A STUDY ON THE CORRELATION BETWEEN SPUTUM SMEAR STATUS AND CD4 COUNT IN CASES OF PULMONARY TUBERCULOSIS WITH HIV COINFECTION

DISSERTATION SUBMITTED TO
THE TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY
IN PARTIAL FULFILLMENT OF THE REGULATIONS FOR THE AWARD OF THE DEGREE OF

M.D. (GENERAL MEDICINE) BRANCH –I

TIRUNELVELI GOVT.MEDICAL COLLEGE & HOSPITAL
TIRUNELVELI

THE TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY
CHENNAI,TAMIL NADU

APRIL 2014

CERTIFICATE

This is to certify that the Dissertation entitled "**A STUDY ON THE CORRELATION BETWEEN SPUTUM SMEAR STATUS AND CD4 COUNT IN CASES OF PULMONARY TUBERCULOSIS WITH HIV COINFECTION**" is the bonafide original work of **Dr.SHARMILA B** in partial fulfilment of the requirements for MD(General Medicine) Branch-I Examination of the Tamil Nadu Dr.M.G.R. Medical University to be held in APRIL 2014.

Prof.Dr.S.S.Nazar M.D.,
VI Medical Unit
Department of Internal Medicine,
Tirunelveli Medical College,
Tirunelveli.

Prof.R.Geetha Rani M.D.,
HOD of Medicine
Department of Medicine,
Tirunelveli Medical College,
Tirunelveli.

.

DR.S.SOUNDARARAJAN M.S.
The Dean
Tirunelveli Medical College,
Tirunelveli - 627011.

TIRUNELVELI MEDICAL COLLEGE

TIRUNELVELI,

STATE OF TAMILNADU, INDIA

PIN CODE:627011

Tel: 91-462-2572733, 2572734 Fax: 91-462-2572944

Estd:1965

Under the Directorate of Medical Education, Government of Tamilnadu.

Institutional Ethical Committee

Certificate of Approval

This is to certify that the Institutional Ethical Committee of this College unanimously approves the Thesis /Dissertation/ Research Proposal submitted before this committee by Dr.SHARMILA B, **A POST GRADUATE IN MD MEDICINE,** Department of **MEDICINE** of Tirunelveli Medical College /Hospital, Tirunelveli titled **"A STUDY ON THE CORRELATION BETWEEN SPUTUM SMEAR STATUS & CD4 COUNT CASES OF PULMONARY TUBERCULOSIS IN HIV PATIENTS"** registered by the IEC as 196/G.M./IEC/2012 dated. 11.07.2012. The Investigator is hereby advised to adhere to all the stipulated norms and conditions of this ethical committee.

Issued on this Date

11.07.2012

Under Seal

TIRUNELVELI MEDICAL COLLEGE ★ TAMILNADU INDIA ★ TIRUNELVELI-11

Secretary

Secretary,
Ethical Committee,
Tirunelveli Medical College.
Tirunelveli-11.

Turnitin Document Viewer - Torch

https://www.turnitin.com/dv?o=379518939&u=1024312108&s=&student_user=1&lang=en_us

The Tamil Nadu Dr. M.G.R. Medic... | Medical - DUE 31-Dec-2013 | What's New

Originality | GradeMark | PeerMark

NEW DISSERTATION

BY 20111199 . M.D. GENERAL MEDICINE SHARMILA B . BRABHAKARAN

turnitin 11% SIMILAR -- OUT OF 0

A STUDY ON THE CORRELATION BETWEEN SPUTUM SMEAR STATUS AND CD4 COUNT IN CASES OF PULMONARY TUBERCULOSIS WITH HIV COINFECTION

DISSERTATION SUBMITTED TO
THE TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY
IN PARTIAL FULFILLMENT OF THE REGULATIONS FOR THE
AWARD OF THE DEGREE OF

M.D. (GENERAL MEDICINE)BRANCH –I
TIRUNELVELI GOVT.MEDICAL COLLEGE & HOSPITAL
TIRUNELVELI

Match Overview

1	www.ncbi.nlm.nih.gov Internet source	4%
2	Padyana, Mahesha; B... Publication	<1%
3	hivinsite.ucsf.edu Internet source	<1%
4	Submitted to iGroup Student paper	<1%
5	www.euro.who.int Internet source	<1%
6	infectioncontrolsociety.... Internet source	<1%
7	tvmc.ac.in Internet source	<1%
8	www.urbanurchins.co.uk Internet source	<1%

PAGE: 1 OF 110

Text-Only Report

Turnitin -... | Turnitin ... | Skype™ - ... | MBlaze A... | Microsoft... | DISSERTA... | COVER P... | Documen...

19:40
20-12-2013

DECLARATION

I solemnly declare that the dissertation titled "**A STUDY ON THE CORRELATION BETWEEN SPUTUM SMEAR STATUS AND CD4 COUNT IN CASES OF PULMONARY TUBERCULOSIS WITH HIV COINFECTION**" was done by me at Tirunelveli Medical College hospital, Tirunelveli during 2012_2013 under the guidance and supervision of **PROF.DR.S.S.NAZAR M.D.,** Professor of Medicine.

This dissertation is submitted to The Tamilnadu Dr. M.G.R.Medical University towards the partial fulfillment of requirements for the award of M.D. Degree (Branch I) in General Medicine.

Place: Tirunelveli
Date:

Dr.SHARMILA B,
Postgraduate Student,
M.D General Medicine,
Department of Medicine,
Tirunelveli Medical College
Tirunelveli.

ACKNOWLEDGEMENT

I take immense pleasure to acknowledge all those who have helped me to make this dissertation possible.

I am grateful to the **Dean, Tirunelveli Medical College** and **Medical Superintendent of Tirunelveli Medical College Hospital** for permitting me to undertake this study and for allowing me to avail the facilities needed for my dissertation work

I express my profound sense of gratitude to **Dr.R.Geetharani M.D.,** my respected teacher, Professor and Head of Department of Medicine, Tirunelveli Medical College, Tirunelveli for approving this study and for her guidance in preparing this dissertation.

I am extremely grateful and indebted to my unit chief **Prof.DR.S.S.Nazar .M.D.,** for his guidance and constant encouragement throughout this study.

I am grateful to **Professor.Dr.Arumugapandian@Mohan M.D**., Nodal officer, ART Plus Centre,Tirunelveli Medical College Hospital for his guidance in doing this study.

I thank **Professor.Dr.K.Krishnamoorthy M.D.**, and **Assistant Professor Dr. G. Sangamithra, M.D.,** Department of Thoracic Medicine for their constant support throughout the study.

I am extremely grateful to my assistant professors **Dr. V. Rajesh Babu M.D., Dr. S. M. Shavana M.D.,** and **Dr. N. Govindarajan M.D.,** for their valuable guidance and constant encouragement.

Finally, I wish to thank all the patients without whom this study would not have been possible.

ABBREVIATION

TB _ Tuberculosis

HIV _ Human Immunodeficiency Virus

PLHA _ People Living With HIV-AIDS

AIDS _ Acquired Immunodeficiency Syndrome

PTB _ Pulmonary Tuberculosis

CXR _ Chest X Ray

CD4 _ Cluster of Differentiation 4

ART _ Anti Retroviral Therapy

ATT _ Anti Tubercular Drugs

RNTCP _ Revised National Tuberculosis Control Programme

NNRTIs _ Non Nucleoside Reverse Transcriptase Inhibitors

DOTS _ Directly Observed Treatment Shortcourse

NACO _ National AIDS Control Organisation

IRIS _ Immune Reconstitution Inflammatory Syndrome

WHO _ World Health Organisation

NRTIs _ Nucleoside Reverse Transcriptase Inhibitors

CMI _ Cell Mediated Immunity

ICTC _ Integrated Counselling and Testing Centre

PIs _ Protease Inhibitors

DMC _Designated Microscopy Centre

CONTENTS

ABSTRACT

TITLE : A study on the correlation between sputum smear status and CD4 count in cases of pulmonary tuberculosis with HIV coinfection

AUTHOR : SHARMILA B

BACKGROUND OF THE STUDY :

Around 2.5 million people are infected with HIV in India. Estimated 40% of the Indian population is infected with M.tuberculosis. Estimated 1 million persons are co-infected with M.tuberculosis and HIV. Risk of developing TB is higher in HIV infected persons. Life time risk of developing TB is 60% in persons infected with both HIV and TB. Surveys in India show 1 to 13% prevalence of HIV among TB patients. Hence the need of more studies in HIV TB coinfection is warranted.

WHO states that sputum positivity decreases as CD4 count decreases. But this expectation has not been substantiated in a few studies done in the past.

Hence this study was undertaken to find the correlation between sputum smear status and CD4 count in cases of pulmonary tuberculosis with HIV coinfection in Tirunelveli medical college hospital ,being a tertiary care referral center.

METHODOLOGY :

In our hospital based cross sectional study,50 patients with HIV infection who developed pulmonary tuberculosis were tested for sputum acid fast smear status and correlated with CD4 count and radiologic findings.

RESULTS :

Sputum smear negative cases were more prevalent in patients CD4 count less than 200 whereas sputum smear positive cases were common with CD4 count more than 200 with a statistically significant difference(p=0.0001) which endorses the fact by WHO that sputum smear negativity increases with increase in the degree of immunosuppression.

CONCLUSION :

Though sputum smear microscopy remains a gold standard method for diagnosing pulmonary tuberculosis in immune compromised host with CD4 count more than 200, there is an urgent need for better diagnostic tools in patients with CD4 count below 200.

KEYWORDS : HIV TB coinfection,WHO,CD4 count.

INTRODUCTION

Tuberculosis and HIV have been associated with each other ever since the emergence of AIDS. Because of the HIV pandemic, a resurgence in TB epidemic has been observed in India for the last few decades. Tuberculosis remains the most common opportunistic infection in HIV seropositive individuals of which the most common manifestation is pulmonary tuberculosis , irrespective of the degree of immunosuppression .

In India, both HIV and TB infection are prevalent maximally in the reproductive age group of 15-49 years ,thus the interface between HIV and TB is increased. The dual infection has been termed "cursed duet"[(1)]. HIV TB co-infection thus presents an urgent and serious public health and an alarming danger to the socioeconomy of our country. Shortening the time for pulmonary TB (PTB) diagnosis and treatment initiation is an important step in decreasing TB-associated mortality and transmission.

One of the major challenges in diagnosing PTB [(2)] is alteration of the presentation of PTB due to HIV infection. TB and HIV infections have an added negative influence on the host immunity. An important marker in assessing the degree of immunosuppression and identifying HIV disease progression is the CD 4 lymphocyte count .

The presentation of TB in individuals with HIV co infection with normal CD4 count is similar to that of HIV-negative patients. However, patients with depressed immunity (low CD4 count) have a pattern that is deviated from the normal, both in clinical features and investigation findings, thus making diagnosis difficult.

In countries with high prevalence of TB ,the most cost effective method of detecting PTB among suspects is sputum smear microscopy. WHO states that sputum positivity decreases as CD4 count decreases. But this finding has not been proven in a few studies done in the past. Hence this study has been done in our institution to better understand the relationship between CD4 count and the status of sputum smear.

AIM OF THE STUDY

1. To study the correlation between sputum smear status and CD4 count in cases of pulmonary tuberculosis with HIV coinfection.
2. To study the time interval between the diagnosis of HIV infection and the occurrence of clinical tuberculosis.

REVIEW OF LITERATURE

EPIDEMIOLOGY:

In 1993, TB was declared a global emergency. The WHO's Director General has recently declared AIDS also as a Global emergency .

World Health Organization (WHO) states that the global prevalence of *Mycobacterium tuberculosis* [3] infection is 33% whereas in India the infection rate is 40% . Among the TB patients of the world, 14.8% have HIV coinfection [4] whereas in India, 5% of the TB patients have HIV coinfection .

. The global prevalence of PLHA[5] is about 33 million of which 2.5 million have coinfection with tuberculosis. In India, the third highest HIV burdened country in the world, there are around 2.5 million HIV infected people of which 1 million have coinfection with tuberculosis[6].

In India, it is foreseen that 50 to 60% of the HIV-infected persons during their life span will develop TB disease[7] .

India remains home for a larger number of tuberculosis (TB) cases in the world accounting for 23% of the global incidence of TB cases i.e. 2 million of the 8.8 million incident cases in 2011 were from

India. Indian surveys show that the prevalence of tuberculosis in India is 14 million, with an annual incidence of 1.8 million cases , of which the number of infectious smear positive cases is 0.82 million.

Persons with *Mycobacterium tuberculosis* infection have about 10% risk of reactivation TB during the rest of their lives. Thus, their annual risk of developing active disease is less than 0.5% while it is 10% for individuals infected with both HIV and TB, imposing a life time risk of 50% to 60%.

Approximately 88% of HIV TB co-infection cases occur in the productive age group of 15 to 49 years. Therefore, this dual epidemic of TB and HIV poses a great hurdle to the development of our country.

IMPACT OF HIV INFECTION ON THE NATURAL HISTORY OF TUBERCULOSIS:

FROM EXPOSURE TO INFECTION:

Exogenous factors mainly determine the risk of acquiring infection with *M.tuberculosis*. *Mycobacterium tuberculosis* is transmitted by droplet infection from persons with infectious PTB, less than 5 microns in size which are aerosolised by coughing, sneezing or speaking. Most infectious patients are those with cavitatory disease and laryngeal TB where sputum contains around 10^5 to 10^7 bacilli.[8]

Cavitation is less likely to occur in persons with HIV TB co-infection. Hence they are be infectious when compared to persons without HIV coinfection.

But evidence is lacking whether HIV patients acquire TB infection more when compared to HIV-seronegative persons when given the same amount of exposure.[2]

FROM INFECTION TO DISEASE:

In immunocompetent persons, following primary infection with *M. tuberculosis*, the organisms are ingested by the macrophages and the mycobacterial antigens are then processed and presented by the macrophages to T lymphocytes. This results in the secretion of lymphokines by the CD4+ T lymphocytes, which increase the phagocytic and bactericidal capacity of macrophages . In majority of individuals , thus the infection is controlled and active disease does not manifest, though a few dormant bacteria may be present in the host[7] . Thus TB can develop through reactivation of latent infection, progression of recently acquired primary infection , or exogenous reinfection[2]. Endogenous factors, such as innate defense mechanisms and function of cell-mediated immunity (CMI) of the individual determine the risk of developing active disease[8].

The risk of acquiring TB following new infection is greatly enhanced by HIV infection. However, this aspect has not been studied in detail in India. *Primary tuberculosis*_tuberculosis disease developing immediately following infection is common among immunocompromised persons because of defective macrophage function to contain the infection.

HIV is one of the major risk factors for the activation of TB infection to clinical illness. HIV infection is characterised by a progressive decline in the number and function of CD4+ T lymphocytes, together with defects in the function of macrophages and monocytes. As the dominant role in anti-mycobacterial defences is played by CD4+ T lymphocytes and macrophages, dysfunction of these cells in HIV infection imposes a greater risk for primary TB as wells as reactivation TB [(7)]. The lifetime risk of developing tuberculosis following *M.tuberculosis infection* in immunocompetent individuals is 10% , with a larger proportion manifesting within 1-2 year of infection whereas in HIV-coinfected persons the risk of developing active disease is 20-30 times amplified compared to non infected persons , with an annual activation rate of 8-10% per year[(2,3)].

