife was also positive by AFB smear examination. All five children were very weak and underweight they were under precaution treatment from RNTCP. Pathological findings are- TLC-13500/mm^3 DLC – Neutrophilia (85%) Hb- 10.0 gm% ESR-80 mm 1^{st} hr. MT- **POSITIVE** ZN Stain of pus- Negative Pus sent for culture-**01 POSITIVE.** TB GOLD- **POSITIVE-01**

Table No-24: Symptomatic clinically strong suspected 11 female patients and their diagnostic findings-

Symptoms	**Number of Patients**	**Diagnostic findings**
Vaginal discharge, abdominal pain, fever, weight loss and one patient was primary infertile	05	TLC- 4500 to 8000//mm^3 DLC-Normal-02, Neutrophilia (75-80%)-02, Lymphocytosis (54%)-01 Hb-6.2 to 9.0 gm% ESR-70 to 120 mm 1^{st} hr. MT- **POSITIVE-02** 5 pap smears were collected by gynecologist and

		done cytological examination. 4 were negative and one was suggestive of tuberculosis. 5 menstrual blood samples were collected and sent for culture-we got **01 POSITIVE** result. All these 5 patient blood samples were collected for TB Gold test. Result TB Gold- **POSITIVE**-02. In these two positives patient one was that primary infertile patient.
Amenorrhea+ fever+ weight loss.	01	Our team visited a home where a women found lying on bed. She was very weak, unable to walk and unable to coughing. She was unmarried and underweight. Her father was old TB patient. Pathological findings were- TLC-5100 DLC-Lymphocyte-62% Hb-6.8 gm% MT- **POSITIVE** AFB Sputum- Unable to give sputum sample. TB Gold- Negative
Coughing and weight loss	03	TLC-11400 to 13700, DLC-Neutrophils-(>82%)--02, Lymphocytosis (57%)-01 MT-**POSITIVE**-01 → X Ray- **POSITIVE-01** Sputum AFB-Negative but TB PCR and TB Gold- **POSITIVE -01** Sputum AFB- **POSITIVE-01** Total **POSITIVE cases-02** TB PCR- Negative-01 TB Gold-Negative-01

Secondary Infertile coughing, history of fever and weight loss.	02	TLC-8600 & 11700 DLC-within normal limit & Neutrophilia-76% Hb-10.8 gm% & 9.4 gm% MT-**POSITIVE-02** Pap smear- Negative-02 AFB-Negative & **POSITIVE** TB PCR-Negative TB Gold 1st -Negative → Gene Xpert- Negative

After analyzing the experiment of the research following finding were observe-

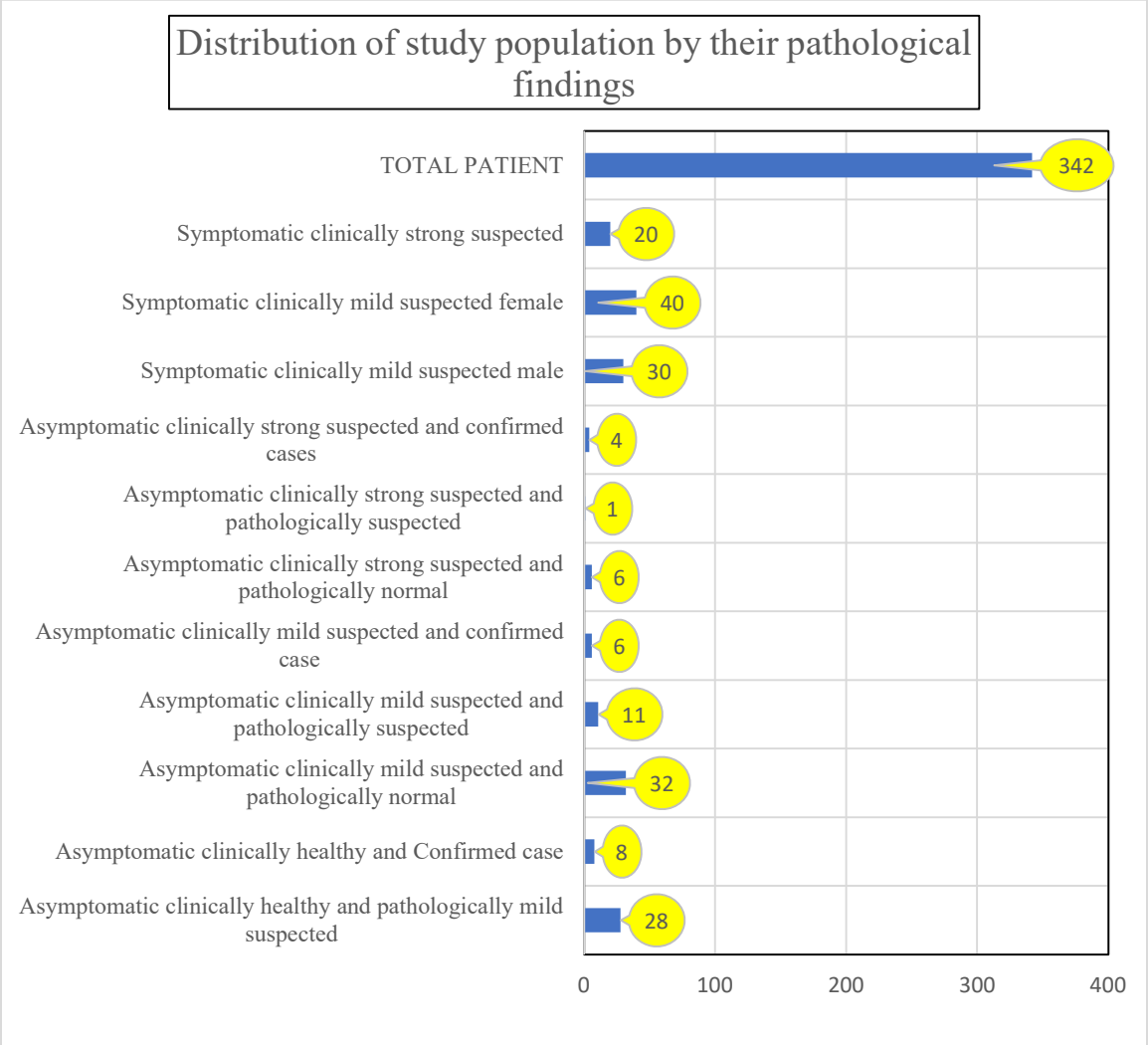


Figure 34- Graph of study population by their pathological findings

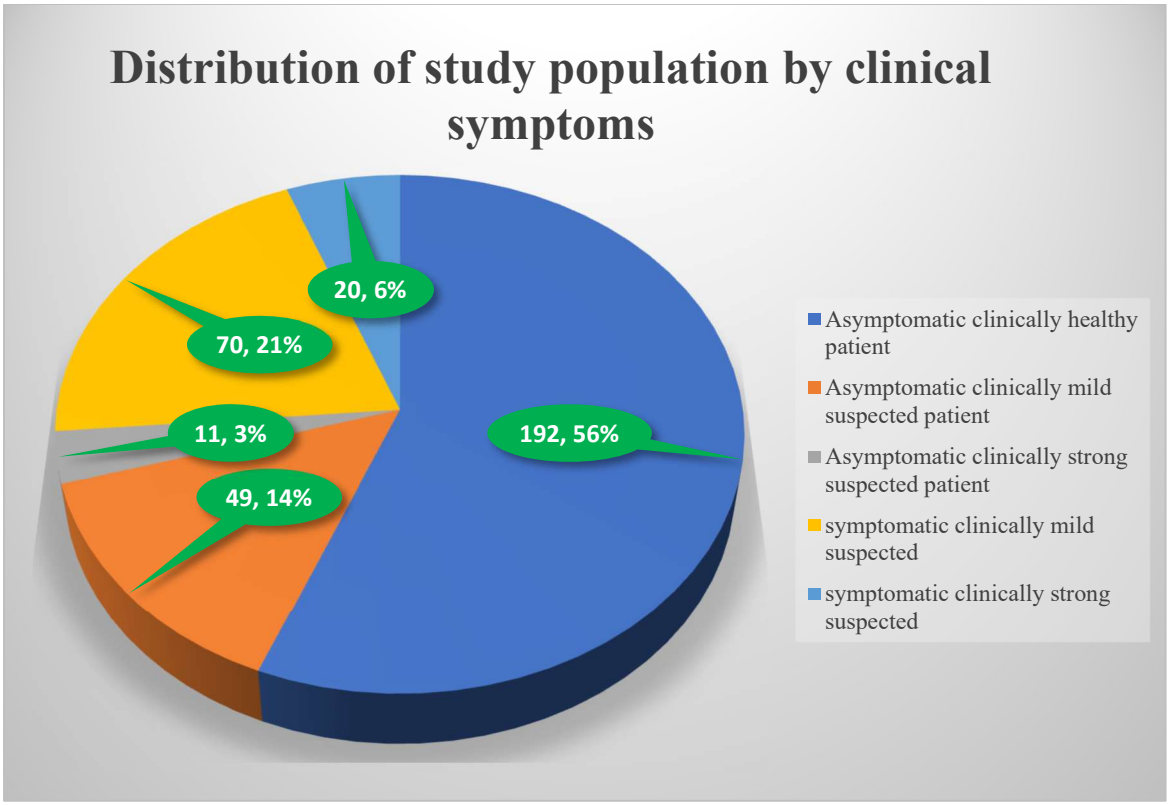


Figure 35: Distribution of study population by clinical symptoms

Description of the figure 35-

Show the different categories of study population. Among study population the highest number of patients, were in asymptomatic clinically healthy group (n= 192,56 %) whereas the lowest number of patients were in asymptomatic clinically strong suspected group (n=11, 3%) and in other groups like asymptomatic clinically mild suspected(n=49,14%), symptomatic clinically mild suspected (n=70,21%), and symptomatic clinically strong suspected (n=20,6%).

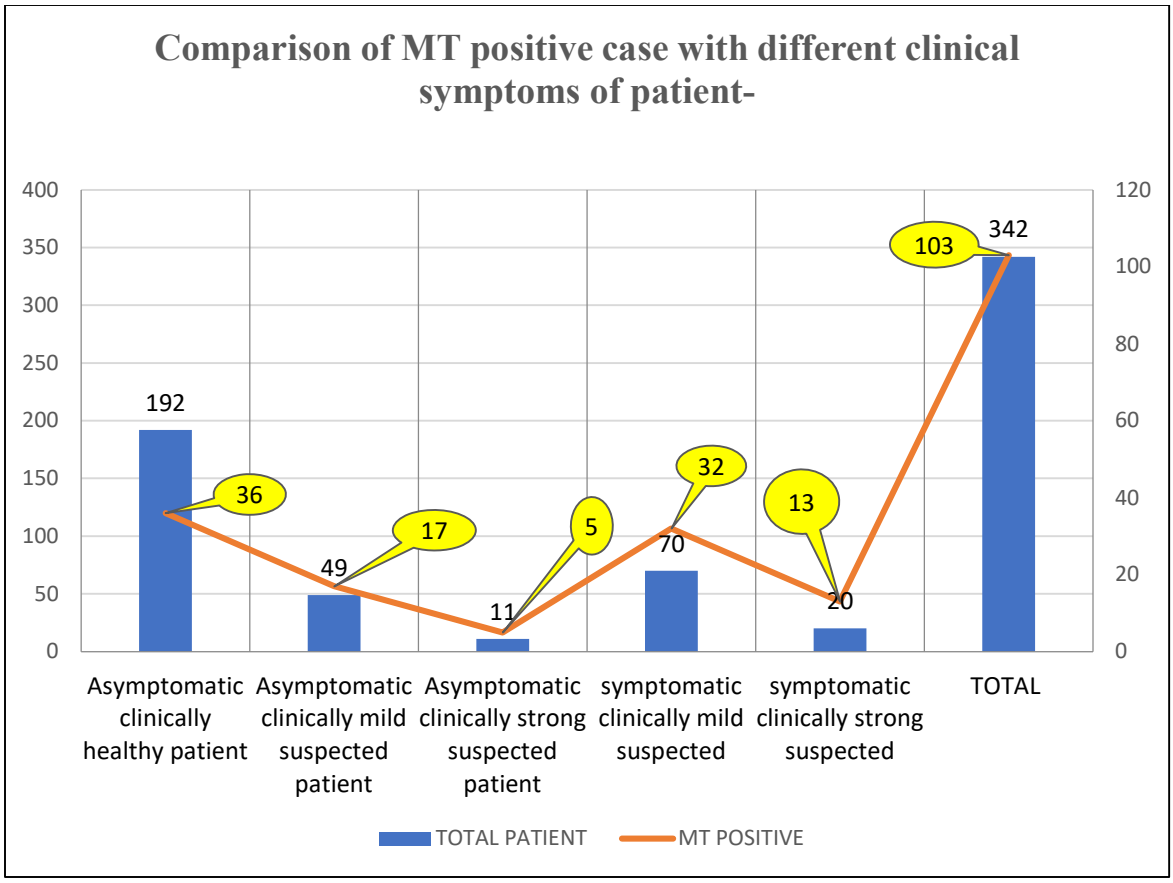


Figure 36: Graph of comparison between different asymptomatic and symptomatic patients with MT positive result.

Description of the figure 36- The comparison of MT-positive cases across different clinical symptom groups shows a clear variation in positivity rates. Among asymptomatic clinically healthy individuals, 18.7% tested MT positive, whereas asymptomatic but clinically mild-suspected patients showed a notably higher positivity of 34.7%. The positivity rate decreased slightly to 45.4% in asymptomatic clinically strong-suspected cases. In symptomatic groups, MT positivity was highest—45.7% in clinically mild-suspected patients and 65% in clinically strong-suspected individuals—indicating a strong association between the presence of symptoms and MT positivity. Overall, out of 103 total MT-positive cases, the results highlight that symptomatic individuals, particularly those with mild to strong clinical suspicion, exhibited the highest proportion of MT detection.

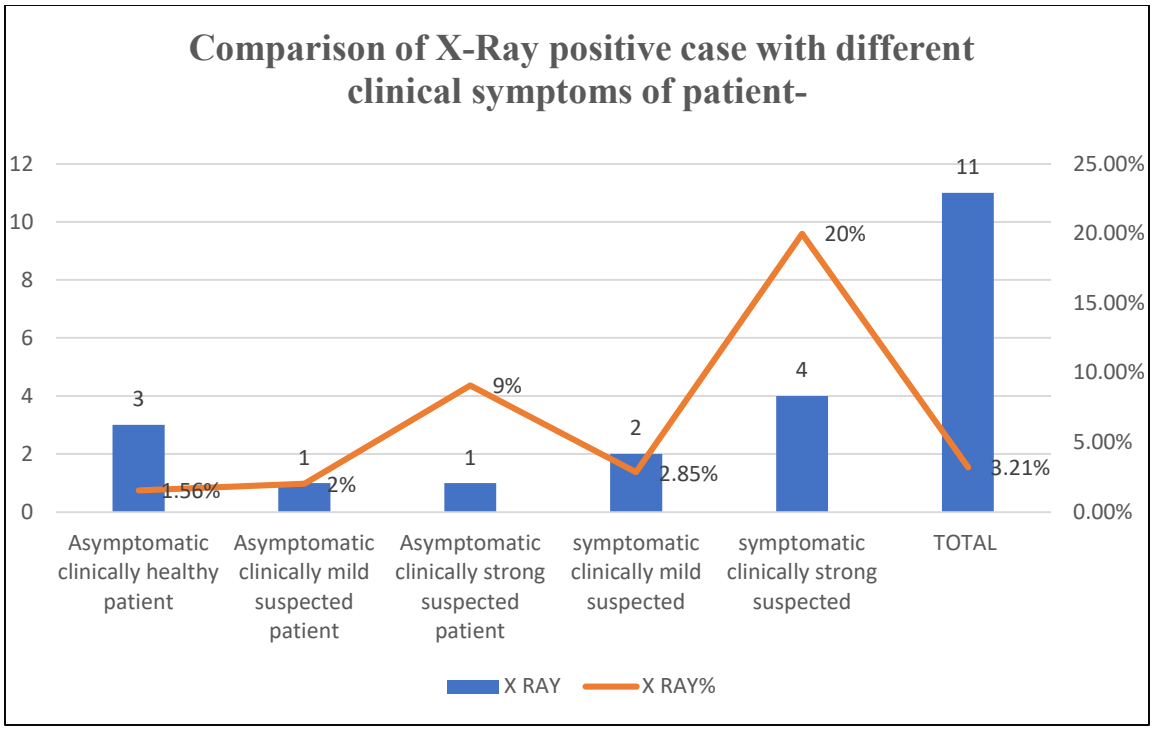


Figure 37: Graph of comparison between different asymptomatic and symptomatic patients with X Ray positive result.