It has been thought that *M tuberculosis* infection in an immunocompetent person confers significant protection against exogenous reinfection. But, regardless of the HIV status of the individual reinfection can occur[(2)].

TB can occur at any stage of HIV disease. This risk of developing tuberculosis rises immediately following HIV infection. Though TB can develop during early stages of HIV infection, as the CD4 count falls , there is a higher risk of developing TB especially disseminated disease.

The rate of recurrent TB disease is increased by HIV infection , which could be because of endogenous reactivation (true relapse) or exogenous re-infection[7].

Thus HIV infection causes an increase in the incidence of active tuberculosis cases exposing the general community to a higher risk of TB .

IMPACT OF TUBERCULOSIS ON THE NATURAL HISTORY OF HIV INFECTION:

Coinfection with *M.tuberculosis* accelerates the rate of progression of HIV disease resulting in higher mortality, especially in individuals with who are not treated for HIV . This is the result of enhanced immune activation and an increase in the expression of CXCR4 and CCR5 coreceptors on CD4 cells[(2)].

The effect of TB on the levels of HIV RNA is doubtful. However , studies have showed increased levels of HIV RNA in the presence of HIV TB-coinfection , which results from multiplication of latent virus residing in macrophages and dysregulated cytokines[(2)].

Thus TB shortens the lifespan of HIV patients . This higher mortality is due to rapid progression of HIV to AIDS rather than TB itself.

CLINICAL PRESENTATION OF TUBERCULOSIS IN HIV INFECTION:

During the course of HIV infection, TB can occur at any time . The most common presentation of TB in HIV TB coinfection is . pulmonary TB .

There is possibility of TB being overlooked in HIV-infected patients because symptoms of fever, malaise and weight loss can be due to HIV infection itself .Patients usually have symptoms over several weeks to months. However, a sudden onset of cough with fever in these individuals suggests a nonmycobacterial pulmonary process.

The clinical presentation of TB is influenced by the severity of immuno-suppression in the patient. In early stages of HIV infection, TB usually manifests as post -primary pulmonary TB with upper lobe involvement, cavitatory lesions and positive sputum smear status. During late stages of HIV infection, PTB often presents as primary tuberculosis with , lower lobe involvement , infiltrative lesions, intra thoracic lymphadenopathy and negative sputum smear status.

The incidence of smear negative pulmonary TB has increased because of the TB- HIV co-epidemic.

HIV infection increases the prevalence of extrapulmonary TB and disseminated TB . As the CD4 cell count decreases, there is an increase in the occurence of extrapulmonary TB and atypical chest radiographic findings, due to defective immune response to contain infection.Manifestations of extrapulmonary TB depends on the organ affected , such as abdominal pain, , meningismus, headache, back pain, abscess formation, , pyuria , lymphadenopathy etc[(2)]

DIAGNOSIS:

Diagnosing TB in the presence of HIV-infection is often challenging.

There is a higher rate of sputum smear negative TB in HIV affected individuals to as high as 66%. Such smear-negative PTB cases may be culture-positive and is seen commonly with advanced immunosuppression.

Generally, the sputum smear positivity rate correlates with the pattern of radiographic changes. For example, individuals with cavitatory disease are more likely to be sputum smear positive , whereas in patients with minimal changes on chest radiograph a negative smear result is .more common . However, in the presence of HIV-infection, patients with little radiographic evidence may have positive sputum smear results.

Other difficulties in establishing the diagnosis of TB in the presence HIV infection are an increased occurrence of extrapulmonary TB and difficulty in differentiating TB from HIV associated neoplastic conditions and other HIV associated infections[(2)].

SYMPTOM SCREENING:

In a TB patient, the conditions that should arouse the suspicion of HIV infection are as follows[9]:

- Oral/ Oesphageal candidiasis
- Oral hairy leukoplakia
- Fever >1 month
- Chronic diarrhea >1 month
- Weight loss of > 10% in the past 6 months
- Recurrent pneumonia
- Herpes Zoster
- Typhoid
- Generalized dermatitis
- Kaposi's sarcoma
- Present or past genital ulceration

Clinical suspicion of HIV infection in TB patients can be confirmed by referring these patients to Integrated Counselling and Testing Centres(ICTC). .

In HIV positive patients , symptoms that should arouse the suspicion of TB are as follows :

- Cough of 3 weeks duration or more
- Fever of more than 2-3 weeks
- Haemoptysis
- Unexplained dyspnoea or chest pain
- Weight loss
- Lymph node enlargement
- Headache, vomiting, alterated sensorium or convulsions
- Fatigue, listlessness

HIV positive patients with clinical suspicion of TB should be referred to the Revised National Tuberculosis Control programme(RNTCP) health centres for establishing the diagnosis of TB.

INVESTIGATIONS:

AFB MICROSCOPY:

Microscopy is the easiest ,oldest ,fastest and least expensive procedure to detect the prescence of acid fast bacilli.

Minimum of 10^5 bacilli per milliliter of specimen must be present to detect tubercle bacilli in stained smears. In contrast, 10 to 100 bacilli are sufficient to give a positive culture. Thus acid fast smear is less sensitive than culture and hence cannot replace culture[(10)].

Smear examination is a rapid procedure making results ready within 24 hours of specimen collection .

However, only the presumptive diagnosis of TB disease can be made with smear microscopy because there are acid-fast bacilli other than *M. tuberculosis*.

Patients with an initial negative AFB smear may have a subsequent positive culture. Therefore TB disease cannot be excluded by a negative smear.

The 3 commonly used procedures for acid-fast staining[11] include:

1. Ziehl-Neelsen method or hot staining method using carbol fuschin dye under light microscope.

2. Kinyoun method or cold staining method using carbol fuschin under light microscope.

3. Truant method_ using the fluorescent dyes like auramine-O and auramine- rhodamine fluorochrome under fluorescent microscope.

Specimens for pulmonary tuberculosis[11]:

- Spontaneous sputum produced by coughing
- Sputum induced by 3 to 5% hypertonic saline
- Bronchoscopy aspirate
- Gastric aspirate

Specimens for extrapulmonary tuberculosis:

- Tissue biopsies
- Body fluids (pericardial,pleural, synovial, ascitic fluid, CSF, bone marrow, blood, pus).
- Urine.

It has been recommended that in persons with suspected pulmonary TB ,minimum of 2 sputum samples should be examined for AFB smear . There is limited increment in the yield by using a third sputum for AFB smear, as low as 2% [2,12,13].

Ziehl Neelsen staining:

- Step 1:slides are fixed by using heat flames.
- Step 2:The entire slide is flooded with carbol fuschin
- Step 3:The slides are heated slowly until they are steaming and the steaming is maintained for 5 minutes by low or intermittent heat
- Step 4: The slide is rinsed with water
- Step 5:The slide is flooded using 3% acid-alcohol or 20% sulphuric acid and then allowed to decolorise for 5 minutes by continued flooding of the slides with till the slides are free of any stain visible to naked eyes
- Step 6:The slides are rinsed thoroughly with water and any excess from the slides is drained.
- Step 7:The slide is flooded with counterstain ,methylene blue and the counterstain is kept on the slide for 1 minute
- Step 8:The slide is rinsed thoroughly with water

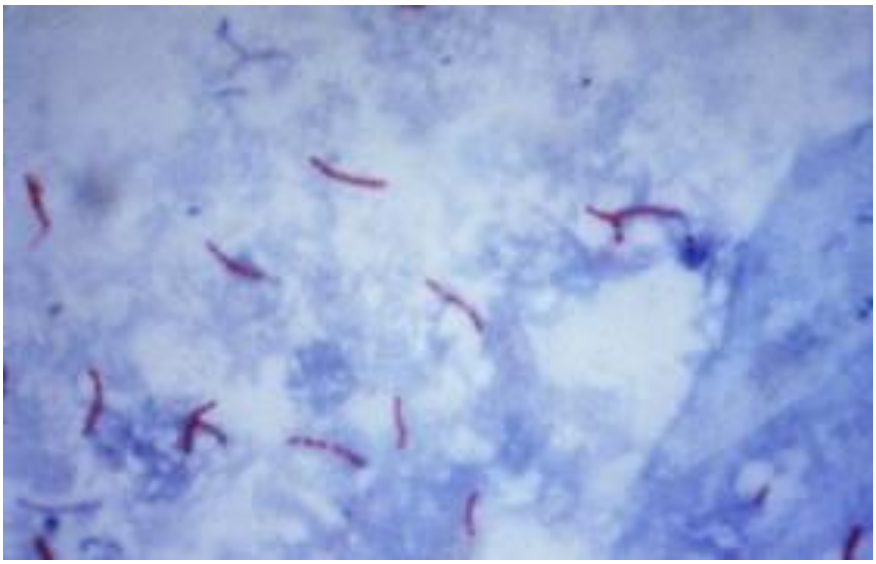
The slide is examined under oil immersion.

Before a smear is being reported as negative,a minimum of 100 fields has to be examined.

An easy method of examination is to have 3 passes along the long axis of the slide or 9 passes along the short axis of the slide so as to view the entire slide.[10]

M. tuberculosis is a thin , rod-shaped, non spore-forming, aerobic bacterium . Acid fastness is due to the high content of mycolic acids, cross-linked long-chain fatty acids, and other lipids present in the cell wall of the organism. Other acid fast microorganisms include *Nocardia* , *Rhodococcus*, the protozoa *Isospora* and *Cryptosporidium* and *Legionella micdadei*. The primary stain (fuchsin) binds to the mycolic acid of cell-wall . Acid alcohol or strong acid used as a decoloriser fails to release fuschin from the cell wall and hence the red colour of carbol fuchsin is retained by mycobacteria – thus acid-fastness.The counterstain, methylene blue gives a contrasting background.

Figure 1 : Acid fast bacilli seen under light microscope



GRADING OF AFB BACILLI:

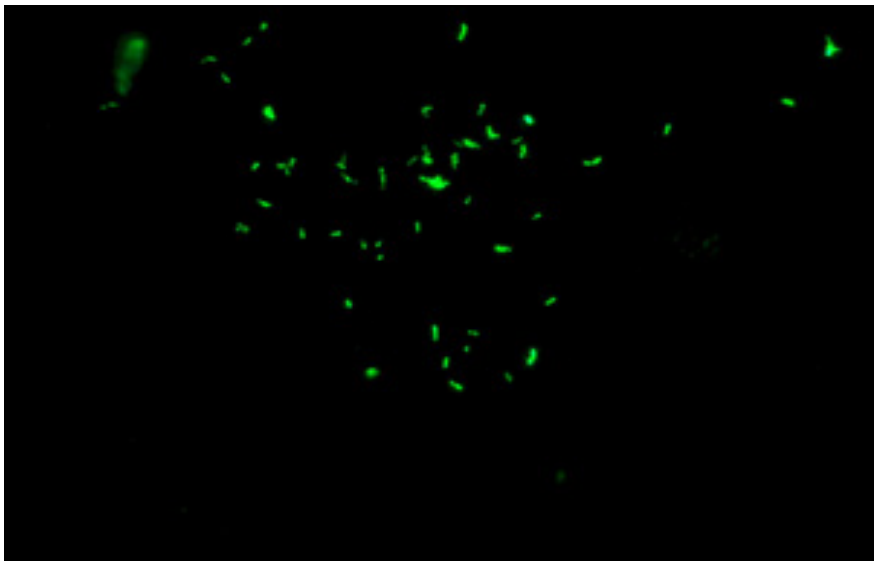
NUMBER OF AFB SEEN	REPORT
0	Negative
1 to 9/100 fields	Doubtful/ Scanty
10 to 99/100 fields	1+
1 to 10/field	2+
>10/field	3+

In the presence of HIV infection, sputum microscopy has a sensitivity that varies between 43% to 51 %, and the sensitivity is further decreased in many resource-limited settings[14] with higher co-infection rates.

Techniques that can increase the yield of smear microscopy are alternative specimen processing techniques[15] like bleach sedimentation, concentration [16] and fluorescence microscopy[17] .

In resource-limited settings, the use of fluorescent microscopes is limited by their higher cost . This problem has been dealt by making use of light-emitting diode bulbs[18,19] that permit the usage of fluorescence microscopes at a lesser price. Another disadvantage of smear microscopy is that drug susceptibility couldnot be tested.

Figure 2 :TB bacilli seen under fluorescent microscope



MYCOBACTERIAL CULTURE:

Mycobacterium tuberculosis is detected by culture with a better sensitivity than smear microscopy and therefore helps in the diagnosis of TB in HIV-infected individuals. Strain characterization and drug susceptibility testing can also be done with the help of culture.

The culture medium that are traditionally used include[20]:

- The L-J medium(Lowenstein-Jenson medium) which is an egg-enriched medium with glycerol and asparagine
- Middlebrook medium which is an agar based medium supplemented with bovine albumin.

Though traditional culture methods are sensitive, they are slow and it may take 6-8 weeks of incubation for the growth to become visible. This causes a delay in the initiation of therapy, which inturn has deleterious effects on the outcome of HIV TB co-infected patients.

Growth characteristics of *M.tuberculosis* in solid media[20] are

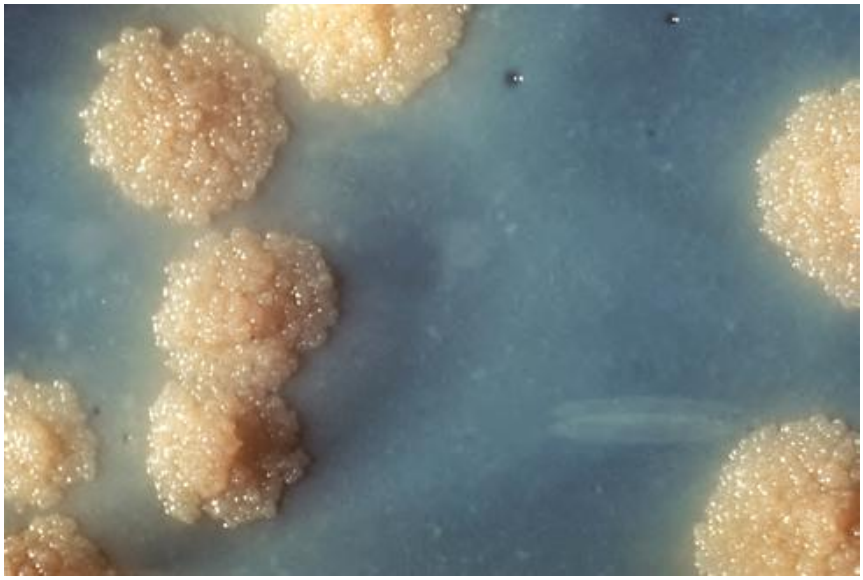
• In primary culture,growth of tubercle bacilli is not visible within a week and usually takes 2 to 4 weeks to give visible growth.

• Colonies appear like breadcrumbs or cauliflower because they are rough and buff coloured.

• Growth is not emulsifiable and appears as granular suspensions.

• Microscopically they appear like serpentine cords or has linear clumps in liquid medium.

Figure 3 : TB colonies grown in culture



Newer methods of culture include **automated liquid culture systems** [21] which can rapidly detect the mycobacterial growth within 1-2weeks by identifying bacterial oxygen consumption or carbon dioxide production with the help of

- colorimetric sensors(MB/ BacT system)
- redox reagents such as Alamar blue[22]
- fluorescent sensors [BACTEC Mycobacteria Growth Indicator Tube (MGIT) 960][23]
- radiometric sensors (BACTEC 460TB)
- pressure sensors(ESP culture system II)

Microscopic observation drug susceptibility(MODS) assay[24],

a cheaper, less commercial method is utilised for the early identification of growth of microcolonies and drug resistance. It has got better sensitivity, rapid growth and less costly compared to regular L-J medium.

Bacteriophage based assays[25] are novel methods employed for TB diagnostics . The FAST Plaque TB assay is one such assay that can identify mycobacteria in 50-65% of smear negative samples with 98%

specificity. The diagnostic accuracy of these assays is increased by performing them on culture isolates. However, in HIV-TB co-infection this method has a lower sensitivity with a higher risk of contamination.

Newer rapid diagnostic technologies that are under pipeline include **colorimetric culture system**[26] using TK medium culture system and **recombinant mycobacteriophages** . These automated systems have increased sensitivity and .require shorter time period for positive culture (9-10 days). Rapid culture results results in faster treatment implementation.

CHEST X RAY:

The cornerstone for the diagnosis of pulmonary TB is chest radiograph. The typical findings in reactivation TB are upper-lobe infiltrates and cavities, whereas lower-lobe disease and intrathoracic lymphadenopathy are evident in primary TB.

HIV-infected individuals having higher CD4 counts ie, >200 cells/μL have radiographic changes mimicking reactivation TB as evident by upper-lobe infiltrates and cavities.

HIV-infected individuals having a higher degree of immunosuppression ie, CD4 count <200 cells/μL have radiographic changes mimicking primary TB as evident by lower-lobe infiltrates, intrathoracic lymphadenopathy and military pattern .

Normal chest radiographs can occur in 21% of culture-positive cases with CD4 count <50 cells/μL. This exemplifies the necessity of a high degree of suspicion while evaluating HIV patients with symptoms suggestive of TB.

Figure 4 : Chest X ray showing cavity in right lower zone in HIV TB coinfection

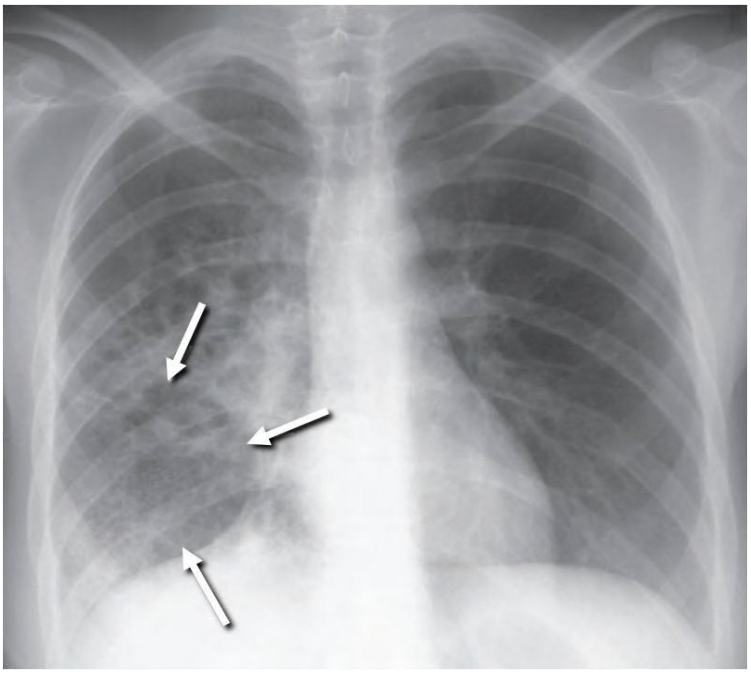
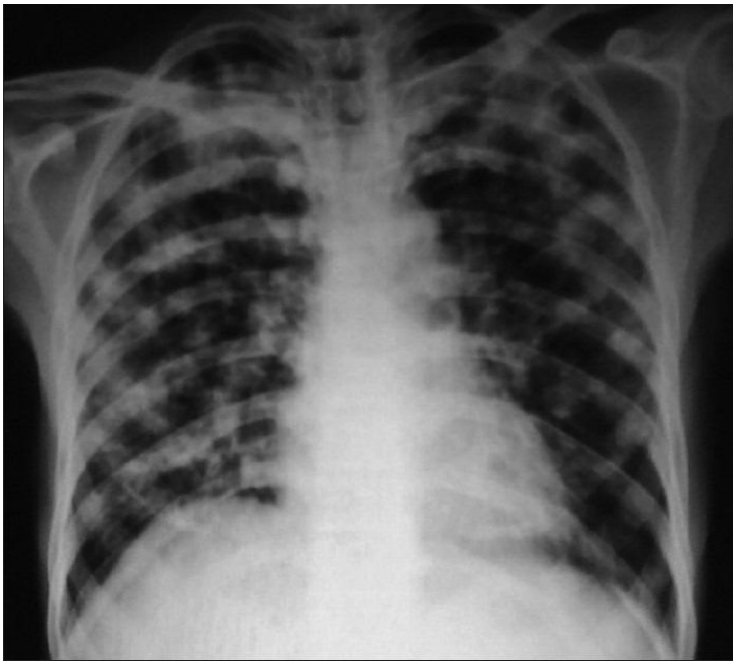


Figure 5 : Diffuse nodular infiltrates seen in CXR of HIV TB coinfected patient



CT CHEST:

CT chest has a role in interpreting doubtful findings on plain chest radiography. It also helps in the diagnosis of some forms of extrapulmonary TB like Pott's disease.

CT chest helps to differentiate old fibrotic lesions from new active lesions. Early active tuberculosis is characterised by lesions in and around the small airways and may be taken as a reliable criterion for disease activity.

MOLECULAR TECHNIQUES:

NUCLEIC ACID AMPLIFICATION TESTING (NAAT):

Nucleic acid amplification testing (NAAT) has increased specificity but variable sensitivity, particularly in paucibacillary disease. Well standardized and reproducible commercial kits are now available. However, there are limitations for their use in resource-limited settings such as lack of data regarding their reliability ,accuracy, higher cost, requirement for strict quality control and requirement of proper laboratory infrastructure.

Various modifications of NAAT[27] include

- Line probe assays (LPA)[28]
- Fluorescence *in-situ* hybridization (FISH)
- Loop-mediated isothermal amplification (LAMP)

Line probe assays have got higher sensitivity of >95% and specificity of 100% especially when culture isolates were used. Line probe assays have been endorsed by WHO for the detection of *M. Tuberculosis* and detection of rifampicin and isoniazid resistance on sputum smear-positive samples and culture growth isolates.

In India, the Intermediate Reference Laboratories governed by the Revised National TB Control Programme (RNTCP) use line probe assays together with culture.

GENEXPERT-RIF:

GeneXpert-Rif has been recently endorsed by WHO for the rapid detection of TB bacilli and detection of rifampicin resistance in TB suspects of PLHA . GeneXpert is an automated, TB-specific ,cartridge-based nucleic acid amplification assay, that has got a completely automated and integrated sample preparation, amplification and detection using real-time PCR, thereby making results available within 100 minutes.

A single direct Xpert MTB/RIF test can detect 92.2% of culture-positive patients whereas a a single direct smear can detect 59.5% of culture positive cases.In addition, a single Xpert MTB/RIF test can also detect 72.5% of smear-negative/culture positive cases whose sensitivity can be increased to 90.2 % by using three samples. Xpert The specificity of this method is 99%. Advantage of this newer technique is that in the presence of HIV co-infection, the sensitivity of direct microscopy is significantly reduced to 47% in this setting , but did not significantly affect the sensitivity of Xpert MTB/RIF[29-33].

In the detecting rifampicin resistance , it has got 99.1% sensitivity and 100% specificity.

Average time taken for diagnosis of tuberculosis using Xpert MTB/RIF is <1 day whereas by microscopy it is 1 day, by liquid culture it takes 17 days and >30 days in solid culture[32,33]. Therefore this technique appears to have the ability to complement the current reference standards for TB diagnostics and enhance its speed and sensitivity.

SEROLOGICAL DIAGNOSIS OF TUBERCULOSIS:

DETECTION OF ANTIBODIES:

The commercial serological tests[34,35] available in the market have not shown adequate specificity and sensitivity to be recommended for diagnostic use. Recently a negative recommendation has been made by WHO against the usage of serological tests for diagnosis of tuberculosis[36]. This is based on information which highlights the fact that these tests could not either replace microscopy or be used as an add-on test to rule out TB. RNTCP has endorsed this fact and is applicable in India, where estimates show that millions of such investigatons are done in the private sector resulting in wastage of resources[37].

DETECTION OF ANTIGEN:

ELISA–based commercial assays can detect mycobacterial antigens like

- *M. Tuberculosis* MPB-64 (TAUNS) antigen in peripheral blood
- lipoarabinomannan (LAM) in the urine
- Early secreted antigenic target 6 in the cerebrospinal fluid[38]

Urinary LAM assays happen to yield better results in HIV TB co infected patients when compared to non HIV TB patients especially when CD4 counts is less than 50 cells/μL, which could be due to the increased occurrence of disseminated TB in them[39,40]. However, urinary LAM assays have a low overall sensitivity (40-60%) in culture-positive TB patients with HIV coinfection but is increased to 67-85% when there is a decline in CD4 count to <50 cells/μL. Specificity in HIV TB coinfection is 99-100%. The combined use of sputum smear microscopy and urine lipoarabinomannan assay needs further studies in high HIV burden settings.

DIAGNOSIS OF LATENT INFECTION:

TUBERCULIN SKIN TESTING:

This test is used widely to screen latent *M*. tuberculosis infection[41] . It consists of an intradermal injection using tuberculin-PPD. This test has got low sensitivity and specificity and it does not differentiate between latent infection and active disease thus limiting its role in the diagnosis of active TB False-negative reactions occur in immunocompromised patients and in individuals with overwhelming TB. False-positive reactions are caused by atypical mycobacterial infections and by BCG vaccination. Despite having TB infection or disease,HIV infected patients can have a negative tuberculin test due to anergy.

INTERFERON GAMMA RELEASE ASSAY (IGRA):

This test used to diagnose latent TB infection is particularly useful in seriously ill patients and those with severe malnutrition.

2 types of IGRA are available[11]:

- QuantiFERON- TB Gold
- T SPOT-TB test.

Both are enzyme- linked immunospot assays that quantify the number of mononuclear cells in peripheral blood producing IFN- γ in response to tuberculosis specific antigen stimulation (ESAT-6 and CFP10).The sensitivity of these tests are similar to that of the tuberculin skin test, but with are costlier.

Disadvantage of IFN-γ assays is that they do not distinguish between active and latent tuberculosis or IRIS and treatment failure.

IGRAs are suitable for serial testing because they can be repeated without boosting. They are not affected by previous BCG vaccination. They need fewer patient visits.

IGRAs are more specific than TST because of lesser cross-reactivity to BCG vaccination and atypical mycobacteria. However, WHO has given negative recommendation against IGRAs for their use in diagnosis of latent or active TB, in resource limited settings[42,43].

TREATMENT OF HIV TB COINFECTION :

There is no cure for HIV/AIDS till date. There are treatment options only for the opportunistic infections arising from the disease. Anti retroviral drugs act by slowing the action of the virus thereby prolonging the life of patients. TB treatment in HIV coinfected individuals is similar to those of TB patients without HIV coinfection[(9)].

ANTI-TB THERAPY:

Standard anti_TB therapy has 4 drugs in the intensive phase namely (H)isoniazid, (R) rifampicin , (Z) pyrazinamide and (E)ethambutol for 2 months and 2 drugs in the continuation phase namely H and R for 4 months[(44)]. RNTCP recommends Category I (2HRZE3/4HR3) , an intermittent thrice weekly regimen for new TB cases. For relapse cases - Category II(2HRZES3/1HRZE3/5HRE3) is given where streptomycin (Sm) is added in the intensive phase and duration of treatment is prolonged to 8 months. The key drug in the treatment of TB in HIV infected individuals is rifampicin because it has the ability to kill both intracellular bacilli and intermittent and slow growers of TB bacilli.

Increasing the treatment duration to 9 months did not improve the outcome of the treatment, however TB recurrences were significantly reduced during the follow up period[45]. Studies are showing that intermittent regimens are associated with an increased risk of failure and a higher probability of rifampicin resistance, particulary in ART naïve individuals . This has led WHO to suggest daily TB regimens (at least in intensive phase) rather than intermittent regimens for HIV-TB patients .Concurrent ART during TB treatment results in better treatment outcomes, lower case fatality rates,lesser failure rates and recurrence rates. As there is no strong evidence to support changeover to daily regimen and to show the superiority of intermittent regimens, this recommendation has not been followed in India.

ANTI-RETROVIRAL THERAPY:

In resource limited settings , WHO recommends the use of two nucleoside reverse transcriptase inhibitors (NRTIs) in combination with one non-nucleoside reverse transcriptase inhibitor (NNRTI) as first line therapy for the treatment of HIV-infected TB patients[46]. In India, a triple regimen consisting of zidovudine or stavudine , lamivudine and efavirenz is recommended by NACO[47].

The cytochrome CYP-450 enzyme system which is located in the liver and intestinal wall is induced by rifamycins, thus accelerating the metabolism of NNRTIs and PIs . This inducing effect is weaker with rifabutin when compared to rifampin. When rifampicin and certain antiretroviral drugs are given in combination, it results in reduced trough levels of the latter , resulting in therapeutic failure[48]. There is a reduction in Nevirapine levels by 40–55 %, delavaridine by 96% ,efavirenz by 18-25 %, and most PIs by 80-90 %.

In India, Efavirenz 600 mg once daily is the NNRTI used in HIV-TB co-infected individuals in India. A triple NRTI regimen or a regimen consisting of two NRTIs and nevirapine is used if patients cannot

tolerate or have contraindications to efavirenz (*e.g.*, pregnancy, psychiatric disturbances). An alternative strategy is to alter the anti-TB regimen with rifampicin replaced by rifabutin – the recommended dose of rifabutin is 300 mg OD twice/thrice-weekly together with nevirapine based ART. Contraindications for rifabutin include leucopenia and thrombocytopenia while uveitis occurs with higher doses .

TIMING OF ART & CONCOMITANT ADMINISTRATION WITH ATT:

Early initiation of ART has the advantages of decline in early mortality, decline in relapses ,better cure rates, decrease in malabsorption thus preventing ATT drug resistance and a decrease in the occurrence of other opportunistic infections besides TB[49].

It has got disadvantages like a higher risk of developing IRIS, cumulative drug toxicity, drug interactions of ART with rifampicin thus restricting the choice of combinations . These in turn can have an adverse impact on the long term compliance of these patients.