Description of the figure 37-

This graph compares the number and percentage of X-ray positive cases among clinical asymptomatic and symptomatic patients. X-ray positivity was observed among both groups, with varying frequencies. The highest percentage of X-ray positive cases (20%) occurred among *symptomatic clinically strong suspected patients*. While asymptomatic clinically mild suspected and asymptomatic clinically strong suspected shows respectively 2.04% and 9% positivity. Mildly suspected symptomatic patients showed a positivity rate of 2.8%. Asymptomatic clinically healthy individuals demonstrated the lowest positivity (1.5%). Overall, a total of **11 X-ray positive cases (3.21%)** were recorded among all categories.

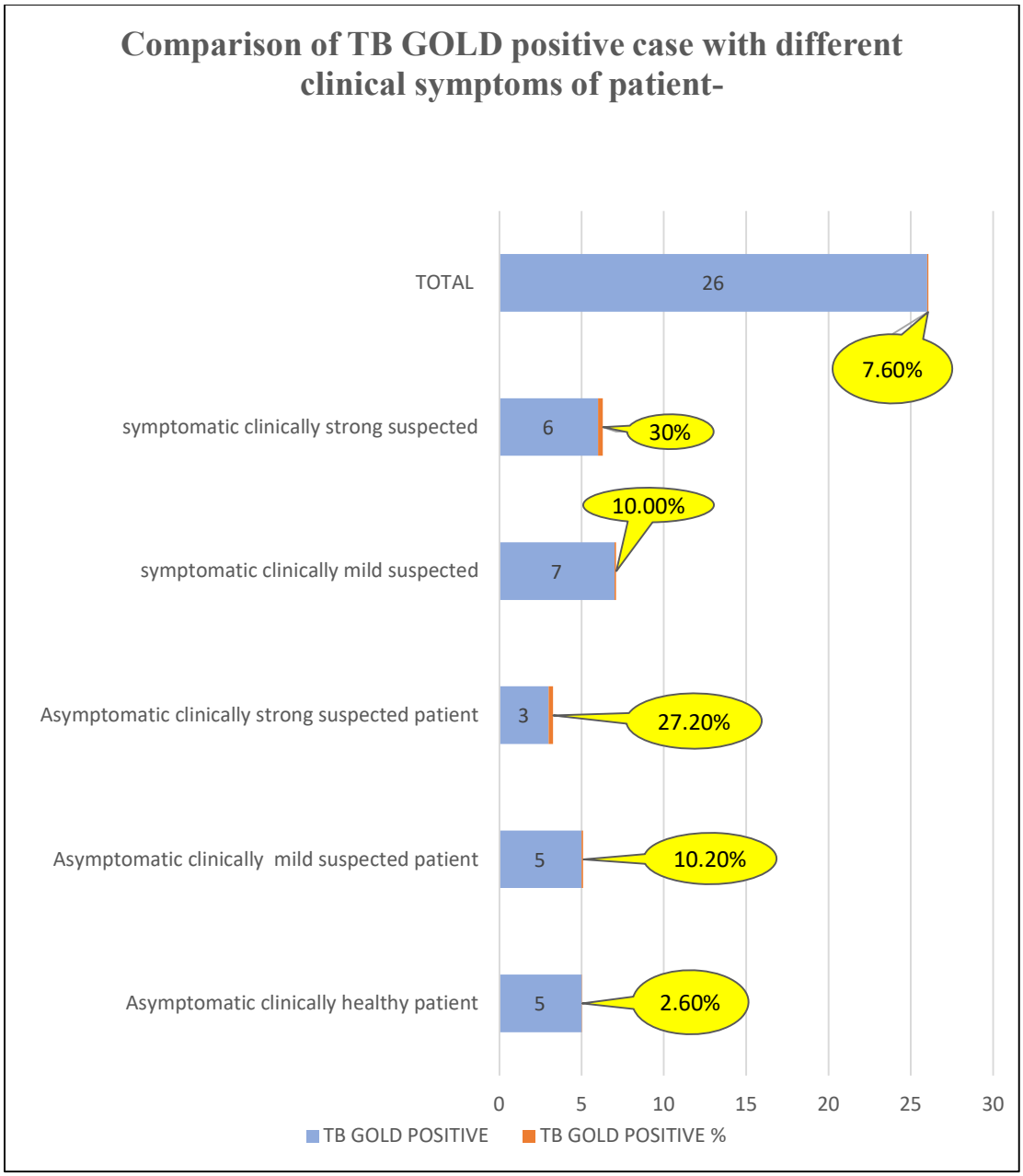


Figure 38: Graph of comparison between TB Gold positive case and different asymptomatic and symptomatic patients

Description of the figure 38-

The graph compares TB GOLD–positive cases among clinical asymptomatic and symptomatic patients. TB GOLD positivity was observed in all groups, with the highest

positivity rate (30%) found among symptomatic clinically strong suspected patients, followed by 27.2% in asymptomatic clinically strong suspected cases. Mildly suspected symptomatic patients also showed a positivity rate of 10%, while asymptomatic clinically mild suspected individuals had an 10.2% positivity rate. The lowest positivity (2.6%) found in asymptomatic clinically healthy patients. Overall, a total of **26 TB GOLD–positive cases (7.6%)** were identified in all categories of clinical cases, which means that TB GOLD positivity is unequally distributed, depending on the severity of clinical suspicion.

Table No-25: **Distribution of Study Participants by Clinical Category, Sex, and MTB Positivity**

PATIENT CATEGORY	Total	Male	Female	Positive male	Positive female
Asymptomatic clinically healthy patient	192	130	62	3	5
Asymptomatic clinically mild suspected patient	49	32	17	2	4
Asymptomatic clinically strong suspected patient	11	4	7	2	2
symptomatic clinically mild suspected	70	30	40	6	9
symptomatic clinically strong suspected	20	9	11	7	5
TOTAL	**342**	**205**	**137**	**20**	**25**

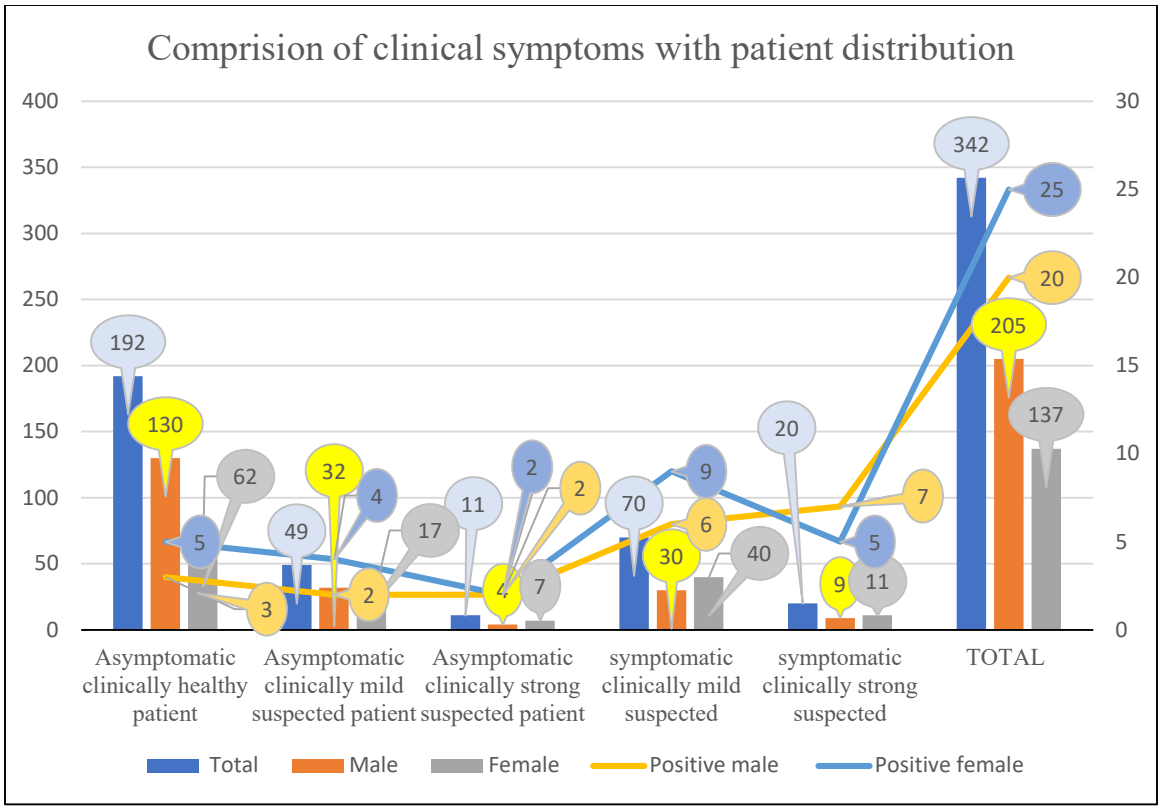


Figure 39: Graph of patient distribution in respect to their clinical symptoms, sex, MTB positive and negative results.

Description of figure 39-

Graph shows the distribution of patients in various clinical symptom categories, the highest number of patients was in the group of asymptomatic clinically healthy patients (n = 192), males (130) prevailed over females (62), and 3 male and 5 female positive cases were detected in the group. Among 32 male and 17 female,2 male and 4 female patients were found to positive from a total of 49 asymptomatic clinically mild suspected patients.

The male category (4) is predominant over the female category (7) in the asymptomatic clinically strong suspected category (n = 11) with 2 males and 2 females testing were positive. There were 70 symptomatic clinically mild suspected cases, of which 30 were males and 40 were females, and 6 and 9 of them were positive respectively.

The symptomatic clinically strong suspected group consisted of 20 patients (9 males and 11 females) in which 7 males and 5 females were positive. In general, when accounting all the categories, the total patient count was 342 out of which 205 were males and 137 females, in which 20 males were positive whereas 25 females were positive.

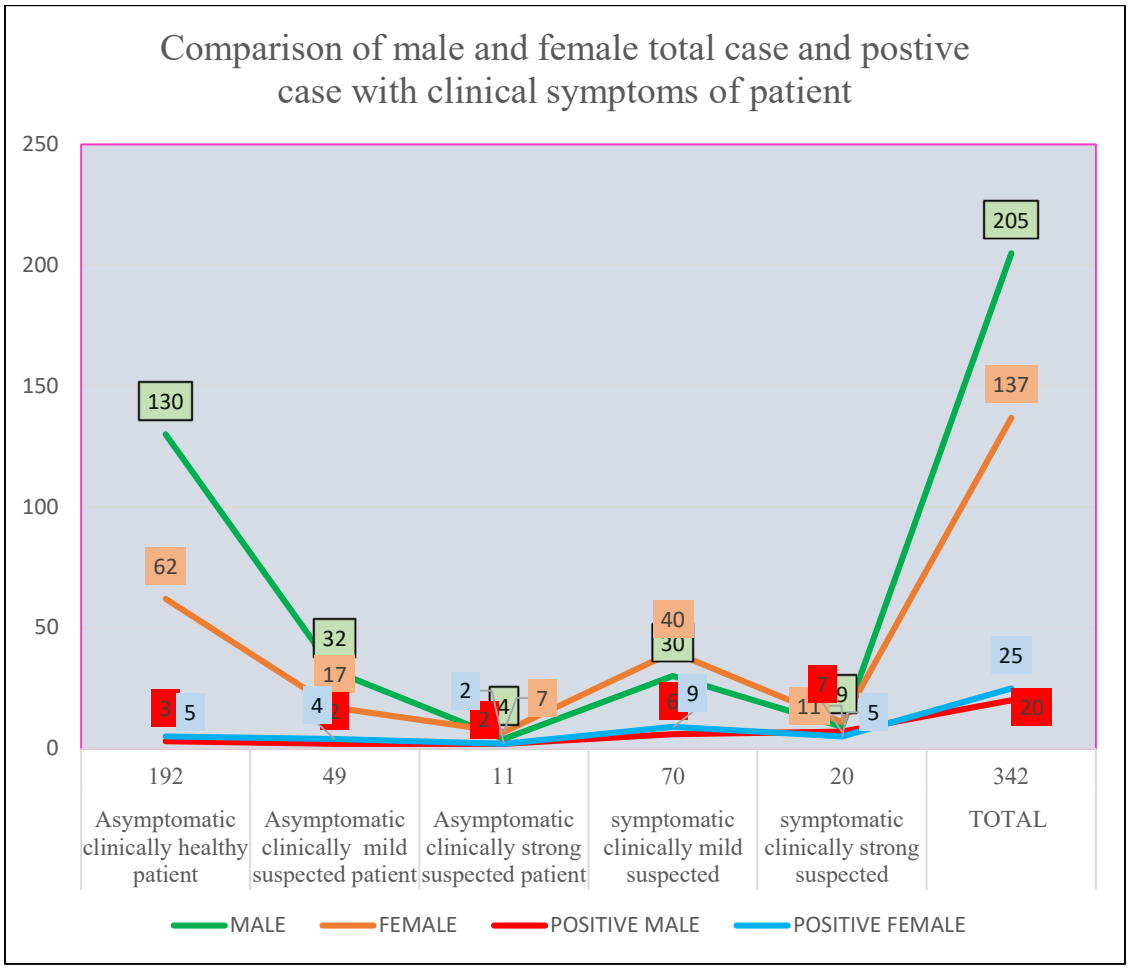


Figure 40: Graph of comparison between total male and female case with male and female positive case in respect to their clinical symptoms.

Description of figure 40-

The graph shows, the distribution of patients, male and female, by their positive cases on various categories of clinical symptoms. Among the clinically health asymptomatic patients (n = 192), males were the most common (130) against females (62), with a positive

result of 3 males and 5 females. In the clinically mild suspected group with no symptoms, having an asymptomatic group (n = 49), 32 males and 17 females were reported, with 2 males and 4 females having a positive report. The strong suspected group (n = 11) that was asymptomatic had 4 males and 7 females with 2 males and 2 females having tested positive.

In symptomatic clinically mild suspected patients (n = 70), females (40) were more than males (30), and positive results were observed in 6 males and 9 females. In another category there were 7 and 5 positive cases in the males and females, respectively in the symptomatic clinically strong suspected group (n = 20). In general, the patient population was 342 (205 men and 137 women), with 20 male and 25 female positive cases of all

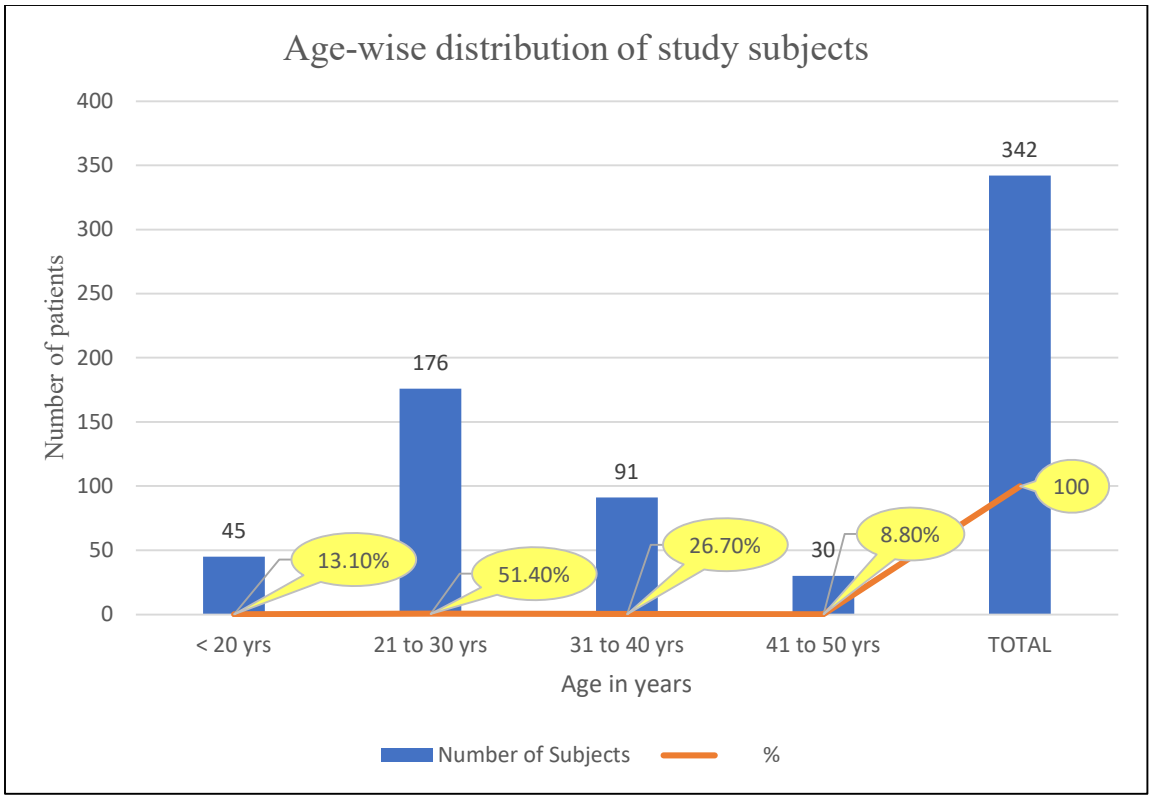


Figure 41: Graph of age-wise distribution of study subjects

Figure- 41 shows that: -

- Most of the study populations were in age group 21 to 30 years.
- Reproductive age groups (20–40 years): Account for 78.1% of cases
- The lowest population found in age group 41 to 50 years.
- The modal age group was 21 to 30 years of this study.