TREATMENT STRATEGY FOR HIV TB COINFECTION

MANIFESTATION	ATT	ART
Extrapulmonary TB (regardless of CD4 count)	Start immediately	ART to be started as soon as ATT is tolerated (between 2 weeks to 2 months)
Pulmonary TB CD4 <200 cells/mm3	Start immediately	ART to be started as soon as ATT is tolerated (between 2 weeks to 2 months)
Pulmonary TB CD4 = 200–350 cells/mm3	Start immediately	ART to be started after completing intensive phase of ATT (start earlier if severely compromised)
Pulmonary TB CD4 >350 cells/mm3	Start immediately	Monitor CD4 count. ART to be considered when CD4 cell count falls below 350 cells/mm3

IMMUNE RECONSTITUTION INFLAMMATORY SYNDROME(IRIS):

IRIS is defined as a transient deterioration of signs and symptoms of tuberculosis or radiological worsening after the initiation of ART, inspite of HIV load reduction (>1 log10 copies/µl) and immunological recovery[50]. Before a diagnosis of IRIS is being made, drug resistance and other opportunistic infections must be ruled out. A peculiar feature of IRIS in tuberculosis is hypercalcemia.

IRIS has two types of presentation:

- unmasking IRIS and
- paradoxical IRIS

The incidence of IRIS in tuberculosis varies from 8 to 43%.

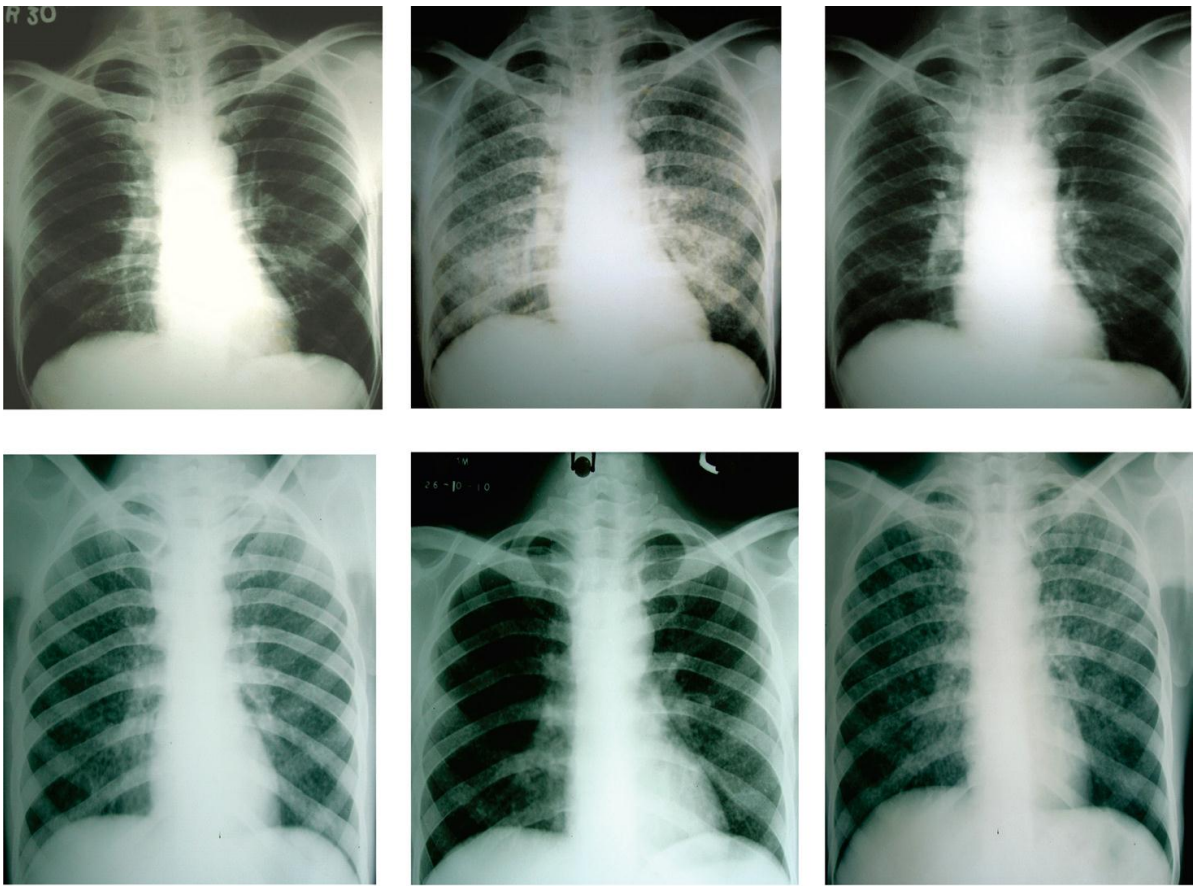
IRIS can manifest as fever, worsening respiratory symptoms and signs, worsening CNS lesions such as tuberculoma and meningitis , psoas abscess, cold abscess and lymph node enlargement[2] .

Figure 6 : Types of IRIS

A, B and C_ unmasking IRIS; D, E, F _ paradoxical IRIS.

(A) Asymptomatic patient when started on ART; **(B)** developed miliary TB after ART –unmaskingreaction; **(C)** After ATT showing resolution; **(D)** Patient with miliary TB at baseline; **(E)** After 1month of ATT treatment; **(F)** After ART showing flare up of lesion (paradoxical reaction)

(A) (B) (C)



(D) (E) (F)

Genetic predisposition(HLA B-44),high bacillary antigen load at the start of treatment, higher viral load at initiation of treatment, lower CD4 cell count, very rapid decline in viral load , starting ART closer to the starting of ATT are probable risk factors for the development of IRIS.

Pathophysiology of IRIS is not yet fully understood, it is thought to be due to increased production of cytokines like IFN-γ or a deficiency of inhibitory immune responses[51].

IRIS is usually managed with anti-inflammatory drugs and steroids. Death is a rarer outcome in IRIS and is commonly seen with CNS IRIS. ART termination is not needed usually.

MATERIALS AND METHODS

INCLUSION CRITERIA:

Adult patients (>12 years) satisfying WHO criteria for the diagnosis of pulmonary tuberculosis , with HIV coinfection[21].

Smear-positive pulmonary tuberculosis

- Acid-fast bacilli (AFB) positive in sputum smear **and**
- HIV infection confirmed in laboratory

Smear-negative pulmonary tuberculosis

- AFB negative in at least two sputum specimens **and**
- Radiological picture suggestive of active tuberculosis **and**
- HIV infection confirmed in laboratory

EXCLUSION CRITERIA:

- Diabetes mellitus
- Chronic renal failure
- Long term steroid/other immunosuppressants intake

METHODOLOGY

This hospital based non randomised cross sectional observational study was conducted in Tirunelveli Medical College Hospital, a tertiary care referral centre located in the southern part of Tamil Nadu. After giving written informed consent , 50 eligible patients admitted with pulmonary tuberculosis and HIV coinfection in the medical wards, ART centre and Thoracic medicine wards of our hospital from August 2012 to August 2013 participated in the study. The research protocol was approved by the Institutional Ethical Committee of Tirunelveli Medical College.

Patients were enquired about symptoms such as cough , fever , hemoptysis, weight loss , loss of appetite, etc. and underwent a thorough clinical examination.

Patients were instructed to produce two expectorated sputumsamples, of which atleast one is an early morning sample. In the laboratory of RNTCP Designated Microscopy Centre (DMC) of our hospital, sputum samples were tested for AFB by Ziehl Neelson method.

100 microscopic fields were visualised in each smear. Smears were categorised as positive or negative. One specimen positive out of the two is enough to declare a patient as smear positive TB.

Sputum density was further graded as

3+ (>10 AFB/oil field) (20 fields to be examined),

2+ (1 to 10 AFB/oil field) (50 fields to be examined),

1+ (10 to 99 AFB/100 oil fields),

Scanty (1 to 9 AFB/100 oil fields).

Chest X ray posteroanterior view was taken for all the patients. In all the smear negative cases, CT chest was taken and expert opinion given by radiologists of our hospital.

In addition to the baseline investigations like Complete Blood Count, blood sugar, urea, creatinine, LFT that were done in biochemistry lab, CD4 count was done in ART plus centre of our hospital by flow cytometry.

STATISTICAL ANALYSIS

The information obtained from study participants were tabulated in an Excel Master Chart. Using **Epidemiological Information Package (EPI 2010)** organised by Centre for Disease Control, Atlanta further the data were analysed using a computer.

By utilising this software means, standard deviations, frequencies, percentages, range and 'p' values were obtained. The significance of difference between quantitative variables was determined by using Kruskul Wallis chi-square test and for qualitative variables Yate's chi square test was used. A 'p' value of less than 0.05 was considered to indicate significant relationship.

OBSERVATIONS AND ANALYSIS

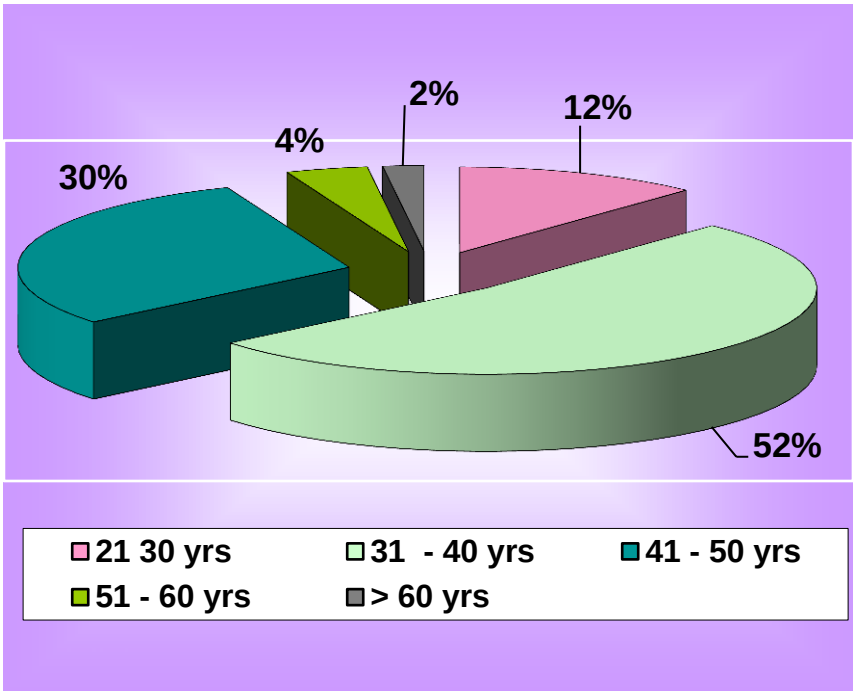
50 patients with pulmonary tuberculosis and HIV coinfection who fulfilled the inclusion criteria participated in the study.

The observations made were as follows:

A. PROFILE OF CASES STUDIED:

AGE:

Chart 1: AGE DISTRIBUTION



Among the study group, the minimum age was 27 years and maximum age was 65 years.

The mean age was 39.3 years.

Table A1 : Age distribution

Age group	Cases	
	No	**%**
13-20 years	-	-
21-30 years	6	12
31-40 years	26	52
41-50 years	15	30
51-60 years	2	4
> 60 years	1	2
Total	50	100
Range	27 - 65 years	
Mean	39.3 years	
SD	8.0 years	

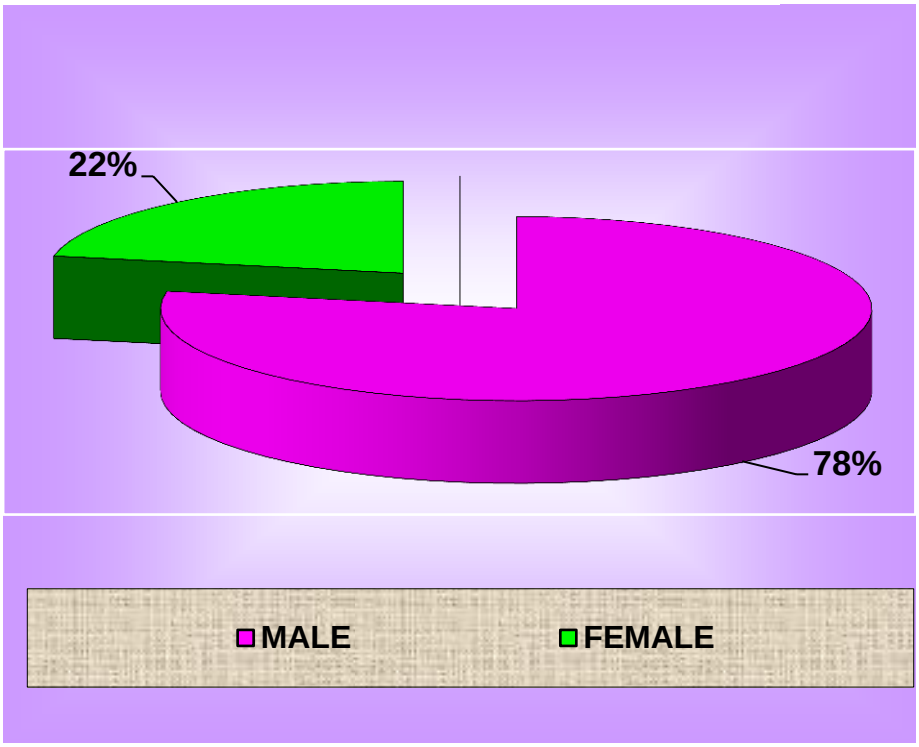
SEX:

Of the 50 patients, 39 were male and 11 were female.

Table A2 : Sex distribution

Sex	Cases	
	No	%
Male	39	78
Female	11	22
Total	50	100

Chart 2: SEX DISTRIBUTION



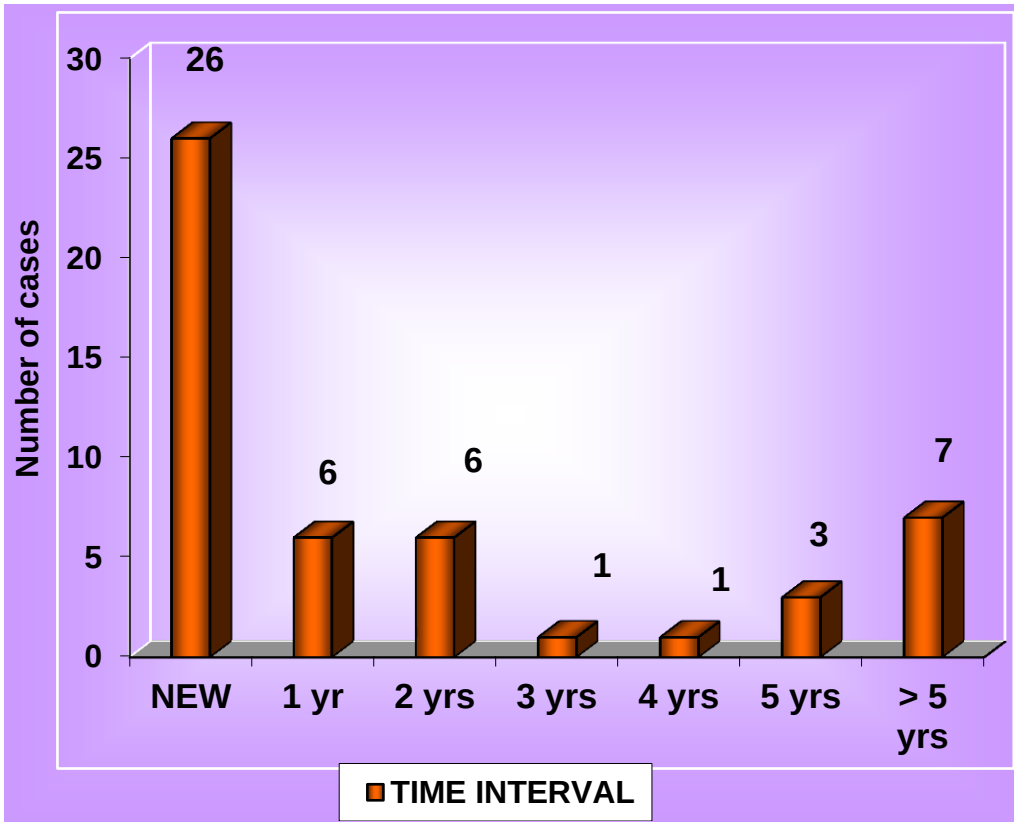
TIME INTERVAL:

Among the 50 patients, 26 patients had pulmonary tuberculosis during the initial diagnosis of HIV infection

Table A3 : Time interval

Time interval	Cases	
	No	**%**
New	26	52
1 year	6	12
2 years	6	12
3 years	1	2
4 years	1	2
5 years	3	6
>5 years	7	14
Total	50	100

Chart 3: TIME INTERVAL



Among the 50 patients , 26 patients had pulmonary tuberculosis during the initial diagnosis of HIV infection.