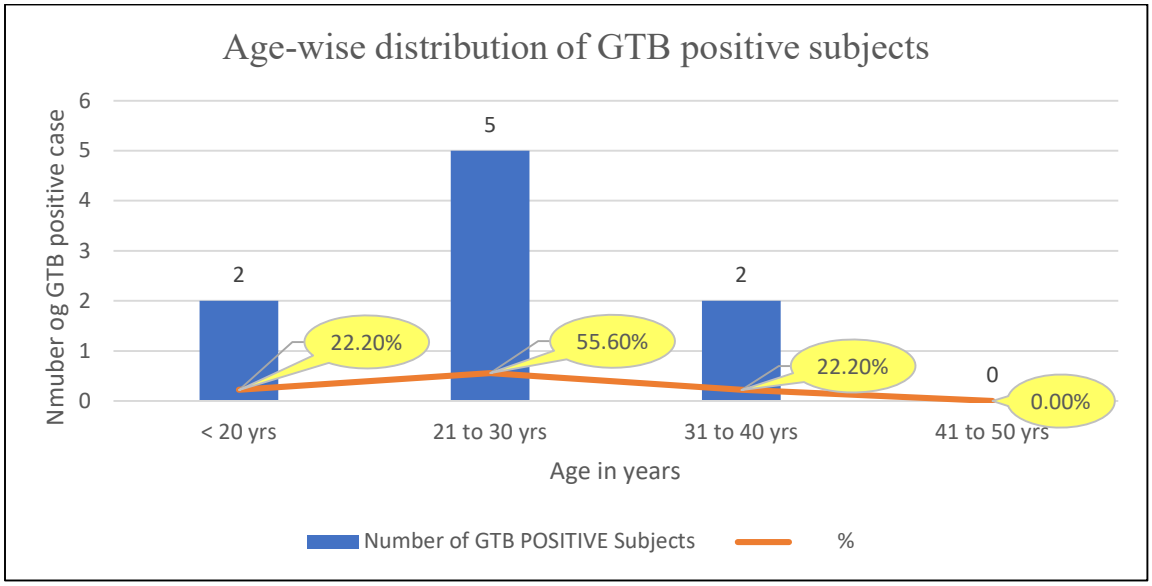


Figure-42- Graph of age-wise distribution of GTB positive subjects in the study population.

Figure 42 shows that: -

- 55.6 % of genital tuberculosis patients were found in age group of 21 to 30 years.
- 22.2 % of GTB subjects were below 20 years.
- 77.8 % of total GTB patient were found below the age of 3o years.

Table 26- Distribution of study population according to economical standard by BG Prasad Scale-

Economic Status	Number of Subjects	%	GTB (+)	GTB (+) %	GTB (+) % in respect to number of subjects
Economically strong	39	11.5	01	11.1	2.56
Economically average	58	16.7	01	11.1	1.72
Economically weak	164	48.2	04	44.4	2.43
Economically very weak	81	23.6	03	33.4	3.45
TOTAL	342	100	09	100	

Table 26- shows that:

- Most of the subject 48.2 % were from economically weak group, whereas total 71.8% were from economically lower class.
- Similarly, most of the GTB positive case were came from economically weak (44.4 %) and economically very weak group (33.4%) total case 7(77.8%) came from economically weak classes.
- We also found that the maximum % of GTB (+) cases were came from economically very weak class (3.45) 3 out of 81 were GTB positive and 7(5.88%) came from economically weak class.

Table No.27- Distribution of study population according to the type of infertility-

Infertility type	Number of patients	%	% v/s 342
Primary infertility	10	83.3%	2.92 %
Secondary infertility	02	16.7%	0.58 %
Total	12	100	

Table 27 Shows:

- Out of 342 patients 10 (2.92%) were primary infertile whereas 02 (0.58%) were secondary infertile.
- In total infertility cases 83.3 % were primary infertile where as 16.7% were secondary infertile patients.

Table No 28- Distribution of study population according to the history of contact with TB.

History of contact TB	**Contact history present**	**Absent**	**Total**
GTB Positive	06 (66%)	03 (34%)	09
GTB Negative	297 (89.1%)	36 (10.8%)	333
Total	303	39	342

Chi-Square (X^2) = 4.34	degree of freedom (df) =1	Value of $P < 0.05$, So null hypothesis (H_0) is rejected. Statistically significant.
$P \approx 0.037$	Value of $P < 0.05$	

Table 28 Shows-

- About 66% of GTB positive patient had previous contact history of TB.
- About 34 % of GTB positive patients had not previous contact history of TB.
- Difference in contact history of TB between GTB positive and GTB negative patients was found to be significant.

Table No-29- Distribution of study population according to the symptoms-

Symptoms	**Symptomatic**	**Asymptomatic**	**Total**
GTB (+)	09 (100%)	00	09
GTB(NEG)	90 (27.02%)	243 (72.98 %)	333
TOTAL	99	243	342

Chi-Square (X^2) = 22.65	degree of freedom (df) =1	Value of P is much smaller than 0.05, So null hypothesis (H_0) is rejected. Statistically highly significant.
$P \approx 2.1x\ 10^{-6}$	Value of $P < 0.05$	

Table 29 shows-

- All GTB Positive patients were symptomatic (100%).
- 90 (27.02%) GTB negative patients were symptomatic.
- Difference in status of symptoms of GTB positive and GTB negative patients were found to be highly significant.

Table-30 Distribution of Risk Factors among study population (n = 342)

Risk Factors	Number	**%**
Male infertility	2	0.6
Female infertility	10	2.9
Total infertility	12	3.5
Previous history of TB	22	6.4
Contact history	303	88.6
Socio economic status	245	71.6
Male GTB (+)	2	0.6
Female GTB (+)	7	2.0
Total GTB Positive	9	**2.6**

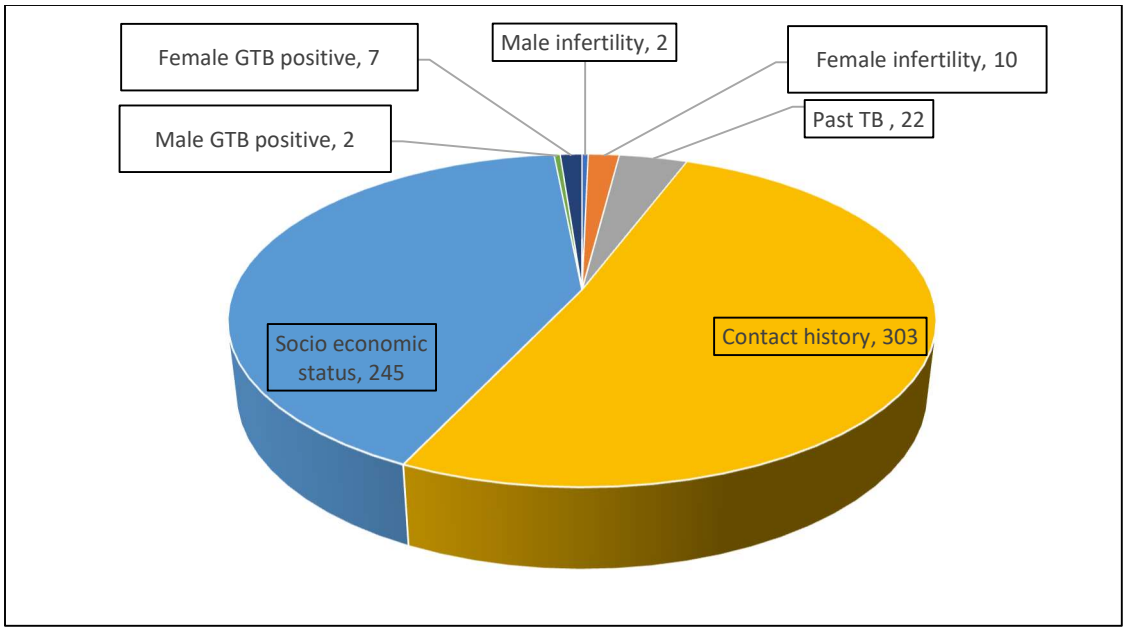


Figure 43- Graph of Risk Factors distribution among study population

Overall finding indicates that contact history, low socioeconomic status, infertility (predominantly female infertility) and past history of tuberculosis are found the major risk factors.

Table No.31- Distribution of study population in respect to the Hb findings: -

Hb%	**Within normal range**	**Low**	**Total**
GTB (+)	02	07	09
GTB Neg	148	185	333
TOTAL	150	192	342

Chi-Square (X^2) = 1.735	degree of freedom (df) =1	Value of P is more than 0.05, So null hypothesis (H_0) is accepted. Statistically no significant. There is no relation between Hb and GTB (+) and GTB negative cases.
P ≈ 0.187	Value of P >0.05	

Table No-32- Erythrocyte sedimentation rate (ESR) of the study population: -

ESR	**Within normal range**	**High**	**Total**
GTB (+)	03 (33.3 %)	06 (66.6%)	09
GTB Neg	223 (67%)	110 (33%)	333
TOTAL	326	116	342

Chi-Square (X^2) = 4.396	degree of freedom (df) =1	Value of P is smaller than 0.05, So null hypothesis (H_0) is rejected. Statistically significant.
P ≈ 0.036	Value of P < 0.05	

Table 32, Shows-

- Difference in ESR value between GTB (+) and GTB negative were significant.
- 66.6% GTB patients had high ESR values.
- 33.03% GTB negative patient had high ESR value.
- ESR value can be both high and low but probability of high value is greater in positive cases.

Table N0 33- Distribution of the study subjects according to the results of AFB-

AFB	**Number of patients**	**%**
Found	10	2.93 %
Not Found	332	97.07 %
Total	342	100

Table 34- Distribution of the study subjects according to the results of TB Gold-

TB Gold	Number of patients	%
TB Gold Positive	26	7.6 %
Total Positive case	45	13.1 %
Diagnostic significance of TB Gold test in tuberculosis detection		57.7 %

Table 35- Distribution of Genital tuberculosis positivity among normal and abnormal total leucocyte counts-

TLC	Within normal range	High	Total
GTB (+)	03	06	09
GTB Neg	270	63	333
Total	273	69	342

Chi-Square (X^2) = 12.78	degree of freedom (df) =1	Value of P is much smaller than 0.05, So null hypothesis (H_0) is rejected. Statistically highly significant. There is statistically significant association between Total Leucocyte Count and GTB.
P ≈ 0.00036	Value of P < 0.05	

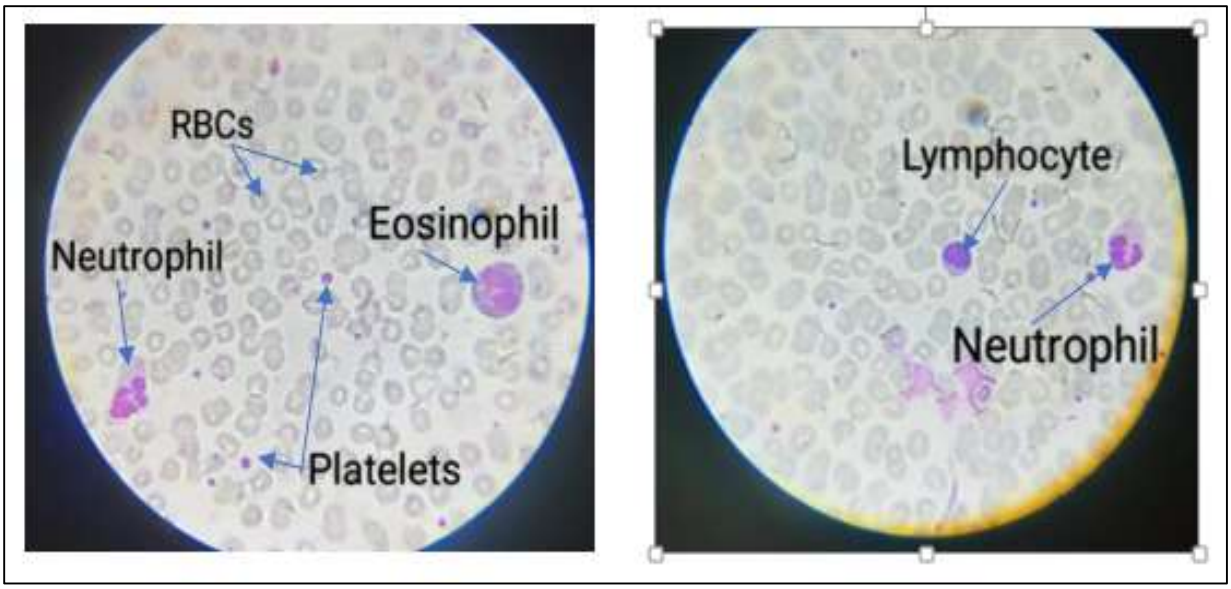


Figure 44: - Microscopic image of peripheral blood smear showing WBCs, RBCs and platelets.

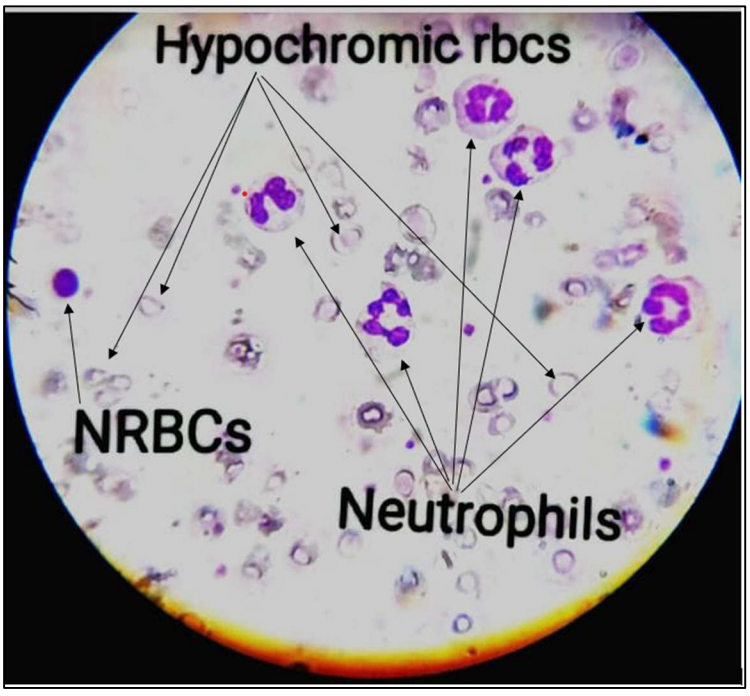


Figure 45: Microscopic picture of peripheral blood smear of a patient having hb 4.0 gm,neutrophilic leucocytosis with hypochromic rbcs.