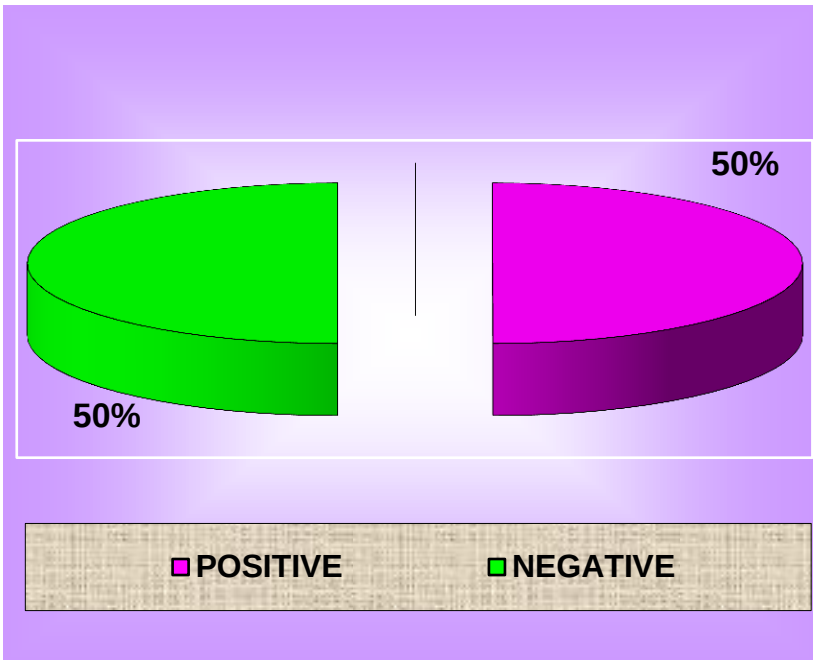
SPUTUM STATUS:

Out of the 50 patients with HIV_TB coinfection , 25 were sputum smear positive and 25 were sputum smear negative.

Table A4 : Sputum status

Sputum status	Cases	
	No	%
Positive	25	50
Negative	25	50
Total	50	100

Chart 4: SPUTUM STATUS



SPUTUM DENSITY:

Among the 50 sputum positive cases, seven cases had scanty positivity, five cases had 1+, six cases had 2+ and six cases had 3+ status.

Chart 5: SPUTUM DENSITY

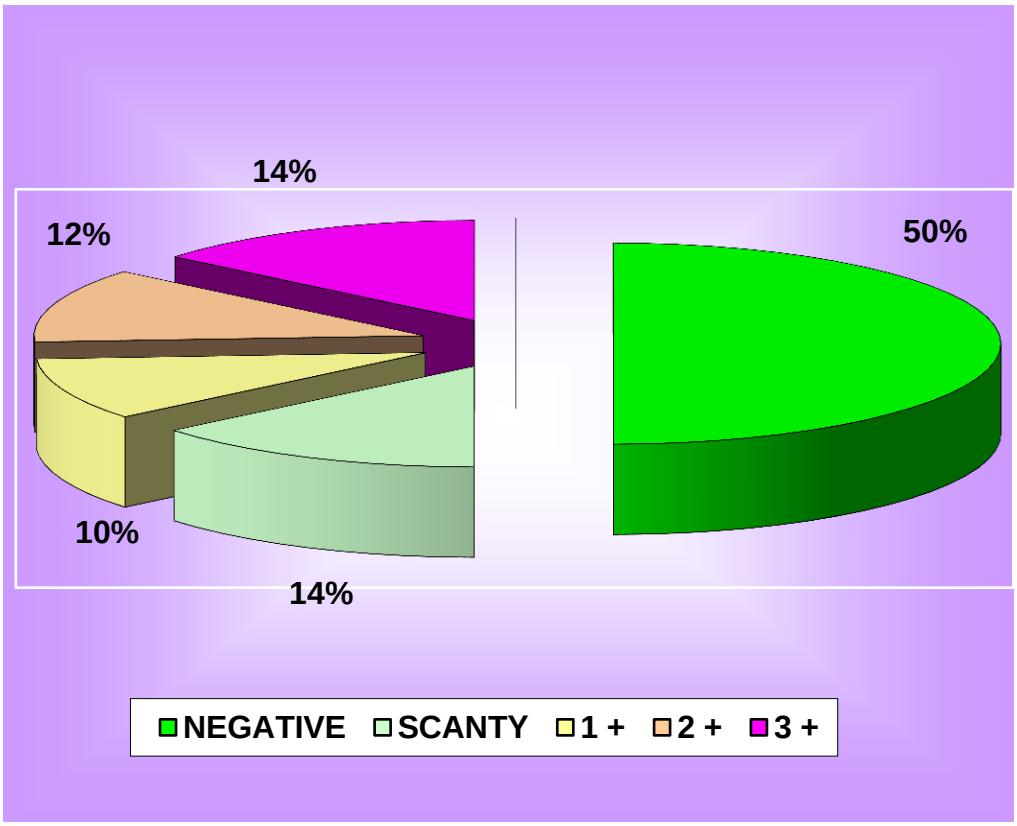


Table A5 : Sputum density

Sputum density	Cases	
	No	**%**
Negative	25	50
Scanty	7	14
1+	5	10
2+	6	12
3+	7	14
Total	50	100

Among the 50 sputum positive cases, seven cases had scanty positivity, five cases had 1+, six cases had 2+ and six cases had 3+ status.

CD 4 COUNT:

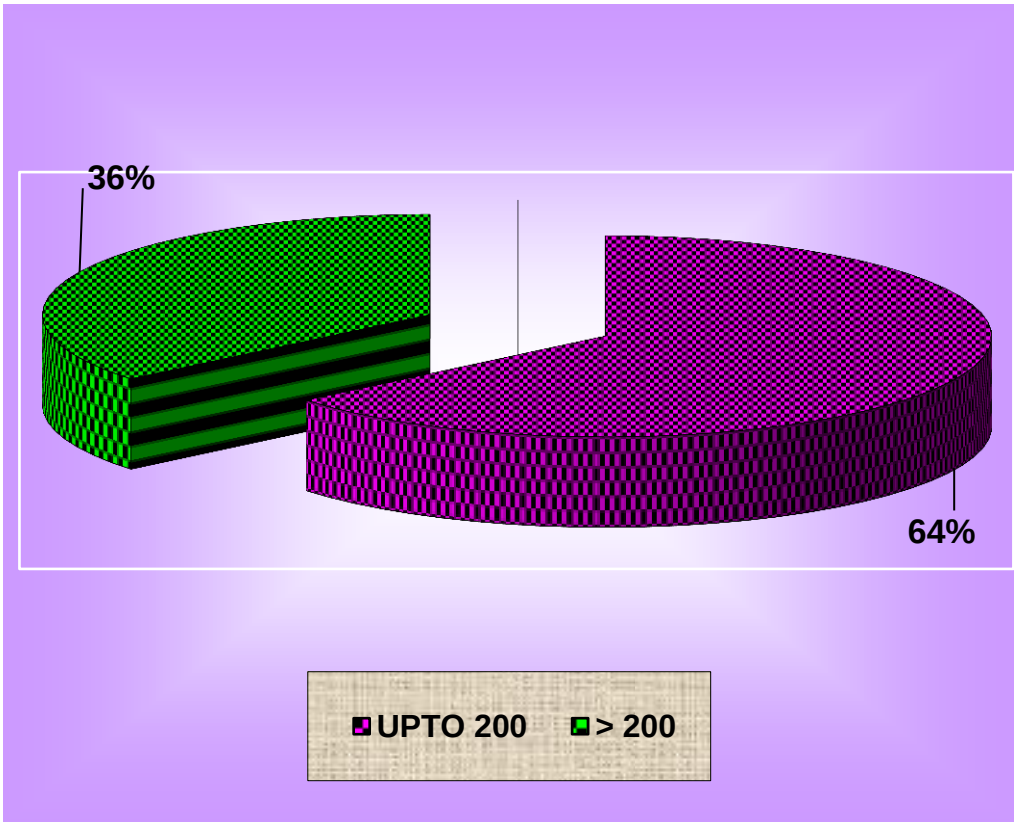
Of the total 50 cases, 32 had CD4 count less than 200 cells and 18 had CD4 count more than 200. Mean CD4 count was 172.9

Table A6 : CD4 count

CD4 count	Cases	
	No	%
0-50	9	18
51-100	7	14
101-150	8	16
151-200	8	16
201-250	4	8
251-300	5	10
301-350	7	14
>350	2	4
Total	50	100

Upto 200	32	64
>200	18	36
Range	18-411	
Mean	172.9	
SD	109.2	

Chart 6: CD 4 COUNT



RADIOLOGICAL FINDINGS:

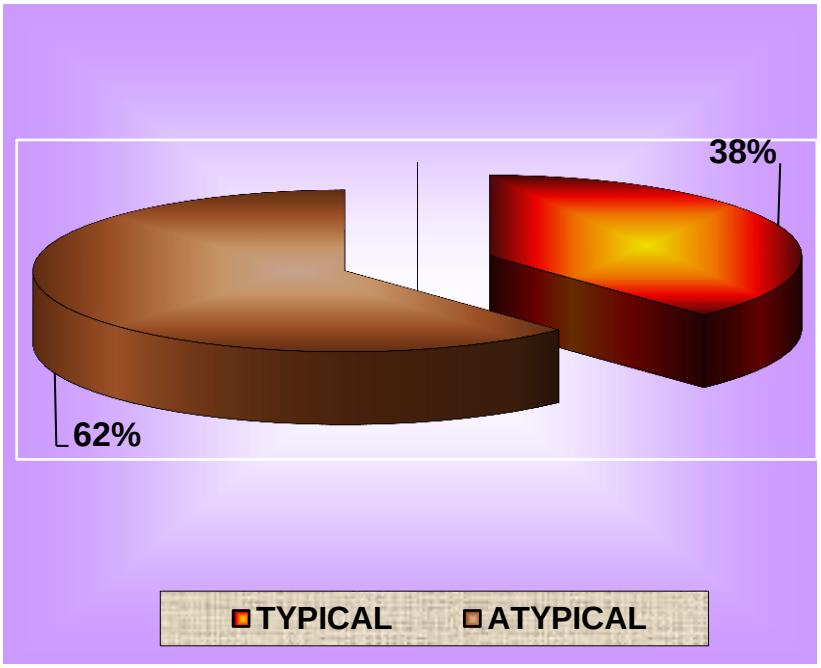
Infiltration was the most common radiologic finding followed by consolidation and cavity .

Table A7 : Radiological findings

Radiological findings	Cases	
	No	%
Upper zone infiltration	14	28
Midzone infiltration	1	2
Mid & lower zone infiltration	8	16
Lower zone infiltration	5	10
Diffuse infiltration	3	6
Upper zone cavity	4	8
Mid zone cavity	1	2
Lower zone cavity	1	2
Miliary	3	6
Consolidation	10	20
Total	50	100

Radiological findings	No	%
Bilateral	20	40
Unilateral	30	60
Radiological findings	**No**	**%**
Upper zone	19	38
Mid zone	7	14
Mid & lower zone	8	16
Lower zone	10	20
Others (military & diffuse)	6	12
Radiological findings	**No**	**%**
Typical findings	19	38
Atypical findings	31	62

Chart 7: RADIOLOGICAL FINDINGS



B:RELATIONSHIP BETWEEN SPUTUM SMEAR STATUS & OTHER VARIABLES

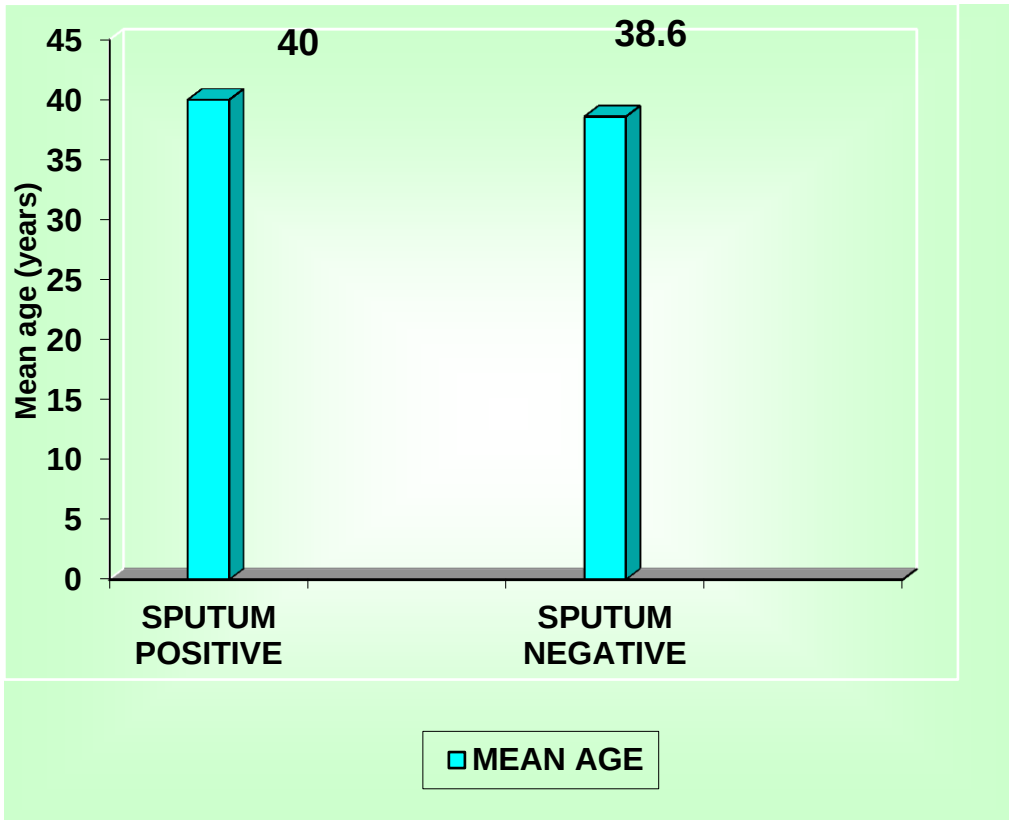
AGE AND SPUTUM SMEAR STATUS:

Table B1 : Age and sputum smear status

Age group	Sputum smear status			
	Positive		Negative	
	No	%	No	%
13-20 years (0)	-	-	-	-
21-30 years (6)	3	50	3	50
31-40 years (26)	11	42.3	15	56.7
41-50 years (15)	9	60	6	40
51-60 years (2)	2	100	-	-
>60 years (1)	-	-	1	100
Age Mean SD	40.0 8.2		38.6 8.0	
'p'	0.5199 Not significant			

Mean age of presentation in sputum positive cases is 40 whereas it is 38.6 in smear negative cases. There was no stastistically significant correlation between age and sputum smear status (p=0.5199).

Chart 8: AGE AND SPUTUM SMEAR STATUS



SEX DISTRIBUTION AND SPUTUM SMEAR STATUS:

Sputum positive and negative cases were equally distributed among males and females.

Chart 9: SEX DISTRIBUTION AND SPUTUM SMEAR STATUS

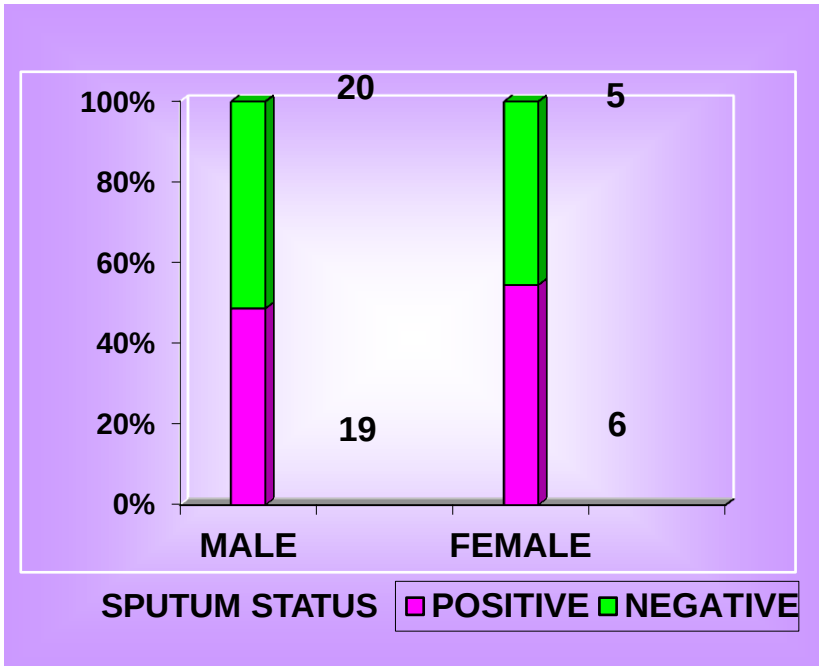


Table B2 : Sex distribution and sputum smear status

Sex	Sputum smear status			
	Positive		Negative	
	No	%	No	%
Male (39)	19	48.7	20	51.3
Female (11)	6	54.5	5	45.5
'p'	0.7354 (Not significant)			

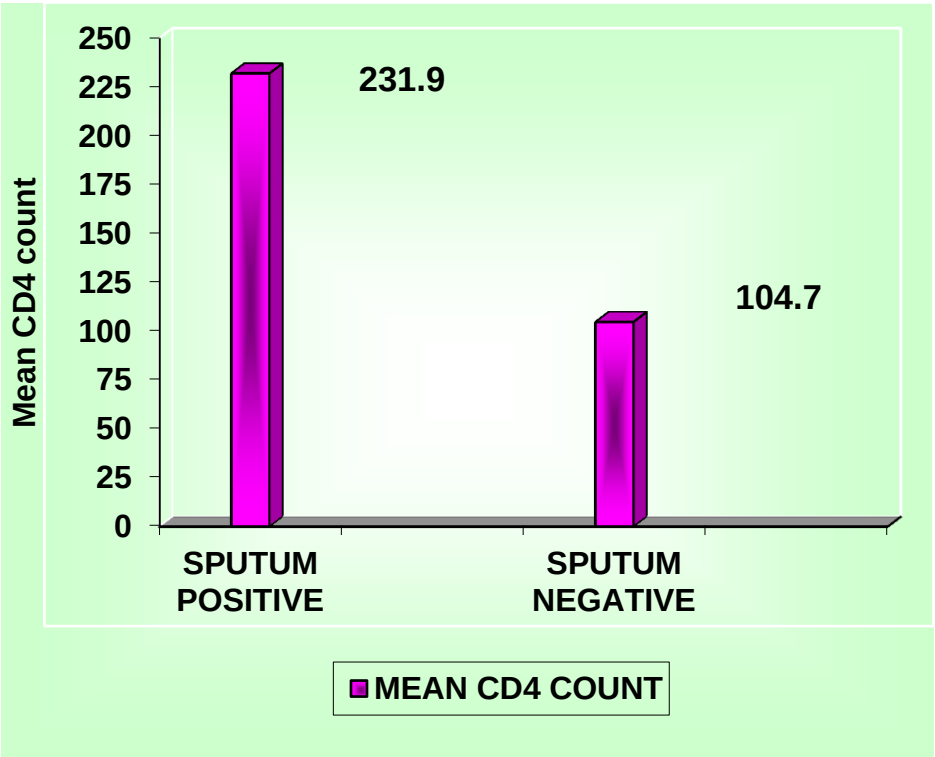
SPUTUM SMEAR STATUS AND CD4 COUNT:

Table B3 : Sputum smear status and CD4 count

CD4 count	Sputum smear status			
	Positive		Negative	
	No	%	No	%
Upto 200 (32)	11	34.4	21	65.6
>200 (18)	14	77.8	4	22.2
CD4 count				
Mean	231.9		113.8	
SD	104.7		78.1	
'p'	**0.0001 (Significant)**			

When CD4 count was less than 200,sputum negative cases were common while sputum positive cases dominated in CD4 count more than 200 with a statistically significant difference (p=0.0001).

Chart 10:CD4 COUNT AND SPUTUM SMEAR STATUS



RADIOLOGICAL FINDINGS AND SPUTUM SMEAR STATUS:

Sputum positivity was common among patients with unilateral radiologic findings and sputum negativity was prevalent among patients with bilateral findings and the difference was stastically significant(p=0.0015).

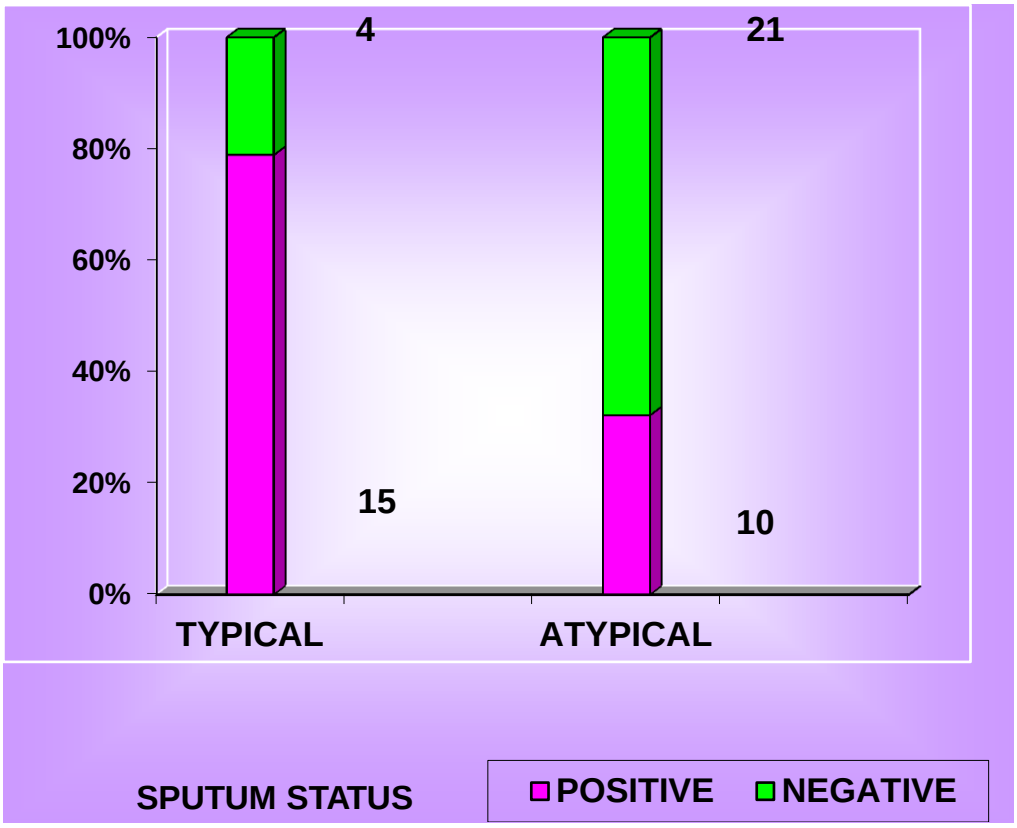
Table B4 : Radiological findings and sputum smear status

Radiological findings	**Sputum smear status**			
	Positive		**Negative**	
	No	**%**	**No**	**%**
Bilateral (20)	4	20	16	80
Unilateral (30)	21	70	9	30
'p'	**0.0015 (Significant)**			

Radiological findings	**Sputum smear status**			
	Positive		**Negative**	
	No	**%**	**No**	**%**
Typical (19)	15	78.9	4	21.1
Atypical (31)	10	32.3	21	67.7
'p'	**0.0036 (Significant)**			

Typical findings include upper lobe infiltration,upper lobe cavity and upper lobe consolidation. Sputum positivity was common among patients with typical radiological findings whereas sputum negativity was seen among patients with atypical radiological findings.

Chart 11:RADIOLOGICAL FINDINGS AND SPUTUM SMEAR STATUS



C : RELATIONSHIP BETWEEN CD4 COUNT AND OTHER VARIABLES

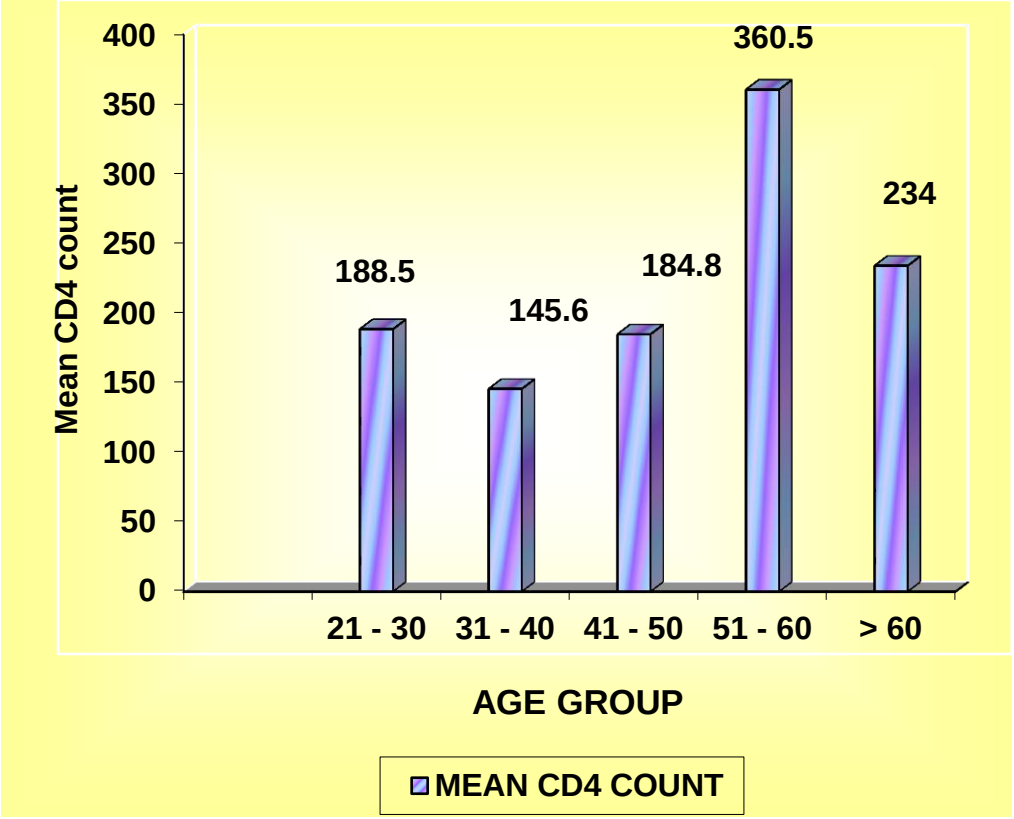
AGE AND CD4 COUNT:

Majority of cases were in the age group of 31 to 40 years with CD4 count less than 200. There is no correlation between CD4 count and age.

Table C1 : Age and CD4 count

Age group (in years)	CD4 Count				Mean	SD
	<200		>200			
	No	%	No	%		
13-20 (0)	-	-	-	-	-	-
21-30 (6)	4	66.7	2	33.3	188.5	108.5
31-40 (26)	19	73.1	7	26.9	145.6	102.6
.941-50 (15)	9	60	6	40	184.8	106.7
51-60 (2)	-	-	2	100	360.5	76.4
1>60 (1)	-	-	1	100	234	-
'p'	0.1604 Not significant					

Chart 12: AGE AND CD4 COUNT



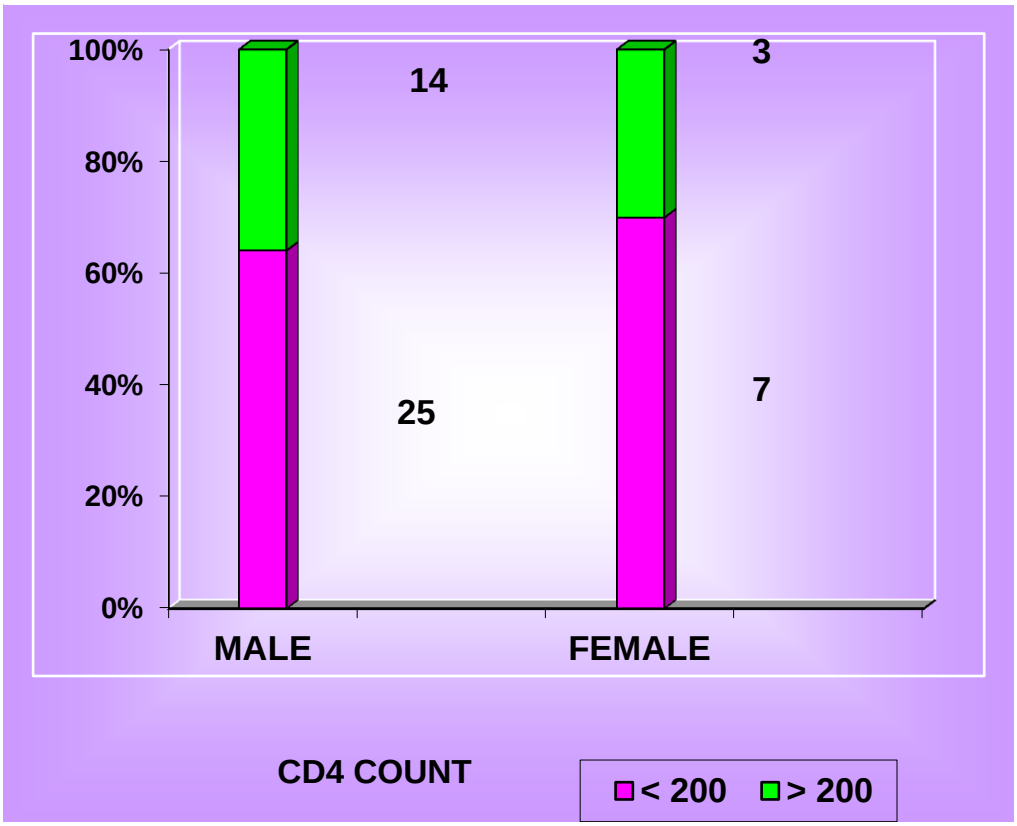
SEX AND CD4 COUNT:

Among males the mean CD4 count was 107.1 whereas the mean CD4 count among females is 119.9.