Table 36- Distribution of the Genital tuberculosis positivity among infertile and non-infertile group-

	Infertile case	**Non infertile case**	**Total**
GTB (+)	04	05	09
GTB Neg	08	325	333
Total	12	330	342

Chi-Square (X^2) = 34.17	degree of freedom (df) =1	Value of P is much smaller than 0.05, So null hypothesis (H_0) is rejected. Statistically highly significant.
$P \approx 5.04 \times 10^{-9}$	Value of $P < 0.05$	

Table No-37: **Distribution of Differential Leucocyte Count (DLC) Abnormalities across Patient Classifications**

Patient classification	Neutrophilia	Lymphocytosis	Eosinophilia	DLC with in normal limit	Total
Asymptomatic clinically healthy patient	32	30	0	130	192
Asymptomatic clinically mild suspected patient	04	0	01	44	49
Asymptomatic clinically strong suspected patient	11	0	0	00	11
Symptomatic clinically mild suspected	24	17	02	27	70

Symptomatic clinically strong suspected	11	06	00	03	20
Total	82	53	03	204	342

Chi-Square (X^2) = 96.40	degree of freedom (df) =12	Value of P is much smaller than 0.05, So null hypothesis (H_0) is rejected. Statistically highly significant.
P ≈ 2.82 x 10^{-15}	Value of P < 0.05	

Table 37, Shows-

This indicates that there is a statistically significant association between patient classification and the differential leucocyte counts (Neutrophilia, Lymphocytosis and Eosinophilia)

Table 38: Differential Leucocyte Count (DLC) Distribution in GTB Positive and Negative Cases-

DLC	WNL	Neutrophilia	Lymphocytosis	Eosinophilia	Total
GTB (+)	01	06	02	00	09
GTB (-)	203	76	51	03	333
Total	204	82	53	03	342

Chi-Square (X^2) = 11.03	degree of freedom (df) =3	Value of P is smaller than 0.05, So null hypothesis (H_0) is rejected. Statistically significant.
P ≈ 0.0116	Value of P < 0.05	

Table 38 Shows-

- There is statistically significant association between GTB status and the distribution of DLC types.
- Among GTB positive patients (n=9):
  6 had Neutrophilia, 2 had Lymphocytosis, 1 was WNL,0 had Eosinophilia
- Among GTB negative patients (n=333):
  203 were WNL,76 had Neutrophilia,51 had Lymphocytosis,3 had Eosinophilia
- This suggests that the DLC distribution differs significantly between GTB (+) and GTB (−ve) individuals.

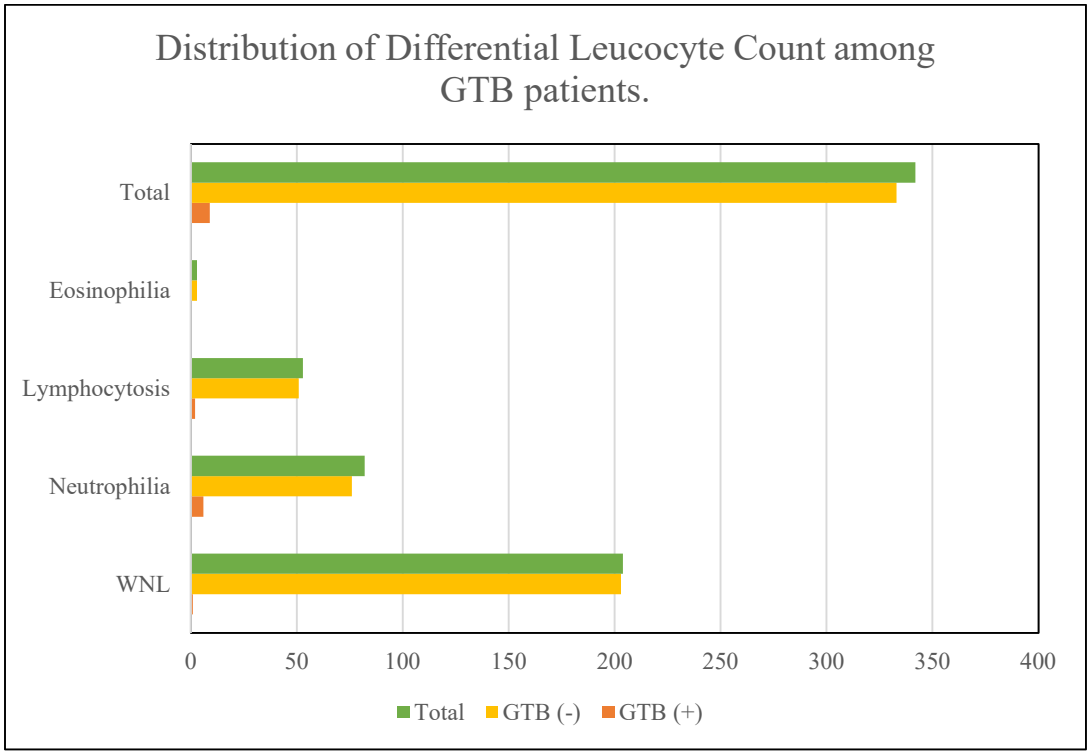


Figure 46-Graph of differential leucocyte counts (DLC) categories between GTB positive and negative individuals.

- Neutrophilia and Lymphocytosis are more prevalent in GTB positive cases compared to study population.

- WNL (normal counts) is predominant in GTB negative individuals.
- Eosinophilia is rare in both groups

Table 39: **Culture Result of *Mycobacterium tuberculosis* obtained in L.J. medium in study population-**

L.J Medium culture	Number	%
Positive	03	15 %
Negative	17	85 %
Total	20	100

Table-40 Distribution of Genital tuberculosis positivity among *Mycobacterium tuberculosis* culture results obtained in L.J. medium

L.J Medium culture	Positive	Negative	Total
GTB Positive	03 (33.3%)	06 (66.6%)	09
GTB Negative	00	17	17
Total	03	23	26

Chi-Square (X^2) = 6.41	degree of freedom (df) =1	Value of P smaller than 0.05, So null hypothesis (H_0) is rejected. Statistically significant. There is statistically significant association between genital tuberculosis and Culture results.
P ≈ 0.011		

Table 41- Comparison of Hemoglobin levels with L.J. medium culture results-

	L.J Media Culture Positive	**L.J Media Culture Negative**	**Total**
Hemoglobin Low	03	06	09
Hemoglobin WNL	0	11	11
Total	03	17	20

Chi-Square (X^2) = 3.32	degree of freedom (df) =1	Value of P smaller than 0.05, So null hypothesis (H_0) is rejected. Statistically significant. There is statistically significant association between Hb level and Culture results.
P ≈ 0.038		

Table 42- Comparison of Total Leucocyte Count with L.J. medium culture results-

Total Leucocyte Count (TLC)	**L.J Media Culture Positive**	**L.J Media Culture Negative**	**Total**
High	03	02	05
WNL	0	15	15
Total	03	17	20

Chi-Square (X^2) = 7.06	degree of freedom (df) =1	Value of P smaller than 0.05, So null hypothesis (H_0) is rejected. Statistically highly significant. There is statistically highly significant association between TLC and Culture results.
P ≈ 0.008		

Table 43- Comparison of Neutrophil % with L.J. medium culture results-

Neutrophil %	**L.J Media Culture Positive**	**L.J Media Culture Negative**	**Total**
High	03	02	05
WNL	0	15	15
Total	03	17	20

Chi-Square (X^2) = 7.06	degree of freedom (df) =1	Value of P smaller than 0.05, So null hypothesis (H_0) is rejected. Statistically highly significant. There is statistically highly significant association between Neutrophil % and Culture results.
P ≈ 0.008		

Table 44- Comparison of Lymphocyte % with L.J. medium culture results-

Lymphocyte %	**L.J Media Culture Positive**	**L.J Media Culture Negative**	**Total**
High	03	01	04
WNL	0	16	16
Total	03	17	20

Chi-Square (X^2) = 9.41	degree of freedom (df) =1	Value of P much smaller than 0.05, So null hypothesis (H_0) is rejected. Statistically highly significant. There is statistically significant relation between Lymphocyte % and Culture results.
P ≈ 0.002		

Table 45- Comparison of ESR level with L.J. medium culture results-

ESR	**L.J Media Culture Positive**	**L.J Media Culture Negative**	**Total**
High	03	07	10
WNL	0	10	10
Total	03	17	20

Chi-Square (X^2) = 3.53	degree of freedom (df) =1	Value of P greater than 0.05, So null hypothesis (H_0) is accepted. statistically no significant. There is statistically no significant relation between ESR and Culture results.
P ≈ 0.060		

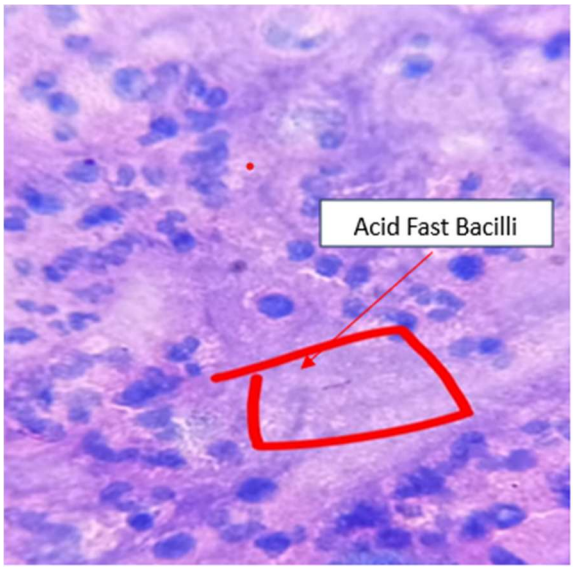


Figure 47: *Mycobacterium tuberculosis* bacilli seen in ZN Stain.

A microscopic analysis of the sputum smear stained with Ziehl-Neelsen stain as shown in Figure shows a large number of inflammatory and epithelial cells, primarily macrophages and lymphocytes, scattered across a proteinaceous background. A few thin, rod-shaped bacilli found in marked area which confirms the presence of *Mycobacterium tuberculosis*. Clusters of epithelioid cells and sporadic lymphocytes were two more signs of chronic granulomatous inflammation in the cellular background. The diagnosis of tuberculosis was confirmed by presence the of acid fast bacilli in this smear.

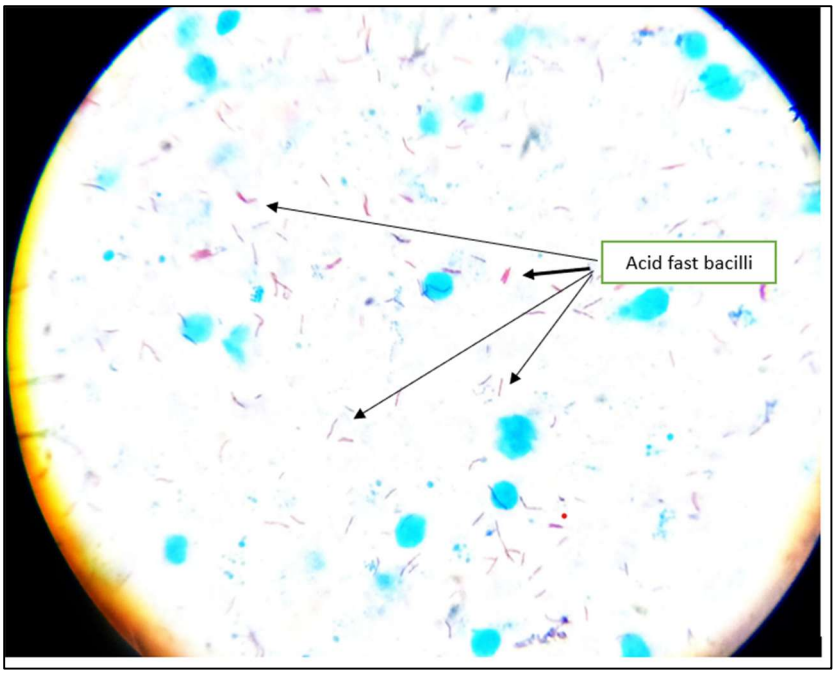


Figure 48: Mycobacterium tuberculosis bacilli seen in ZN Stain (+++).

Ziehl-Neelsen staining provided additional confirmation, revealing acid-fast bacilli (AFB) as thin, bright red rods set against a blue backdrop of inflammatory and epithelial cells (Figure 48). *Mycobacterium tuberculosis* infection was confirmed by the presence of these acid-fast organisms and confirmed the diagnosis of tuberculosis.

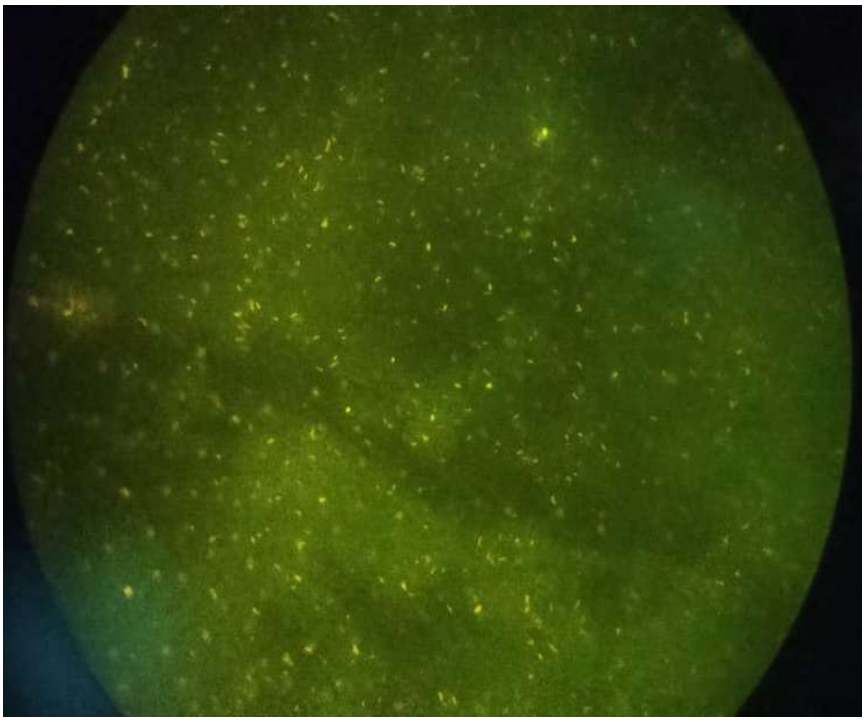
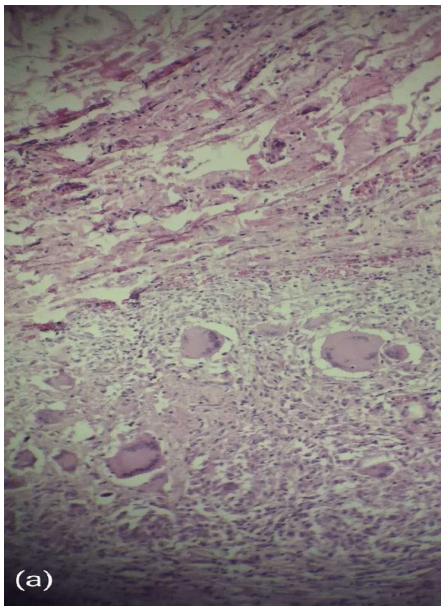


Figure 49- AFB image under fluorescent microscope

The presence of numerous bright yellowish-green, rod-shaped fluorescent bacilli against a dark background was revealed by fluorescent microscopy of the smear stained with auramine–rhodamine fluorescent dye (Figure 49). Acid-fast Mycobacterium tuberculosis is characterized by these fluorescent bacilli. Comparing the Auramine–Rhodamine stain to traditional Ziehl–Neelsen staining, the sensitivity for identifying tubercle bacilli was increased. The presence of an active tuberculous infection in the examined specimen is confirmed by the strong fluorescence seen, which suggests a high bacterial load.



50 (a) and (b) Figures display biopsy specimens illustrating granuloma formation characterized by Langhans giant cells and regions of necrosis, findings that align with tuberculosis.

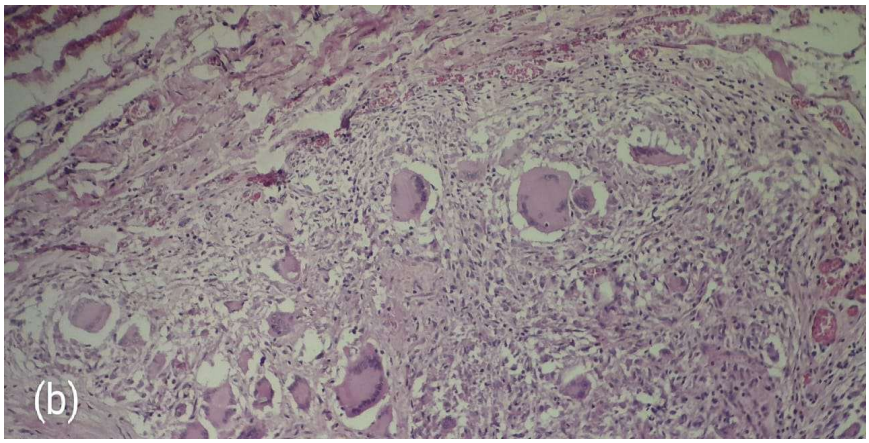
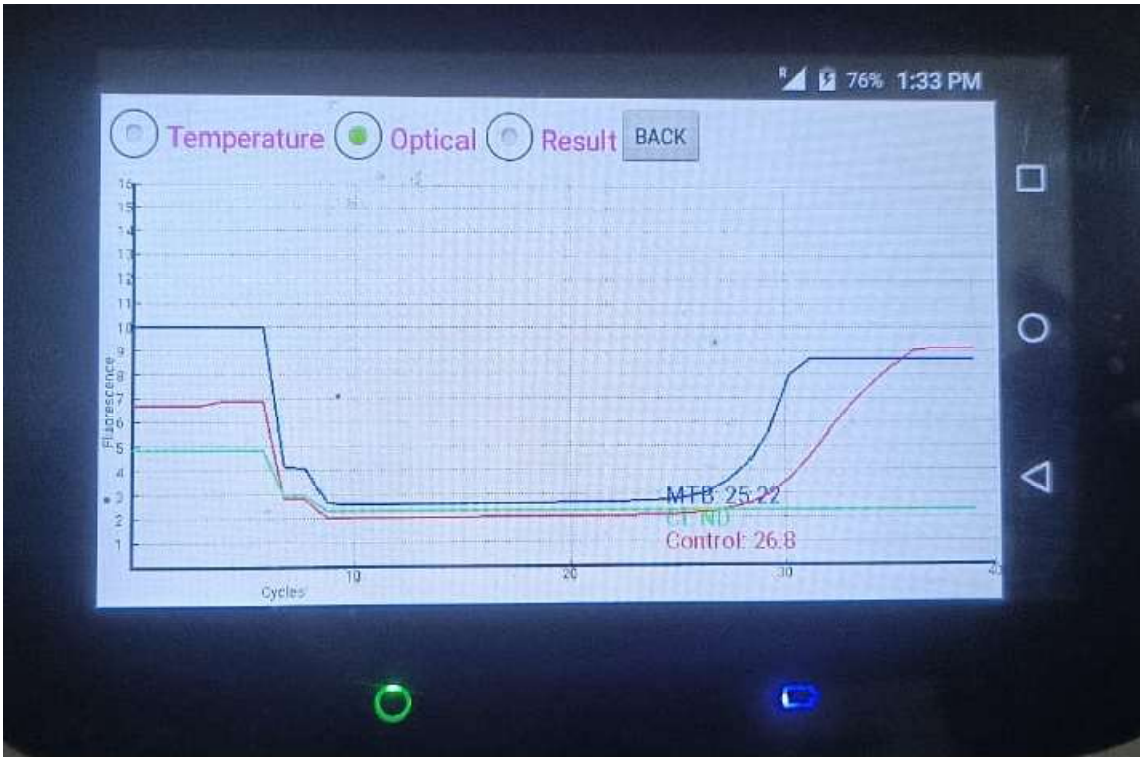


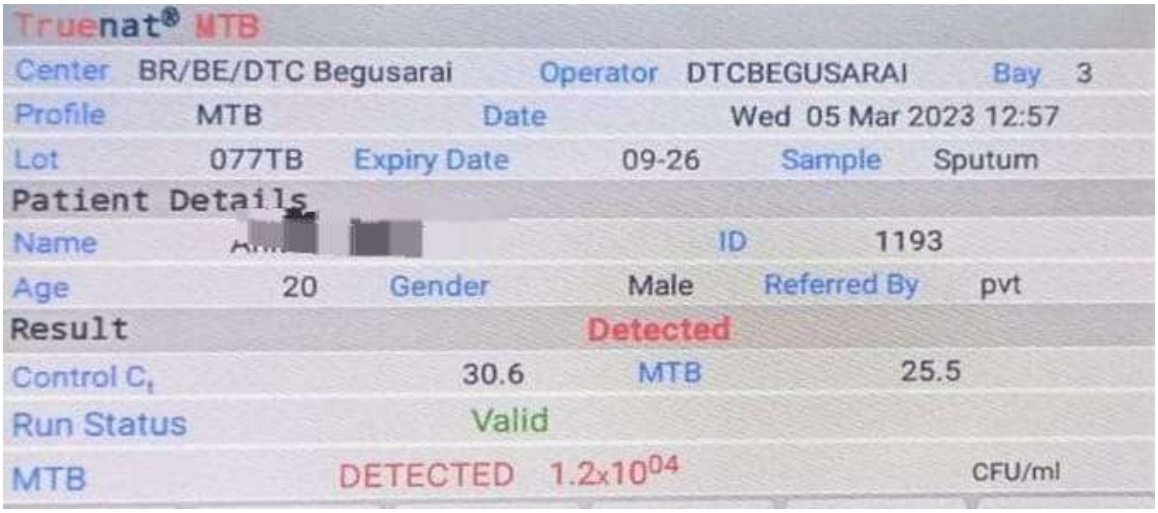
Figure-50 (a) & (b) Biopsy specimen slides showing granuloma, Langhans giant cells and necrosis suggestive of tuberculosis


Truenat® MTB
Center BR/BE/DTC Begusarai
Operator DTCBEGUSARAI
Bay 1
Profile MTB
Date Tue 25 Mar 2022 12:18
Lot 077TB
Expiry Date 09-26
Sample Sputum
Patient Details
Name
ID 1567
Age 45
Gender Female
Referred By PVT
Result Not Detected
Control C_t 30.4
MTB ND
Run Status Valid
MTB NOT DETECTED

51 (a)


76% 1:33 PM
Temperature
Optical
Result
BACK
Fluorescence
Cycles
MTB 25.22
Control 26.8

51 (b)



51 (c)

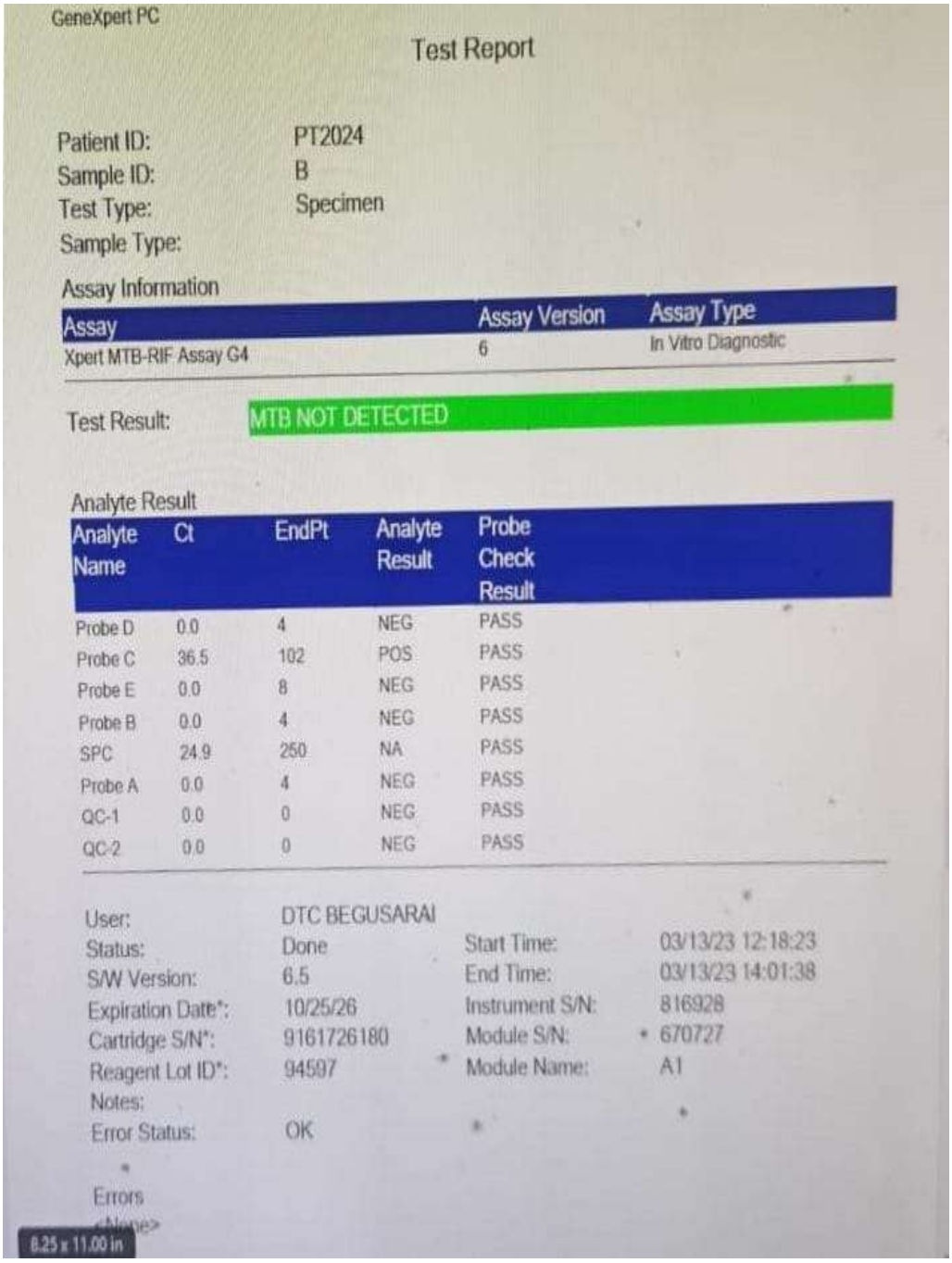
Figure 51 (a) MTB PCR negative, (b) and (c)- positive result.

Sputum samples collected from suspected cases were assayed using the Truenat MTB PCR assay for molecular detection of MTB. The test, therefore, was rapid and automated with high specificity. Figure 51(a) shows a "Not Detected" result of the Truenat MTB test conducted for one of the patients, aged 45 years, indicating that M. tuberculosis DNA was not detected in the specimen. Figure 51(b) shows a positive graph and 51 (c) presents a "Detected" result in another patient, aged 20 years, and the quantification value is 1.2×10^4 CFU/mL, indicating the presence of M. tuberculosis DNA in the sample.

All runs were valid because the internal controls were within the acceptable Ct value range, thus proving that the results were reliable. These findings highlight the diagnostic accuracy of the Truenat MTB test in distinguishing between MTB-positive and MTB-negative patients. Also, Both Rifampicin-sensitive and Rifampicin-resistant cases were detected by the Truenat MTB RIF assay.

Figure 52- (a) & (b) Rifampicin resistance not detected and detected.

A sample with confirmed Rifampicin resistance is shown in Figure 52(b), while a sample with Rifampicin resistance not detected is shown in Figure 52(a).



GeneXpert PC

Test Report

Patient ID: PT2024
Sample ID: B
Test Type: Specimen
Sample Type:

Assay Information

Assay	Assay Version	Assay Type
Xpert MTB-RIF Assay G4	6	In Vitro Diagnostic

Test Result: MTB NOT DETECTED

Analyte Result

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
Probe D	0.0	4	NEG	PASS
Probe C	36.5	102	POS	PASS
Probe E	0.0	8	NEG	PASS
Probe B	0.0	4	NEG	PASS
SPC	24.9	250	NA	PASS
Probe A	0.0	4	NEG	PASS
QC-1	0.0	0	NEG	PASS
QC-2	0.0	0	NEG	PASS

User: DTC BEGUSARAI
Status: Done
S/W Version: 6.5
Expiration Date*: 10/25/26
Cartridge S/N*: 9161726180
Reagent Lot ID*: 94597
Notes:
Error Status: OK

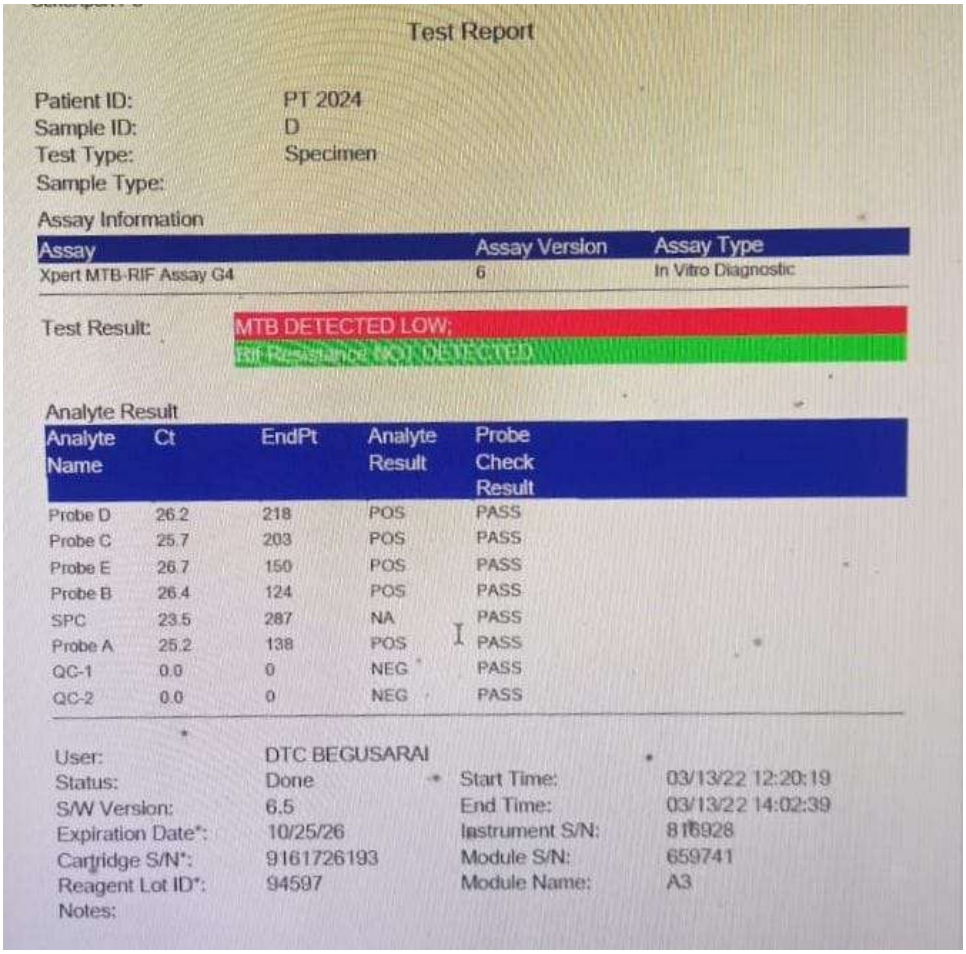
Start Time: 03/13/23 12:18:23
End Time: 03/13/23 14:01:38
Instrument S/N: 816928
Module S/N: 670727
Module Name: A1

Errors
<None>

8.25 x 11.00 in

Figure 53-showing MTB negative in Gene-Xpert

Below picture 54 and 55 shows *Mycobacterium tuberculosis* (MTB) detected in specimens with a low bacterial load by the GeneXpert MTB/RIF assay. Figure 54 shows showed MTB detection without drug resistance, whereas figure 55 shows MTB detected with Rifampicin-resistant.