Table C2 : Sex and CD4 count

Sex	CD4 Count					
	<200		>200		Mean	SD
	No	%	No	%		
Male (39)	25	64.1	14	35.9	167.8	107.1
Female (11)	7	63.6	3	36.4	190.6	119.9
'p'	0.5582 (Not significant)					

Chart 13: SEX AND CD4 COUNT



TIME INTERVAL AND CD4 COUNT:

Majority of the newly diagnosed cases of pulmonary tuberculosis had a CD4 count less than 200. But there was no statistically significant difference between time interval and CD4 count.

Table C3 : Time interval and CD4 count

Time Interval	CD4 Count				Mean	SD
	<200		>200			
	No	%	No	%		
New (26)	19	73.1	7	26.9	154.4	109.5
1 year (6)	5	83.3	1	16.7	112.3	121.6
2 years (6)	3	50	3	50	226.2	80.3
3 years (1)	-	-	1	100	240	-
4 years (1)	-	-	1	100	304	-
5 years (3)	1	33.3	2	66.7	241	80.9
>5 years (7)	4	57.1	3	42.9	190.1	114.7
'p'	0.2171 Not significant					

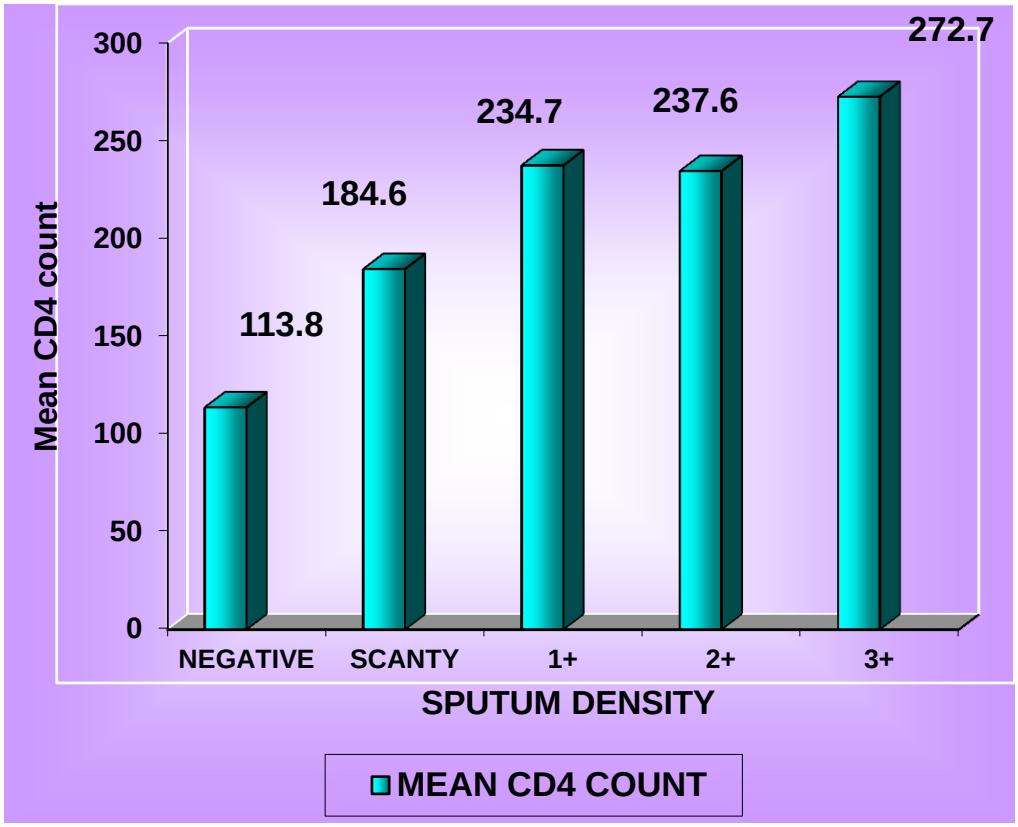
SPUTUM DENSITY AND CD4 COUNT:

Sputum negative pulmonary tuberculosis was common when CD4 count was less than 200.

Table C4 : Sputum density and CD4 count

Sputum density	CD4 Count				Mean	SD
	<200		>200			
	No	%	No	%		
Negative (25)	21	84	4	16	113.8	78.1
Scanty (7)	4	57.1	3	42.9	184.6	99.1
1+ (5)	2	40	3	60	237.6	72.5
2+ (6)	2	33.3	4	66.7	234.7	108.1
3+ (7)	3	42.9	4	57.1	272.7	127.3
'p'	**0.0006** **Significant**					

Chart 14: CD4 COUNT AND SPUTUM DENSITY



As the CD4 count increases, sputum positivity with higher density rises. There was a statistically significant difference between the two groups(p=0.0006).

RADIOLOGICAL FINDINGS AND CD4 COUNT:

Though bilateral findings were commonly seen with CD4 count<200, the difference was not statistically significant(p=0.4697).

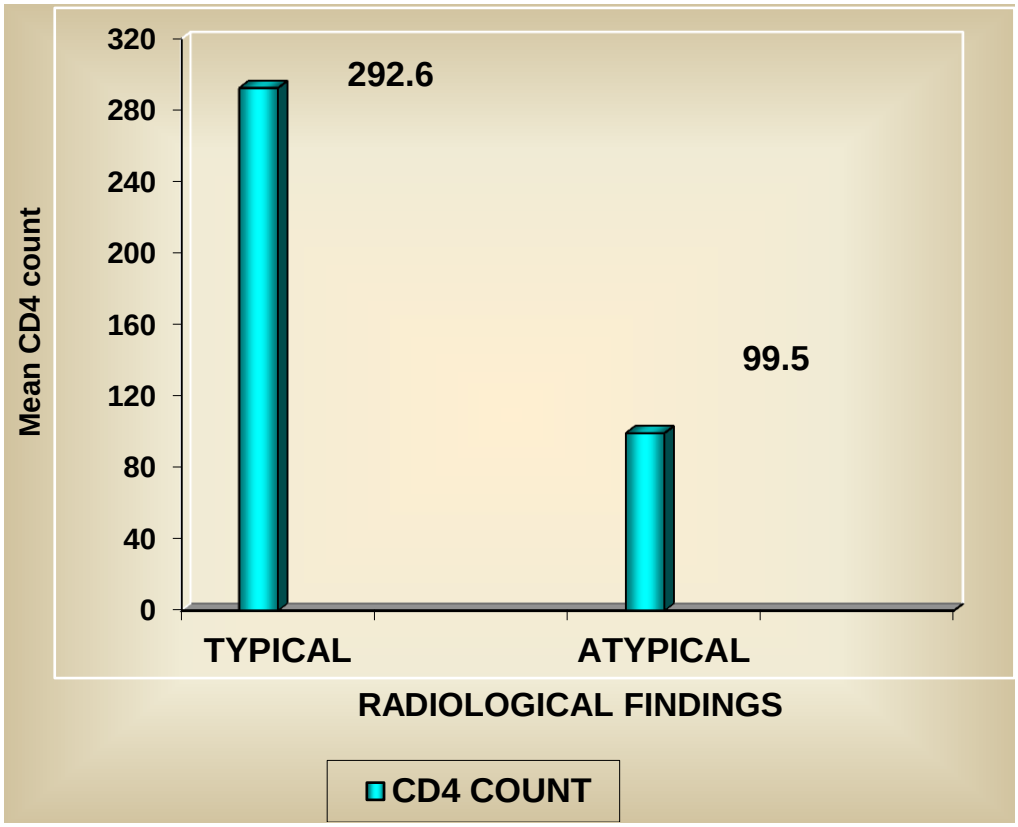
Table C5 : Radiological findings and CD4 count

Radiological findings	CD4 Count					
	<200		>200		Mean	SD
	No	%	No	%		
Bilateral (20)	13	65	7	35	160.4	107.3
Unilateral (30)	19	63.3	11	36.7	181.2	111.4
'p'	0.4697 (Not significant)					

Radiological findings	CD4 Count					
	<200		>200		Mean	SD
	No	%	No	%		
Typical (19)	1	5.3	18	94.7	292.6	56.3
Atypical (31)	31	100	-	-	99.5	53.9
'p'	**<0.0001 (Significant)**					

All patients who had a CD4 count less than 200 had atypical chest X ray findings with a statistically significant difference($p<0.0001$).

Chart 15 : CD4 COUNT AND RADIOLOGICAL FINDINGS



DISCUSSION

While many HIV-related opportunistic infections manifest at lower CD4 counts, tuberculosis is one infection which occurs irrespective of CD4 counts, although its incidence increases with increasing immunosuppression. Several studies done in HIV-TB coinfection showed that they have severe immunosuppression at presentation, possessing CD4 counts of less than 200. In our study , a larger proportion of cases were found to be in the CD4 count range of less than 200(32/50,64%). This confirms the fact that tuberculosis is the most common opportunistic infection in HIV patiens with CD4 count less than 250.

In our study,26 cases(52%) had pulmonary tuberculosis at the initial diagnosis of HIV infection. This undoubtedly proves the necessity of intensive case finding measures to screen for pulmonary tuberculosis in all HIV patients . The reason for this higher incidence of tuberculosis at the time of initial presentation may be explained by larger number of patients having a CD4 count of less than 200.

Among the 26 cases, CD4 count less than 200 was present in 19(73.1%) . In our study , significant number of TB cases(6 cases_12%) were diagnosed in the first year after HIV infection. This finding confirms

the fact that the incidence of tuberculosis is higher in the first year of HIV seroconversion.

Our study also showed a declining trend in the occurrence of TB in the third, fourth and fifth year after diagnosing HIV infection. This may be substantiated by the fact the mean CD4 count in these cases was on a higher range,240,304 and 241 respectively.

In a country like India with resource limited settings, sputum smear microscopy and chest X ray remains the cornerstone methods for diagnosing pulmonary tuberculosis.

Various studies done in the past have shown that sputum negativity is common in HIV_TB coinfection. In our study , we found that sputum negative and sputum positive cases occurred with equal frequency with 25 among the 50 cases having sputum smear negative pulmonary tuberculosis(50%).

Studies by Pitchenick[52] et al showed that HIV_TB coinfected individuals are less likely to have sputum smear positivity(50/74,68%) compared to HIV seronegative individuals(172/215,80%). Also sputum culture positivity for M.tuberculosis was less in the presence of HIV infection(61/74,82%) compared to non_HIV infected individuals(196/215,91%).

Smith et al[53] observed that acid fast smears occur with equal frequency among HIV infected and non_HIV infected patients.

Klein et al[54] showed a decreased sensitivity of sputum smears in pulmonary tuberculosis among HIV patients(45% versus 81%).

Long et al[55] found 66% sputum smear positivity among HIV infected individuals compared to 78% among non_HIV infected individuals.

Studies by Praveen kumar[56] et al showed a sputum smear positivity of 21.4% among HIV_TB coinfected individuals.

Rajasekaran et al[57] reported 15.3% patients as smear positive.

Thus there have been wider variations in the occurrence of sputum smear positivity in HIV_TB coinfection.

Hence we tried to analyse the relationship between the sputum smear status and the severity of immunosuppression expressed in terms of CD4 count. Among the 25 sputum negative cases, CD4 count less than 200 was present in 21 cases whereas CD4 count more than 200 was present only in 4 cases. Among the 25 sputum positive cases, CD4 count less than 200 was present in 11 cases whereas CD4 count more than 200 was present in 14 cases. Applying chi square test there was a statistically significant correlation between sputum smear status and CD4 count. Thus we

concluded that sputum smear positivity decreases as the CD4 count decreases. This endorses the fact by WHO that sputum negativity increases with increase in the degree of immunosuppression.

The findings of our study were indifferent from those done by Sameer singhal et al[58] who showed that acid fast smear positivity to negativity was 1:1 when the CD4 count is between 0 to 200 whereas it was 3:1 in cases with CD4 count above 200.

However ,our study had the limitation of missing few sputum positive cases as sputum culture was not done in our study which has better sensitivity than sputum smear microscopy.

We also derived additional information supporting this fact by comparing the sputum bacillary density with CD4 count which clearly showed a statistically significant correlation between sputum density and CD4 count.

Among the sputum negative cases the average CD4 count was 113.8 whereas in patients with scanty positivity,1+,2+,3+ the average CD4 count was 184.6, 237.6, 234.7, 272.7 respectively. As the CD4 count dropped, sputum density decreased. This finding corroborated with the findings of previous study done by Muqusi F et al[59].

Hence a high degree of clinical suspicion is needed to diagnose pulmonary tuberculosis among HIV infected individuals especially when CD4 count is less than 200.

In our study we also tried to analyse the radiological findings among HIV_TB coinfected individuals.

Tuberculosis usually involves the upper lobes unilaterally manifesting as infiltration,cavity,consolidation,fibrosis and volume loss. Mahesha Padyana et al[5] showed in their study that among patients with HIV_TB coinfection, bilateral lung involvement was common . 27.7% bilateral consolidation, 17% miliary pattern occurred in TB /HIV versus 12% pulmonary bilateral consolidation, 4.7% miliary pattern in of HIV negative tuberculosis patients.

Studies by Pearlman *et al* showed infiltrates among 67%, cavity in 20%, pulmonary nodule in 20%, interstitial disease in 17%, pleural effusion in 10% and lymphadenopathy in 7%. 3% of patients had a normal X ray.

. Observations made by Maniar et al[60] in patients with HIV TB coinfection, showed unilateral involvement in 71.8% patients and bilateral involvement in 28.2% cases .There was involvement of upper zone in 3.7%, middle zone in 62.5% and lower zone in 33.8% of patients.

In our study, we found that the most common manifestation of pulmonary tuberculosis among HIV infected individuals in chest X ray was infiltration(62%) followed by consolidation(20%) and cavity(12%).This clearly shows that the occurrence of cavity is uncommon with HIV_TB coinfection. Miliary pattern occurred in 3 cases(6%) in our study.