Test Report

Patient ID: PT 2024
Sample ID: D
Test Type: Specimen
Sample Type:

Assay Information

Assay	Assay Version	Assay Type
Xpert MTB-RIF Assay G4	6	In Vitro Diagnostic

Test Result: MTB DETECTED LOW;
Rif Resistance NOT DETECTED

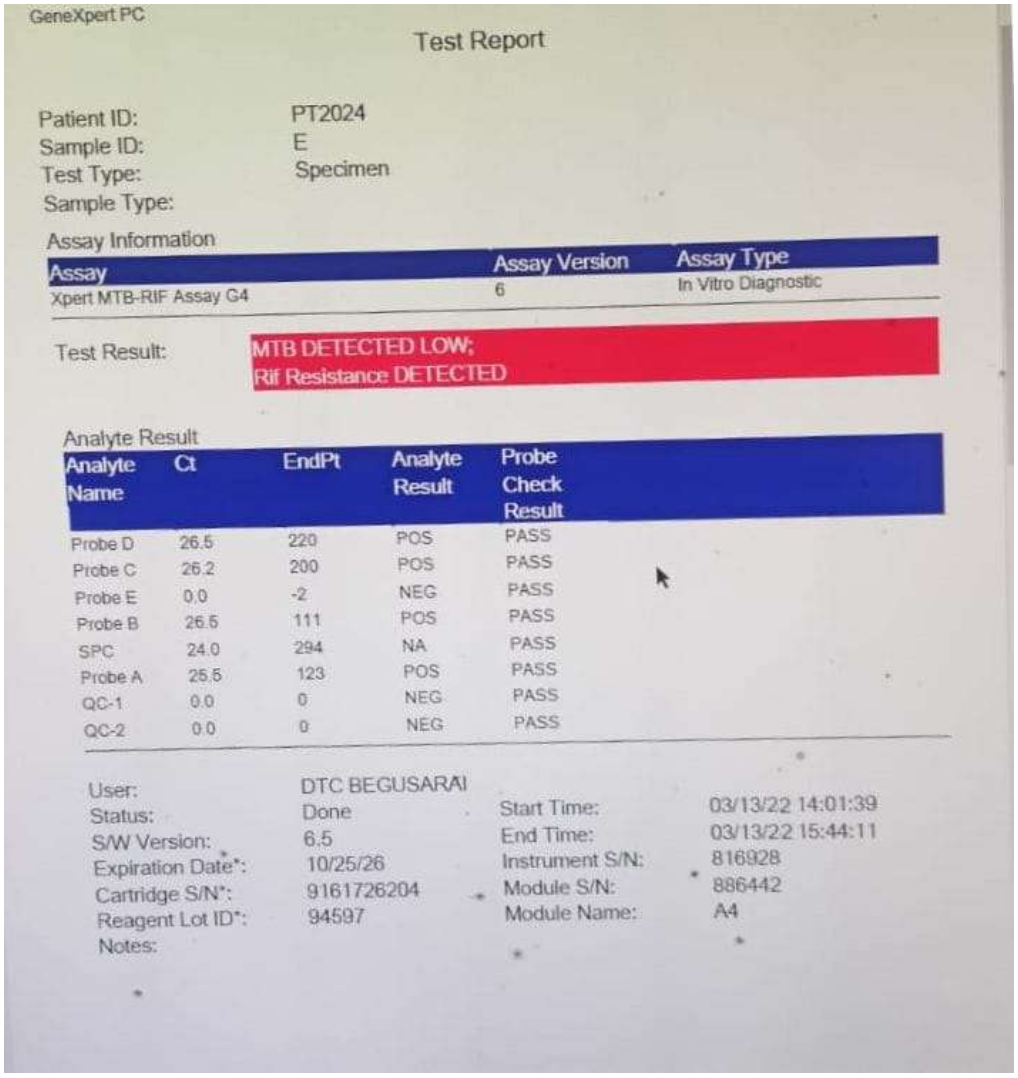
Analyte Result

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
Probe D	26.2	218	POS	PASS
Probe C	25.7	203	POS	PASS
Probe E	26.7	150	POS	PASS
Probe B	26.4	124	POS	PASS
SPC	23.5	287	NA	PASS
Probe A	25.2	138	POS	PASS
QC-1	0.0	0	NEG	PASS
QC-2	0.0	0	NEG	PASS

User: DTC BEGUSARAI
Status: Done
S/W Version: 6.5
Expiration Date*: 10/25/26
Cartridge S/N*: 9161726193
Reagent Lot ID*: 94597
Notes:

Start Time: 03/13/22 12:20:19
End Time: 03/13/22 14:02:39
Instrument S/N: 816928
Module S/N: 659741
Module Name: A3

Figure 54-MTB positive and rifampicin resistance not detected in Gene-Xpert



GeneXpert PC

Test Report

Patient ID: PT2024
Sample ID: E
Test Type: Specimen
Sample Type:

Assay Information

Assay	Assay Version	Assay Type
Xpert MTB-RIF Assay G4	6	In Vitro Diagnostic

Test Result: MTB DETECTED LOW;
Rif Resistance DETECTED

Analyte Result

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
Probe D	26.5	220	POS	PASS
Probe C	26.2	200	POS	PASS
Probe E	0.0	-2	NEG	PASS
Probe B	26.5	111	POS	PASS
SPC	24.0	294	NA	PASS
Probe A	25.5	123	POS	PASS
QC-1	0.0	0	NEG	PASS
QC-2	0.0	0	NEG	PASS

User: DTC BEGUSARAI
Status: Done
S/W Version: 6.5
Expiration Date*: 10/25/26
Cartridge S/N*: 9161726204
Reagent Lot ID*: 94597
Notes:

Start Time: 03/13/22 14:01:39
End Time: 03/13/22 15:44:11
Instrument S/N: 816928
Module S/N: 886442
Module Name: A4

Figure 55- MTB positive and rifampicin resistance detected in Gene-Xpert

Ziehl–Neelsen staining of the lymph node aspirate demonstrated in the figure 56 shows the presence of acid-fast bacilli (AFB), confirming tuberculous infection

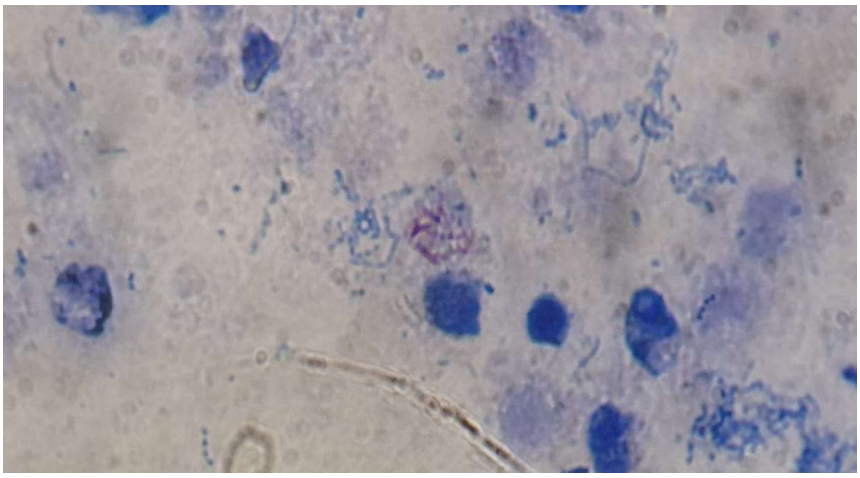
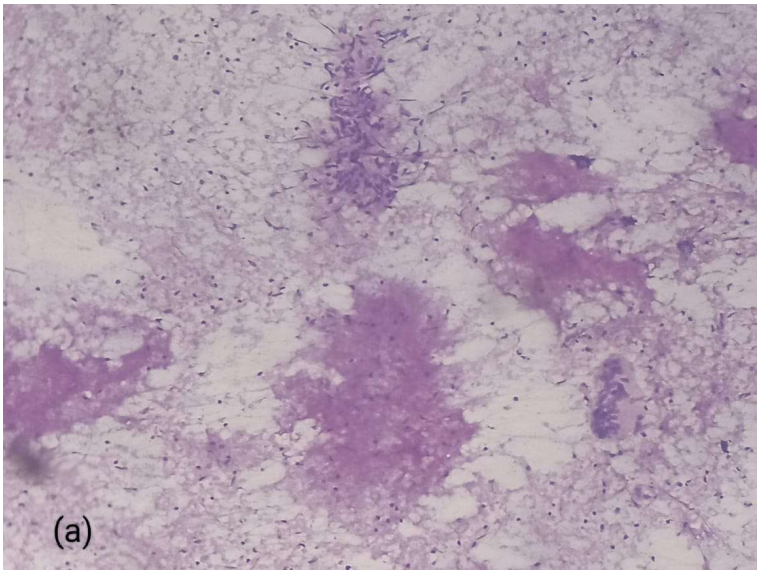


Figure 56- ZN stain in lymph node aspirate showing AFB

Below Fig 57 (a) and (b) shows Fine needle aspiration cytology (FNAC) of the lymph node indicated the presence of caseous necrosis, epithelioid cell granulomas, and several foreign body–type giant cells, indicative of granulomatous inflammation consistent with a tuberculous etiology.



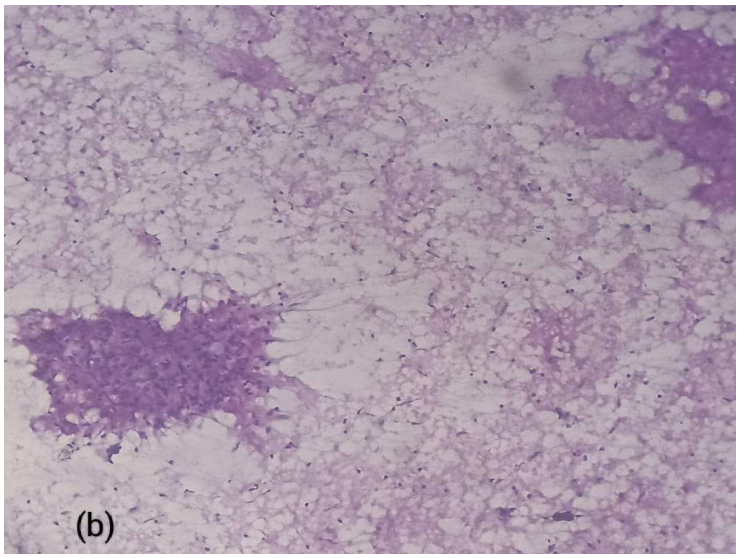


Figure-57 (a) & (b) FNAC lymph node aspirate showing caseous necrosis, epithelioid granulomas, caseous necrosis and few foreign body giant cells.

Colony growth of MTB in L.J medium.

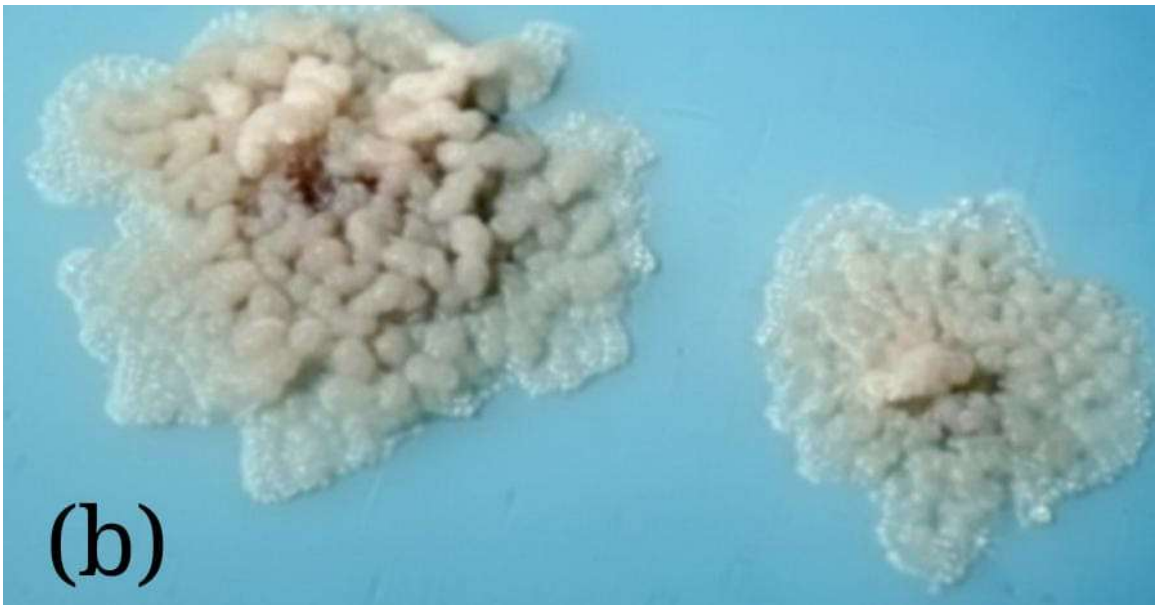


Figure 58- a and b - Mycobacterium tuberculosis colonies as seen in L.J. medium.

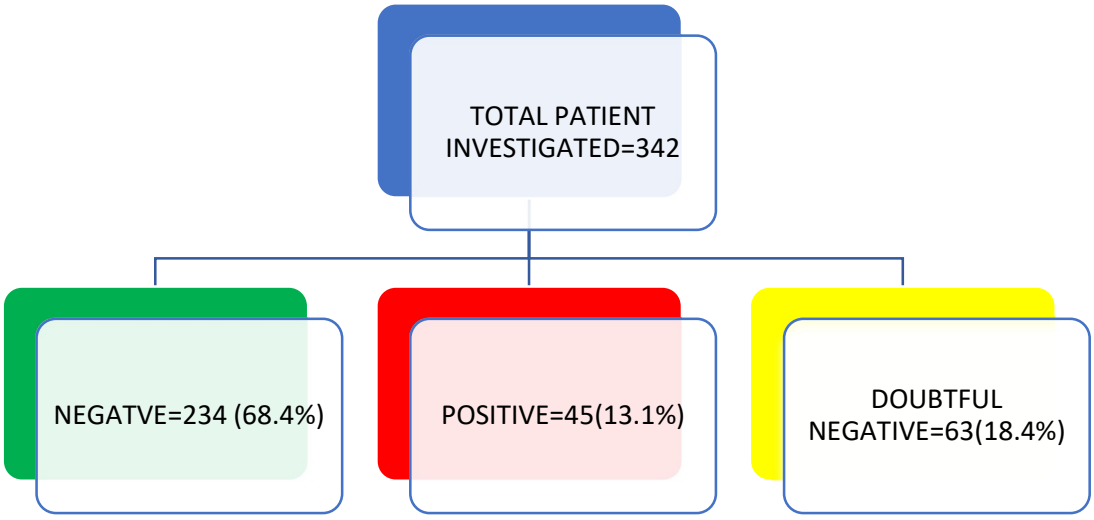


Figure 59: Classification of study population based on pathological findings.

- 45 patients are positive out of 342 = **13.1%**
- 20 males out of 342 are Positive = **5.8%**
- 25 females out of 342 are Positive = **7.2%**
- 20 males out of 207 males are Positive = **9.6%**
- 25 females out of 135 females are Positive = **18.5%**
- Asymptomatic confirmed positive case 11 out of 252 **=4.36%**
- **Total confirmed case of genital tuberculosis = 09 (2.6 %)**
- Male genital tuberculosis = 02 = **0.58%**
- Female genital tuberculosis = 07 = **2.04 %**
- 2 out of 207 males are suffering with genital tuberculosis = **0.96 %**
- 7 out of 135 females are suffering with genital tuberculosis = **5.18%**
- **TOTAL INFERTILE PATIENT=12 (3.5 %)**
- **MALE=02 (0.58 %)**

- **FEMALE=10 (2.92%)**
- **Infertility due to tuberculosis in Male=0**
- **Infertility due to tuberculosis in Female=4 (1.16%)**

Doubtful Negative Cases (63): -

Pathological and other investigations were indicating that these 63 patients may had tuberculosis infection. Most of all have family history of tuberculosis and all were MT positive.

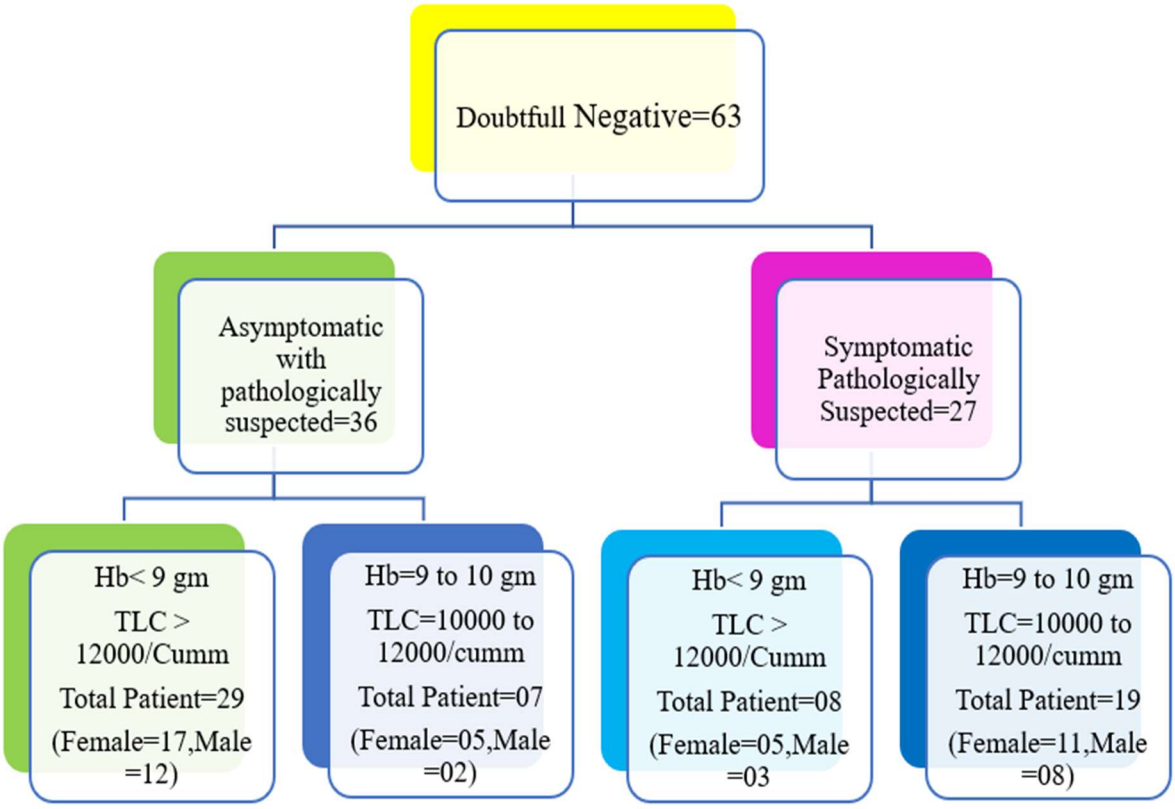


Figure 60: Distribution of doubtful negative cases based on symptoms, hemoglobin levels and total leucocyte count

Asymptomatic with pathologically suspected –

29 patients (male=12 and female=17) from asymptomatic with pathologically suspected group had family history and were MT POSITIVE, hemoglobin less than 9 gm total leucocyte count was also increased more than 12,000 per mm^3.

7 patients Hb was between 9 to 10 gm and total leucocyte count was increase between 10,000 to 12,000 per mm^3. Out of 7 there were two males and 5 females.

Symptomatic pathologically suspected-

08 patients (male=3, female=5) having Hb < 9 gm and total leucocyte count > 12000 per mm^3 whereas 19 patients (male=8 and female=11) having Hb between 9 to 10 gm and total leucocyte count between 10,000-12,000 per mm^3.

But AFB, X ray, TB gold, Gene xpert were negative. All investigations had done again after 4 months but all were again negative. Confirmatory tests were negative but clinical history and pathological findings were suggestive of tuberculosis.

7. DISCUSSSION-

In this study researcher observed that, tuberculosis of any form should be ruled out in shortest duration of time to escape from different types of complication like infertility, PID, tissue damage etc. It has also observed that contact history, low socioeconomic status, infertility, and past history are major risk factors. At present there is not a single test which can diagnose tuberculosis in its initial stage. Even sometimes it was found that when all clinical symptoms were suggestive of tuberculosis in other hand all investigations were negative.

The current paper established a prevalence rate of 2.6% genital tuberculosis (GTB) in 342 patients who were tested due to family history and symptoms like coughing, fever, weight loss, gynecological related complaints etc. in the Northern part of Bihar. The observation is in line with reports from other Indian states, which show that microbiologically confirmed genital TB is between 2–6% in general gynecological populations. The

prevalence observed describes a small yet clinically relevant burden, and it is still possible to claim that GTB is an underappreciated cause of female infertility in this area. Similar tertiary-based hospital studies have reported comparable prevalence rates.

Recent microbiological studies accomplished in Faridabad, Haryana, they also found the prevalence of 2.73% using CBNAAT and culture of suspected female genital TB cases. A study in Central India (2020) noted a prevalence of 1.25%, in Ajmer, Rajasthan, suggested a prevalence rate of 5.5% using a combination of PCR and histopathological tests. The prevalence found in the present study corresponds to the lower-moderate section of this national range. This similarity confirms that the disease burden in Northern Bihar mirrors that of other northern Indian states, where infection remains silent and affects women in their reproductive age group.

The prevalence is considerably lower compared with cohorts consisting solely of infertile women. noted that 48.5% of women presenting to an IVF center in North India were positive for GTB, and recorded GTB as a significant cause of infertility in Delhi, with rates between 20–30%. Similarly, a meta-analysis by affirmed a pooled prevalence of 34.86% in infertile women in India. The increased rates in these cohorts could be due to referral bias and inclusion of patients with long-standing or treatment-resistant infertility. Conversely, the current study involved a more heterogeneous population, which included both infertility and non-infertility gynecologic presentations, resulting in a lower overall prevalence.

The diagnosis in this study was primarily based on AFB, TB GOLD, CBNAAT, FNAC, Culture and histopathology, which are highly specific but may fail to detect early or latent infections. Therefore, the true prevalence in Northern Bihar might be slightly higher if more sensitive molecular or laparoscopic diagnostics were used. Sociodemographic factors and healthcare accessibility also contribute to the relatively low observed prevalence.

Northern Bihar, being a resource-deficient region, likely has a high prevalence of latent TB infection, but limited laboratory facilities reduce the number of clinically confirmed genital TB cases. The absence of specialized gynecologic TB diagnostic departments and under-

reporting further contribute to underestimation of the actual disease burden. Nevertheless, the health impact is significant. Even at a prevalence of 2.6%, the potential reproductive morbidity is considerable because genital tuberculosis often causes irreversible tubal and endometrial damage, leading to secondary infertility.

This study also highlights the importance of routine GTB screening in women presenting with infertility, menstrual irregularities, or persistent pelvic pain in TB-endemic areas such as Bihar.

Considering national trends and current findings, GTB remains an overlooked but significant cause of infertility in India. The 2.6% prevalence in Northern Bihar represents a substantial burden in a population. Strengthening regional laboratory networks, increasing clinician awareness, and promoting early referral for diagnostic evaluation can reduce infertility as well as Genital and other form of tuberculosis.

Our work is not only to find out genital tuberculosis infected people based on their pathological and radiological investigations but also work to aware people about genital and other tuberculosis infection and treatment by camping and organizing programs by the help of RNTCP and IIH (Innovators in health) on what to do and not do. Because there are a number of problems which we found in our study. Female members ignored to go for treatment. They send working male person for treatment and waiting for their recovery and when ATT has completed then they send other person for treatment. We found during this study that a number of non-medico persons prescribed medicine to the economically weak people. It was found that Rifampicin is widely used without any pathological or radiological findings leads to MDR TB. In our study a number of female subjects were found under age (below 18 years) but had 2 to 3 children. About all have menstruation related problem. We found 3 cases of death at the time of delivery. Some have miscarriage history and their children are physically very weak and underweight. Population density is very high in the area of study. Most of the houses were made up of soil and there was only one door and one small window in each room, not enough for proper ventilation. Rooms

were dark. In one room 5 to 10 people were living. Due to this type of living conditions tuberculous infection spread rapidly.

A major challenge faced during the study was resistance from certain community members toward counselling and testing. Despite multiple efforts, some individuals refused participation, stating, “Please leave us in our situation. We can handle it; you don’t need to worry about us.” This reflects a deep-rooted mistrust and lack of awareness, which hinder early diagnosis and treatment. The experience highlights the need for culturally sensitive health education and stronger community engagement in future interventions. Most of people were not aware about personal hygiene. Females were at high risk about all were using cloth instead of sanitary pads, leads to major health issues. Smoking is very dangerous and it becomes more dangerous in case of “beedi”. In villages beedi is easily available at very low cost. Also manufactured by local worker at door steps. The percentage of women in local worker were very high because they work at very low labor cost. During working about all were habituate to smoke beedi. A number of women more than 20 years to older age were smoking beedi at their home in front of kids and other family members and also sharing among themselves. Now due to several government and NGO initiated programme every corner of Bihar has well equipped health care facilities. Where all basic investigations and treatment are available free of cost. But availability of gynecologist was limited. Female patients had to go higher center for their gynecological problems. Sometimes they had to go to private clinic for treatment. This was also a major reason for ignorance and acceptance of the disease. During patient counselling it was observed that some families reported child deaths during or soon after birth. This fact has no direct links to the aims of the current study on tuberculosis, but it was considered important to mention here to display the overall public health and socio-economic status among the study population. This information could be used in future to reveal the cause and gain an insight into public health, maternal and child health and possible risk factors. Our team is not sure that it is due to tuberculosis but there is a big probability that it should be due to tuberculosis. Supporting investigations were missing due to ignorance by patients as well as by consultants. Some patients had 2 to 3 child death incidents.

8. CONCLUSION AND FUTURE PERSPECTIVES-

In this present study scholar found out the prevalence, risk factors and associated clinical features of genital tuberculosis (GTB) in the population of Northern part of Bihar. The findings provide valuable insights into the demographic and pathological distribution of GTB and highlights several major gaps in diagnosis and awareness that need to be taken seriously. Out of 342 study participants, 9 individuals (2.6%) were confirmed positive for genital tuberculosis. Among these, 7 were females (5.18%) and 2 were males (0.96%). A notable concentration of GTB-positive cases (77.8%) was observed in individuals below the age of 30 years, with the age group of 21–30 years showing the highest susceptibility. This reflects the vulnerability of the reproductive-age population, especially women, to genital tuberculosis. The study also found a statistically significant association between GTB and past contact history of tuberculosis, with 66% of GTB-positive cases reporting have such history. This highlights the importance of history-taking during clinical assessment.

An important observation from this research was that all GTB-positive individuals were symptomatic, presenting clinical features such as menstrual disturbances, infertility, pelvic pain, or abnormal vaginal discharge. In comparison, only 27.02% of GTB-negative individuals were symptomatic, showing a statistically significant difference. Moreover, a higher proportion of GTB-positive patients exhibited high erythrocyte sedimentation rate (ESR), with 66.6% having above-normal values, compared to 33.03% in GTB-negative individuals. Similarly, abnormalities in differential leucocyte counts (DLC)—particularly neutrophilia and lymphocytosis—were more prevalent in GTB-positive cases, further supporting the use of hematological parameters as an important indicator for GTB.

This study also linked between genital tuberculosis and infertility. 12 (3.5 %) cases of infertility as found in this study population, 4 female cases (1.16%) were directly linked to GTB. This finding reveals the silent and damaging role of this undiagnosed GTB in reproductive health, particularly among women from economically backgrounds.

A striking part of this study is the identification of 63 “doubtful negative” cases having strong pathological and clinical evidences of tuberculosis but negative results in confirmatory tests such as AFB staining, X-ray, TB Gold, PCR, Culture and GeneXpert. These findings show a significant diagnostic limitation, suggesting that current diagnostic tools are not sufficient alone to rule out GTB, specially in early and latent stages. It is a crucial need to adopt a more comprehensive diagnostic protocol that incorporates clinical signs, pathological findings, family history, and socioeconomic aspects.

This study not only highlights the burden of genital tuberculosis in Northern Bihar but also exposes the multifaceted challenges in its detection and management. Poor living conditions, illiteracy, lack of awareness, and unregulated medical practices contribute significantly to the persistence and spread of the disease. To effectively diagnose GTB, a multidisciplinary approach is necessary, involving early detection strategies, public awareness campaigns, improved diagnostic methods, and accessible reproductive health services. The results of this study can use as a basis for future research and policy development aimed at reducing the incidence of GTB and its associated complications like infertility particularly in high-risk populations**.**

Overall study tells that there are many hidden cases which are responsible for tuberculosis spread. Some hidden cases were due to public ignorance and also due to absence of accurate diagnostic tools. Even confirm positive patients are not aware how to protect their love ones. We also found in some cases, person had coughing from last 6 months or more and live with all family members, who get found 3 (+) positives in AFB smear. We also found most of the confirmed cases people were hand to mouth. They haven’t food to eat, how can we expect for healthy food. Health care facilities are available all corners of our state with all essential diagnostic facilities and treatment. But even after availability of these facility TB cases are gradually increases. Now government supply healthy food items by own or through NGO. But this is not enough to fulfill their basic needs. We also find that the infected person from economically lower class have comparatively long success treatment period and successful treatment ratio is very low in comparison to economically higher class.

Future perspectives-

The findings of this study have highlighted several critical aspects of genital tuberculosis (GTB) in the Northern Bihar population, but they also open up important ways for future research, clinical improvement, and public health strategies. Despite advances in diagnostic technologies, there remains a significant diagnostic gap, particularly in asymptomatic or clinically suspected cases where confirmatory tests fail to detect *Mycobacterium tuberculosis*. Future research should focus on the development of more sensitive and specific diagnostic tools that can accurately identify GTB at the earliest stage, especially in latent or extra pulmonary cases.

For public health concern this study put a important role of socioeconomic factors and healthcare facilities. Future efforts must include community-based awareness and education campaigns, particularly in rural areas where early marriage, poor hygiene, overcrowding, and unregulated medical practices contribute to TB spread and delayed diagnosis and increase MDR TB. Training based programmes that trained local healthcare workers to recognize early GTB symptoms and to refer patients for proper investigation can improve the early detection and spreading of the disease.

Lastly, future policy and programmatic interventions should consider integrating GTB screening with existing maternal and child health services, ensuring that women are not only treated for TB but also supported in restoring reproductive health. Research evaluating the impact of integrated reproductive health and TB care models could inform national strategies to curb the spread of GTB and improve quality of life outcomes.

In summary, this study lays the groundwork for future work aimed at refining diagnostics, methods enhancing awareness, improving patient management, and ultimately reducing the burden of genital tuberculosis in under-resourced communities.

9. APPLICABILITY OF THE DISSERTATION WORK-

Our work helps to know more about the current prevalence of tuberculosis and root cause of its spreading. Now we can make strategies which can help to prevent the disease. Also,

in our study we use different tools and correlate them with clinical symptoms. It enhances our knowledge about advantage and limitations of the investigations. What we are lacking in our techniques? Now we can establish the relationship between infertility and tuberculosis. Our work tells the importance of clinical history in diagnosis of tuberculosis. Our study details how socio economic back ground people are very difficult to cure in comparison of economically higher classes. Our study also tells the impact of economic status on patient treatment.

In our study we found that early signs and symptoms are usually ignored by people because these symptoms are not atypical. Also, even when symptoms are stronger suggestive of the disease they ignore to go for treatment.

Longer the time of living with infected person have higher chance of getting infected as well as higher the chance of infertility and it is much higher in case of female. Because her contact time is much higher than male because males stay at home for a short time period as compare to female. Female have to stay in home for house hold activity.

10. RECOMMENDATIONS-

In our study symptoms and results of all types of investigations are summarized in one place. After analyzing all results and symptoms we found that history taking and routine investigations are very important tools to diagnose the infected person. About all available diagnostic tools have their own limitations and usually fails to detect disease in initial stages. So that further study should be carried out to develop technology which can ruled out the disease in its initial stages with accuracy even from asymptomatic infected person. During counselling it was found that in most of the cases female members were ignoring for treatment. So, awareness campaign at block level should be done to aware all people especially female.

11. ACKNOWLEDGEMENTS-

Centre for Research Degree Programmes Lovely Professional University Phagwara Punjab

Ganga Diagnostic Centre, district tuberculosis center Begusarai and Khagaria for technical support.

12. CONFLICT OF INTEREST - No conflict of interest

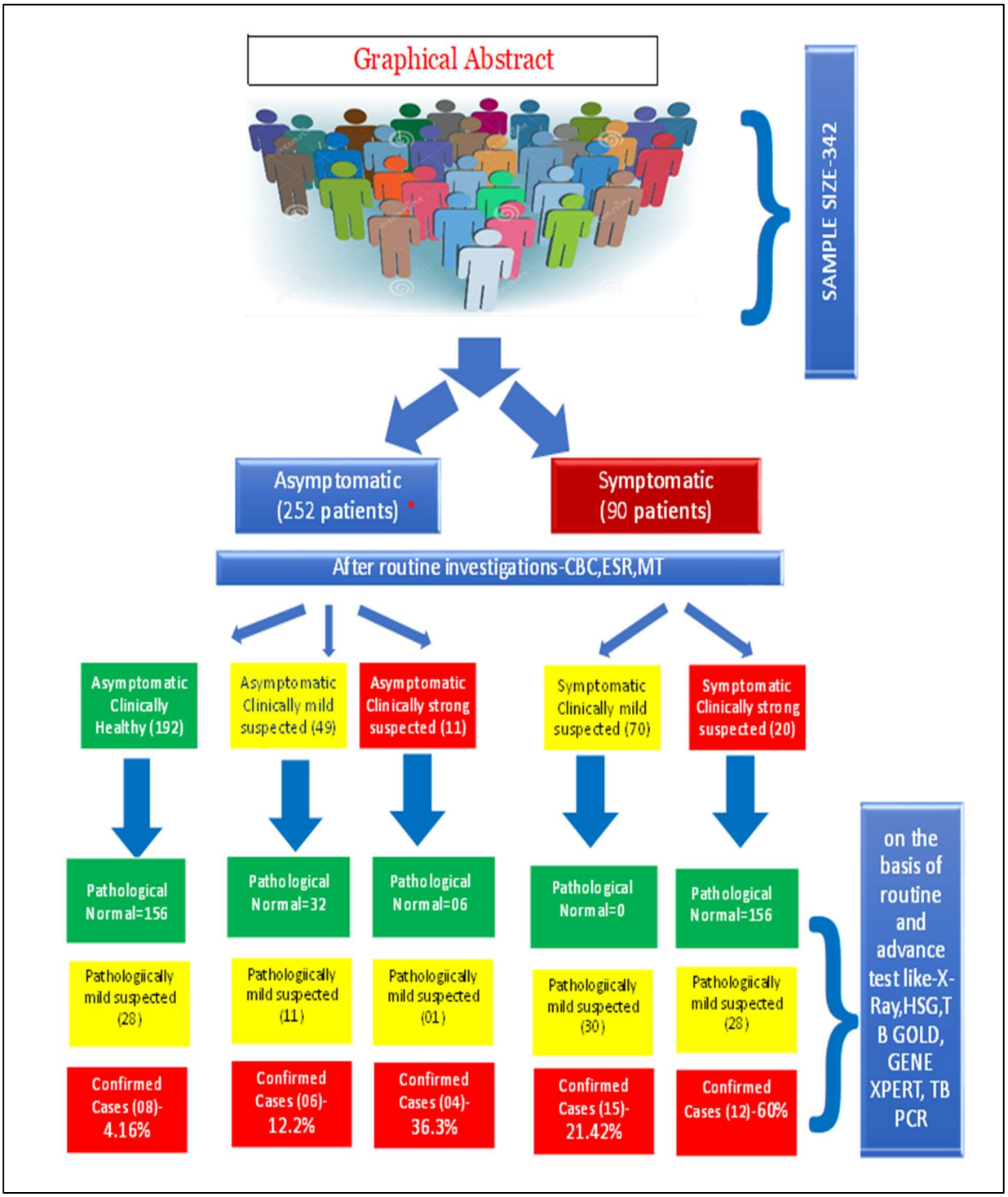
13. DETAIL OF RESEARCH PUBLICATION-

(a) Journal of Applied Bioanalysis, June. 2025, p.785-789. http://doi.org/10.53555/jab.v11i3.294 (ISSN2405710X) Vol.11, No.3 (Tuberculosis in Northern Bihar: Epidemiological and Clinical Aspects.)

(b) South Eastern European Journal of Public Health,24th may 2024, Vol-XXIII S3 2024. https://doi.org/10.70135/seejph.vi.1592 (Tuberculosis its Prevalence, Complications and Clinical Aspects among the Population of Northern Part of Bihar)

(c) International Journal of Environmental Sciences, ISSN: 2229-7359,Vol. 11 No 11s,2025. Artificial Neural Networks In Early Diagnosis Of Neurological Disorders: A Review Of Models, Biomarkers, And Clinical Integration

14. Graphical Abstract



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16. ANNEXURES-

Detail of Research publications-

JOURNAL OF APPLIED BIOANALYSIS, June. 2025, p. 785-789.
http://doi.org/10.53555/jab.v11i3.294 (ISSN 2405-710X)
Vol. 11, No. 3

OPEN ACCESS

Tuberculosis in Northern Bihar: Epidemiological and Clinical Aspects

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Abstract:

Background: Tuberculosis (TB) has been a worrying issue to the population health especially in the under-resourced areas like Northern Bihar in India. The disease remains a heavy burden on national health despite many attempts being made because the disease is only diagnosed late and there is non-compliance as well as socio-economic constraints.

Purpose: This research paper attempts to examine the existing prevalence, clinical presentation, and complications of tuberculosis in the region of Northern Bihar and specifically the social and environmental factors related to it.

Research Design: A cross-sectional study was carried out in a series of several different public health facilities in Northern Bihar in a period of 24 months. The data was gathered on patients by use of structured interviews, clinical assessment, and laboratory tests. The data was analyzed by using descriptive and inferential statistics.

Results: A total of 524 patients were confirmed of TB out of which 63.7 percent were male and most of them (71.8 percent) were at the lower socio-economic levels. The majority of Pulmonary TB was found (76.3%), but the extra-pulmonary manifestations were also lymphadenitis, pleural effusion, and the involvement of skeletal areas. The disease severity and complications were strongly related to malnutrition, HIV-co-infection, and poor sanitation.

Conclusion: Tuberculosis in North Bihar indicates the complicated interaction of clinico-social and water-to-land-to population aspects. A combined method is necessary in addressing them, which includes early detection, support via socio-economic barriers and effective community-based health measures. The results of this paper may be used in the intentions of policy-making on the local level and will help to implement the national plan of TB elimination by 2025.

Keywords: Tuberculosis, Northern Bihar, Pulmonary TB, Socioeconomic conditions, TB outcomes, Epidemiology, HIV co-infection, Public health response

1. Introduction:

Tuberculosis (TB) remains one of the most striking national health concerns, not only in low- and middle-income economies. In spite of that, TB is a treatable and preventable disease that causes the highest rates of morbidity and mortality in the world, with 10.6 million incidences and 1.6 million deaths being reported in 2022 alone [1] ,[2]. The greatest TB burden is place in India, with almost 28% of all cases worldwide (WHO, 2023).

The highly populated and socioeconomically deprived northeastern state of Bihar experiences the disease by increasing the transmission risk (Singh et al., 2020; Kumar & Das, 2021) [3].

Recently, India has witnessed a series of efforts by the Government where the Revised National Tuberculosis Control Program (RNTCP [12]) and the National Tuberculosis Elimination Program (NTEP), designed to eliminate TB by 2025, are introduced. Nonetheless, it is not clear whether such programs can succeed in rural and deprived areas like the Northern Bihar areas because of structural and behavioral factors.

In this study, which attempts to compile the

Tuberculosis its Prevalence, Complications and Clinical Aspects among the Population of Northern Part of Bihar

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KEYWORDS
Genital Organs, Tuberculosis, Infertility, AFB, Genital Tuberculosis

ABSTRACT
Introduction: It is commonly known as death causing disease of reproductive age group people. It is one of the top five death causing disease. It effects upon every age group people. But dangerous impact of tuberculosis occurs on immunocompetent people. There are a number of complications occur only due to one disease that is tuberculosis. it effects almost all organs and impaired their vital functions. Even if we leave death, even after tuberculosis proved to be most dangerous disease. In this study our main aim to explore the aspects, complications and prevalence of genital tuberculosis. In this study we also explore the ancient technics as well as the modern technics and their methods to ruled out the disease and improvement of treatment from ancient time to modern time. Method: This study was carried out between January 22 to April 23. 342 subjects are selected by inclusion and exclusion method. Subjects are classified in to 3 groups according to their physical appearance clinical symptoms and family history. Further patients are again classified in to negative, positive and doubtful negative patients after conducting routine and special investigations. Results: After all pathological investigation we found that 45 (13.1%) subjects have found positive in which 5.8% were male and 7.2 % were female. Conclusion: Tuberculosis is present in different forms and should be ruled out in early stage to safe from tissue damages. But unfortunately, we haven't any tools which can detect the disease in its early stage accurately. All the investigation has their own limitations and contamination errors. There are a number of patients in which some can easily identified by their clinical symptoms and related routine examination, some are asymptomatic and identified by advanced diagnostic tools where are some such patient are also found who were strong symptomatic but even not only routine but also advance investigations are fail to detect. In our study we found the root cause of the disease, which are high population density, economically lower standard, poor hygiene life style, compromise food habits, illiteracy, marriage in early stage, multiple marriage, multiple children, non- skilled medical practices are causing and enhancing the incident of tuberculosis.

1. Introduction

Even after availability of modern and advance diagnostic tools tuberculosis is still a major health related problem and rapidly growing disease. Tuberculosis is most dangerous disease of the world. This single disease

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 11s, 2025
https://theaspd.com/index.php

Artificial Neural Networks In Early Diagnosis Of Neurological Disorders: A Review Of Models, Biomarkers, And Clinical Integration

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Abstract

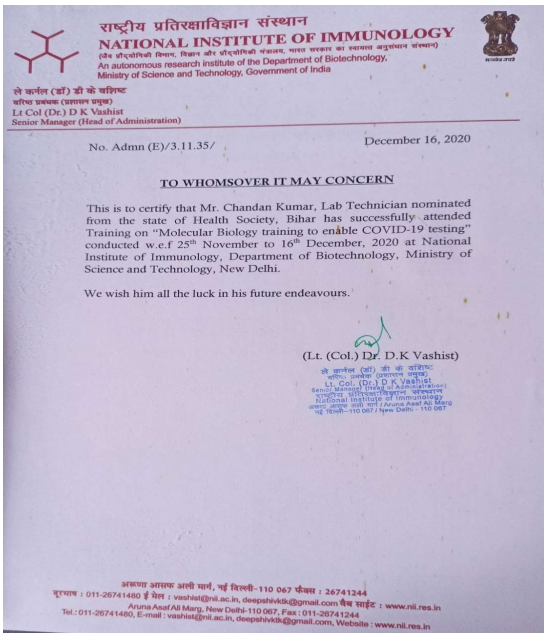
Neurological disorders, ranging from Alzheimer's disease and Parkinson's disease to multiple sclerosis and epilepsy, pose significant global health challenges due to their progressive nature and delayed diagnosis. Early detection is critical for effective intervention and management. Traditional diagnostic techniques, although advanced, often lack the sensitivity and scalability required for early-stage recognition. In recent years, Artificial Neural Networks (ANNs), a branch of artificial intelligence inspired by the human brain, have shown immense promise in enhancing the early diagnosis of these disorders. This review synthesizes current advancements in ANN-based models for early detection of neurological disorders and explores their integration with clinical data and neurobiomarkers such as EEG signals, MRI scans, and genetic data. We first discuss the foundational architecture and learning mechanisms of ANNs that make them suitable for handling complex biomedical data. Following this, the paper examines various ANN architectures–including feedforward networks, convolutional neural networks (CNNs), and recurrent neural networks (RNNs)–and their applications in diagnosing disorders such as Alzheimer's, Parkinson's, epilepsy, and autism spectrum disorders. The paper also highlights the emerging role of hybrid models combining ANN with fuzzy logic, support vector machines, and ensemble learning to improve diagnostic accuracy. A significant focus is placed on biomarkers, including neuroimaging, cerebrospinal fluid analysis, and electrophysiological indicators, and how ANN models are trained to identify diagnostic patterns within them. We explore real-world clinical trials, datasets, and ANN-integrated diagnostic systems currently in use or under development. The challenges of interpretability, data heterogeneity, ethical considerations, and real-time clinical integration are critically assessed. Finally, the review presents future directions emphasizing the need for explainable AI, longitudinal data utilization, and patient-specific modeling. By integrating deep learning with clinical neuroscience, ANN-based systems can revolutionize the landscape of neurological diagnosis. This review underscores their transformative potential while acknowledging the hurdles that must be overcome to achieve seamless clinical adoption.

Conference Attend during the study-

1. **AIIMS NEW DELHI ON 17th July 2022 as a Lecturer.**

2. **AIIMS NEW DELHI ON 21ST AND 22ND OCTOBER 2024.**

Training Attend During the study-



राष्ट्रीय प्रतिरक्षाविज्ञान संस्थान
NATIONAL INSTITUTE OF IMMUNOLOGY
An autonomous research institute of the Department of Biotechnology, Ministry of Science and Technology, Government of India

ले कर्नल (डॉ) डी के वशिष्ट
वरिष्ठ प्रबंधक (प्रशासन मुख्यालय)
Lt Col (Dr.) D K Vashist
Senior Manager (Head of Administration)

No. Admn (E)/3.11.35/ December 16, 2020

TO WHOMSOVER IT MAY CONCERN

This is to certify that Mr. Chandan Kumar, Lab Technician nominated from the state of Health Society, Bihar has successfully attended Training on "Molecular Biology training to enable COVID-19 testing" conducted w.e.f 25th November to 16th December, 2020 at National Institute of Immunology, Department of Biotechnology, Ministry of Science and Technology, New Delhi.

We wish him all the luck in his future endeavours.

(Lt. (Col.) Dr. D.K Vashist)

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GOVERNMENT OF INDIA
MINISTRY OF HEALTH & FAMILY WELFARE
DIRECTOR GENERAL OF HEALTH SERVICES
REGIONAL OFFICE FOR HEALTH & FAMILY WELFARE
PATNA, BIHAR

Certified that *Mr. Chandan Kumar, Lab Technician*

of *Sadar Hospital, Khagaria , Bihar.*

has successfully completed the Training Cum Workshop for Lab. Technician's on

LF Microscopy for Night Blood Survey (NBS)

on 08th December, 2023 at Regional Office for Health and Family Welfare, Govt. of India, Patna.

Date of Issue: 08th December, 2023

Kailash Kumar

Dr. Kailash Kumar
Senior Regional Director
RoH&FW, Patna

जीवन बिहार...
सपना हो साकार

Department of Health Government of Bihar
STATE INSTITUTE OF HEALTH & FAMILY WELFARE

Certificate of Participation

This Certificate is awarded to.....CHANDAN KUMAR.....
From district.....KHAGARIA.....for participation in Residential Training from..28.01.2025..to..29.01.2025.on "Diagnosis and Management of Gestational Diabetes Mellitus" organised at State Institute of Health & Family Welfare, Bihar.

State Program Officer
Maternal and Child Health
State Health Society, Bihar

Director
State Institute of Health & Family Welfare, Bihar

STATE HEALTH SOCIETY, BIHAR

बिहार सरकार

Government of Bihar
Chief Malaria Office (VBDCP)
Swasthya Bhawan, Sultanganj, Patna

CERTIFICATE

This is to certify that Mr./Ms.....Candan Kumar.....Laboratory Technician, Health Dept. (GoB) attended successfully "Two days Reorientation Training on PKDL" held at Rajendra Memorial Research Institute of Medical Sciences, Agamkuan, Patna from 12th Feb. to 13th Feb. 2018.

Dr. P. Das
Director
RMRIMS, Patna

Dr. M.P. Sharma
Additional Director Cum SPO
VBDCP, Bihar, Patna

Ethical Approved Certificate

Independent Research Ethics Society
Regn No. : S/2L 25358 of 2014-15 under W. B. Society Act 1961

Independent Ethics Committee (Clinical Research) India

Regd. Office: 119 Rajdanga Gold Park
Near Tribarna Sangha, RB connector
Kolkata-700 107
Phone: 24414948/7044089242
Mail: ireskol107@gmail.com

PROJECT APPROVAL LETTER

Ethics Committee	**Independent Ethics Committee (Clinical Research) India**		
Address	**119, Rajdanga Gold Park, RB Connector, Kolkata – 700107**		
Date of Approval	**08.04.2023**	**Approval No.**	**IECCRI/23-24/02**

Protocol Title	**Prevalence of genital tuberculosis, its risk factors and associated clinical features among the population of northern part of Bihar**
Principal Investigator	**Mr. Chandan Kumar**

The Independent Ethics Committee has reviewed the following document submitted for the above – mentioned Experimental and Analytical Study

Name of documents reviewed	RV	AP
1. Permission from Health Dept.	✓	✓
2. Research Proposal	✓	✓
3. CV and certificates	✓	✓

RV denotes Reviewed; AP denotes Approved

MEMBERS REVIEWED THE PROJECT

Name of member	Position in ethics committee	Qualification
Dr. S. K. Bandyopadhyay	Chairperson	MD (Pharmacology)
Dr. Pawan Kumar Sharma	Member Secretary	MD (Ayu), MBA
Ms. Moumita Sharma	Member	MBA

The approval is valid from **08.04.2023 till 07.04.2024** and the renewal of this study is subject to review of "Preliminary Study Report" submitted to this Ethics Committee by the Investigator.

The research proponents are here by informed that the Independent Ethics Committee (IEC) will require the following:

- The progress report to be submitted to the IEC at least after 1 month.
- Upon completion of the study, a final study status report needs to be submitted to the IEC.

Independent Research Ethics Society
Regn No. : S/2L 25358 of 2014-15 under W. B. Society Act 1961

Independent Ethics Committee (Clinical Research) India

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RECOMMENDATIONS AND INSTRUCTIONS:

- The approval is given on the basis of requirements for fulfillment of Ph.D Thesis (Clinical Microbiology, Reg.No. 41800702) under Lovely Professional University, Punjab.
- Since, the work will be done on blood and tissue samples available from different hospitals. NECESSARY DOCUMENTS MUST BE PROVIDED regarding obtaining of samples along with the FINAL STUDY REPORT submitted to IEC(CR)India.
- WRITTEN INFORMED CONSENT from the PATIENTS and PERMISSION from the INSTITUTION (local Hospital) at which the STUDY RELATED PROCEDURE and COLLECTION OF SAMPLES FROM PARTICIPANTS will be done, the DOCUMENTS MUST BE SUBMITTED to IEC(CR)I.

DECISION

IEC(CR)I

Opinion of the Independent Ethics Committee for Research

Approval: **APPROVED with Recommendations and Instructions**

Date of approval: **08.04.2023**

Signature:

Dr. Pawan Kumar Sharma
Member Secretary
IEC(CR)I