We also observed that bilateral findings occurred in increasing incidence among HIV infected individuals(40%). While upper zone involvement was seen in 38% cases, midzone ,lowerzone and diffuse involvement dominated the scenario(62%). Thus atypical findings were common compared to typical findings(62% versus 32%).

Observations of our study correlated with the findings of previous studies done by Praveen kumar et al[56] which showed typical versus atypical findings(30.3% versus 69.7%).

We also tried to analyse the correlation between radiological findings and sputum smear status. We observed that atypical findings were common with sputum smear negative cases. Among the 25 smear negative cases,21 had atypical findings and the difference was statistically significant. This was explained by the fact that all the cases with atypical findings occurred among individuals with CD4 count <200 among whom sputum negativity increased. In contrary to the expectations, we also found that sputum negativity was common among cases with bilateral radiologic findings.

Therefore our study explains the fact that in early HIV clinical profile of pulmonary tuberculosis mimics post primary tuberculosis whereas the presentation in advanced HIV mimics primary tuberculosis.

CONCLUSION

1. HIV TB coinfection is often associated with reduced CD4 counts.
2. Pulmonary tuberculosis in HIV infected individuals has lower sputum AFB density. As the CD4 cell count count decreases,sputum AFB density decreases.
3. The most common radiological findings in HIV TB coinfection are infiltration, consolidation and cavity. Atypical findings such as diffuse involvement, mid zone and lower zone involvement and bilateral findings are common in HIV TB coinfection.
4. Our study endorsed WHO's fact that sputum smear negativity increases as the CD4 count falls below 200. Though sputum smear microscopy remains a gold standard method for diagnosing pulmonary tuberculosis in immunocompromised host with CD4 count more than 200,there is an urgent need for better diagnostic tools in patients with CD4 count below 200.

BIBLIOGRAPHY

1. Murray, J.F.: Cursed Duet: HIV Infection and Tuberculosis. Respiration; 57:210-220. Herzog. H,S. Karger, A.G. Basel.
2. Annie Luetkemeyer ,Tuberculosis and HIV,HIV in site knowledge base chapter January 2013.
3. WHO report 2011:Global Tuberculosis control 2011.
4. TB India 2010: RNTCP Status Report Central TB Division, Directorate General of Health Services, Ministry of Health and Family Welfare, Nirman Bhawan, New Delhi-110001, 2010.
5. Global Report. UNAIDS report on the global AIDS epidemic 2010.
6. NACO Annual Report 2010-2011.
7. S.P. Agarwal, Dipanjan Roy, L.S. Chauhan TB-HIV Co-infection:A Lethal Combination tb control in india chapter 16 145 to 154.
8. Harrison's principles of internal medicine 18 th edition chapter 165:1340.
9. Preetish S. Vaidyanathan1 & Sanjay Singh TB-HIV co-infection in india NTI Bulletin 2003, 39 / 3&4, 11-18.
10. Guidelines for Quality Assurance of smear microscopy for diagnosing tuberculosis. Central TB Division, Directorate General of Health

Services, Ministry of Health and Family Welfare, Nirman Bhawan, New Delhi-110001. 2005.

11. TB CDC guidelines 2009
12. Monkongdee P, McCarthy KD, Cain KP, et al. Yield of acid-fast smear and mycobacterial culture for tuberculosis diagnosis in people with human immunodeficiency virus. Am J Respir Crit Care Med. 2009 Nov 1;180(9):903-8.
13. Yassin MA, Cuevas LE. How many sputum smears are necessary for case finding in pulmonary tuberculosis? Trop Med Int Health 2003; 8 : 927-32.
14. Elliot AM, Namaambo K, AllenBW, Luo N, Hayes RJ, Pobee JO, et al. Negative sputum smear results in HIV positive patients with pulmonary tuberculosis in Lusaka, Zambia. Tubercle Lung Dis 1993; 74 : 191-4.
15. Steingart KR, Ng V, Henry M, Hopewell PC, Ramsay A, Cunningham J, et al. Sputum processing methods to improve the sensitivity of smear microscopy for tuberculosis: a systematic review. Lancet Infect Dis 2006; 6 : 664-74.
16. Cattamanchi A, Dowdy DW, Davis JL, Worodria W, YooS, Joloba M, et al. Sensitivity of direct versus concentrated sputum smear microscopy in HIV-infected patients suspected of having pulmonary tuberculosis. BMC Infect Dis 2009; 9 :53.

17. Steingart KR, Henry M, Ng V, Hopewell PC, Ramsay A, Cunningham J, et al. Fluorescence versus conventional sputum smear microscopy for tuberculosis : a systematic review. Lancet Infect Dis 2006; 6 : 570-81.
18. Dorman S. New diagnostic tests for tuberculosis: bench, bedside, and beyond. Clin Infect Dis 2010; 50 (Suppl 3): 173-7.
19. FIND study trials at a glance: Primo Star iLED microscope World Health Organization. Improving the diagnosis and treatment of smear negative pulmonary and extrapulmonary tuberculosis among adults and adolescents: recommendations for HIV-prevalent and resource-constrained settings. Geneva:World Health Organization; 2007.
20. Revised National TB Control Programme Training Manual for Mycobacterium tuberculosis Culture & Drug susceptibility testing April 2009 Central TB Division Directorate General of Health Services Ministry of Health and Family Welfare, Nirman Bhawan, New Delhi 110011.
21. WHO (2007) Improving the diagnosis and treatment of smearnegative pulmonary and extrapulmonary tuberculosis among adults and adolescents: Recommendations for HIV-prevalent and resource-constrained settings.
22.. Farnia P, Mohammadi F, Mirsaedi M, Zarife AZ, Tabatabee J,Bahadori K, et al. Application of oxidation reduction assay for

monitoring treatment of patients with pulmonary tuberculosis. J Clin Microbiol 2004; 42 : 3324-5.

23. Lee JJ, Suo J, Lin CB, Wang JD, Lin TY, Tsai YC. Comparative evaluation of the BACTEC MGIT 960 system with solid medium for isolation of mycobacteria. Int J Tuberc Lung Dis 2003; 7 : 569-74.

24. Moore DA, Mendoza D, Gilman RH, Evans CA, Hollm Delgado MG, Guerra J, *et al*. Microscopic observation drug susceptibility assay, a rapid, reliable diagnostic test for multidrug resistant tuberculosis suitable for use in resourcepoor settings. J Clin Microbiol 2004; 42 : 4432-7.

25. Kalantri S, Pai M, Pascopella L, Riley L, Reingold A. Bacteriophage based tests for the detection of Mycobacterium tuberculosis in clinical specimens: a systematic review and meta-analysis. BMC Infect Dis 2005; 5 : 59.

26. Baylan O, Kisa O, Albay A, Doganci L. Evaluation of a new automated, rapid, colorimetric culture system using solid medium for laboratory diagnosis of tuberculosis and determination of anti-tuberculosis drug susceptibility. Int J Tuberc Lung Dis 2004; 8 : 772-7.

27. Chaudahary M, Gupta S, Khare S, Lal S. Diagnosis of tuberculosis in an era of HIV pandemic: A review of current status and future prospects. Indian J Med Micro 2010; 28 : 281-9.

28. World Health Organization. Molecular line probe assays for rapid screening of patients at risk of multidrug resistant tuberculosis.
29. World Health Organization and STOP TB department. Roadmap for rolling out Xpert MTB/RIF for rapid diagnosis of TB and MDR-TB.
30. . Rachow A, Zumla A, Heinrich N, Rojas-Ponce G, Mtafya B, Reither K et al. Rapid and accurate detection of Mycobacterium tuberculosis in sputum samples by Cepheid Xpert MTB/ RIF assay – a clinical validation study. PLoS One 2011; 6 : e20458.
31. Van Rie A, Page-Shipp L, Scott L, Sanne I, Stevens W. Xpert(®) MTB/RIF for point-of-care diagnosis of TB in high- HIV burden, resource-limited countries: hype or hope? Expert Rev Mol Diagn 2010; 10 : 937-46.
32. . Zeka AN, Tasbakan S, Cavusoglu C. Evaluation of the GeneXpert MTB/RIF Assay for the Rapid Diagnosis of Tuberculosis and detection of RIF-resistance in Pulmonary and Extra pulmonary Specimens. J Clin Microbiol 2011; 49 : 4138-41.
33. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, et al. Rapid molecular detection of tuberculosis and rifampin resistance. N Eng J Med 2010; 363 : 1005-15.
34. Gennaro ML. Immunologic diagnosis of tuberculosis. Clin Infect Dis 2000; 30 : S243-6.

35. Chan ED, Heifets L, Iseman MD. Immunologic diagnosis of tuberculosis: A review. Tuberc Lung Dis 2000; 80 : 131-40.
36. Morris K. WHO recommends against inaccurate tuberculosis tests. Lancet 2011; 377 : 113-4.
37. Dowdy DW, Steingart KR, Pai M. Serological testing versus other strategies for diagnosis of active tuberculosis in India: a cost-effectiveness analysis. PLoS Med 2011; 8 : e1001074.
38. Kashyap RS, Ramteke SS, Morey SH, Purohit HJ, Taori GM, Daginawala HF. Diagnostic value of early secreted antigenic target-6 for the diagnosis of tuberculous meningitis patients. Infection 2009; 37 : 508-13.
39. Mutetwa R, Boehme C, Dimairo M, Bandason T, Munyati SS, Mangwanya D, et al. Diagnostic accuracy of commercial urinary lipoarabinomannan detection in African tuberculosis suspects and patients. Int J Tuberc Lung Dis 2009; 13 :1253-9.
40. Shah M, Variava E, Holmes CB, Coppin A, Golub JE, McCallum J, et al. Diagnostic accuracy of a urine lipoarabinomannan test for tuberculosis in hospitalized patients in a high HIV prevalence setting. J Acquir Immune Defic Syndr 2009; 52 :145-51.
41. Swaminathan S, Subbaraman R, Venkatesan P, Subramanyam S, Kumar SR, Mayer KH, et al. Tuberculin skin test results in HIV-

infected patients in India: Implications for latent tuberculosis treatment. Int J Tuberc Lung Dis 2008; 12 : 168-73.

42. Pai M, Riley LW, Colford JM Jr. Interferon-gamma assays in the immunodiagnosis of tuberculosis: a systematic review.Lancet Infect Dis 2004; 4 : 761-76`.
43. Pai M. Alternatives to the tuberculin skin test: interferon γ assays in the diagnosis of Mycobacterium tuberculosis infection. Indian J Med Micro 2005; 23 : 151-8.
44. Revised National Tuberculosis Control Programme-DOTS plus guidelines 2010.
45. Swaminathan S, Narendran G, Venkatesan P, Iliayas S, Santhanakrishnan R, Menon PA, et al. Efficacy of a 6-month versus 9-month intermittent treatment regimen in HIV-infected patients with tuberculosis: a randomized clinical trial. Am J Respir Crit Care Med 2010; 181 : 743-51.
46. Antiretroviral therapy for HIV infection in adults and adolescents - World Health organization 2010.
47. Antiretroviral Therapy Guidelines for HIV-infected Adults and Adolescents Including Post-exposure Prophylaxis. National AIDS Control Organization, Ministry of Health and Family Welfare, Government of India 2007.

48. McIlleron H, Meintjes G, Burman WJ, Maartens G. Complications of antiretroviral therapy in patients with tuberculosis: drug interactions, toxicity, and immune reconstitution inflammatory syndrome. J Infect Dis 2007; 196: S63-S75.
49. Blanc F, Sok T, Laureillard D, Borand L, Rekacewicz C, Nerrienet E, *et al*. Earlier versus later start of antiretroviral therapy in HIV-infected adults with tuberculosis. N Engl J Med 2011; 365 : 1471-81.
50. Meintjes G, Lawn SD, Scano F, Maartens G, French MA, Worodria W, et al. International network for the study of HIV-associated IRIS. Tuberculosis associated immune reconstitution inflammatory syndrome: case definitions for use in resource-limited settings. Lancet Infect Dis 2008; 8 : 516-23.
51. Lawn SD, Bekker LG, Miller RF. Immune reconstitution disease associated with mycobacterial infections in HIVinfected individuals receiving antiretrovirals. Lancet Infect Dis 2005; 5 : 361-73.
52. Pichenik AE,Rubinson HA. The radiographic appearance of tuberculosis in patients with the Acquired Immune Deficiency Syndrome(AIDS) and pre-AIDS.Am Rev Respir Dis 1985;131(3):393-6.
53. Smith RL,Yew K,Berkowitz KA and Aranda CP. Factors affecting the yield of acid fast sputum smears in patients with HIV and Tuberculosis.Chest 1994;106:684-6.

54. NC Klein, FP Duncanson, TH Lenox,3rd ,A Pitta,SC Cohen and GP Wormser. Use of mycobacterial smears in the diagnosis of pulmonary tuberculosis in AIDS/ARCpatients. Chest 1989;95:1190-2.

55. Long R,Scalcini M,Manfreda L,Baptiste MJ and Hershfield E. The impact of HIV on the usefulness of sputum smears for the diagnosis of Tuberculosis. Am J Public health 1991;81:1326-8.

56. Praveen Kumar, Niraj Sharma, N.C. Sharma and Sudhakar Patnaik Clinical Profile of Tuberculosis in Patients with HIV Infection/AIDS Indian J Chest Dis Allied Sci 2002; 44 : 159-163.

57. Rajasekaran S, Uma A, Kamakshi S, *et al.* Trend of HIV infection in patients with tuberculosis in rural South India. Indian J Tub 2000; 47 : 223-26.

58. Sameer Singhal S, Mahajan SN, Diwan SK, Gaidhane A, Quazi ZS. Correlation of sputum smear status with CD4 count in cases of pulmonary tuberculosis and HIV co-infected patients--a hospital based study in a rural area of Central India. indian J Tuberc. 2011 Jul;58(3):108-12.

59. Mugusi F, Villamor E, Urassa W, Saathoff E, Bosch RJ, Fawzi WW. HIV TB co-infection, CD4 cell counts and clinical correlates of bacillary density in pulmonary tuberculosis. Int J Tuberc Lung Dis. 2006 Jun;10(6):663-9.

60. Maniar JK, Kamath RR, Mandalia S, Shah K, Maniar A. HIV and tuberculosis: Partners in crime. Indian J Dermatol Venereol Leprol 2006;72:276-82.

PROFORMA

Name:

Age:

Sex: Male/Female

Address:

Occupation:

History of present illness:

1. cough
2. fever
3. hemoptysis
4. weight loss
5. loss of appetite

Past history:

Diabetes mellitus / Hypertension / chronic kidney disease / CAHD /

drug intake/HIV

General Examination:

Systemic Examination:

CVS:

RS:

PER ABDOMEN:

CNS:

Investigations:

CBC

Blood sugar

Urea

Serum creatinine

LFT

Sputum AFB _A

_B

ICTC

CD4 count

Chest X ray

CT chest

MASTER CHART

SL. NO.	NAME	AGE	SEX	DURATION	SPUTUM STATUS	SPUTUM DENSITY	CD4 COUNT	RADIOLOGICAL FINDINGS
1	Sheikh jamal	32	M	New	Positive	1+	195	Left lower zone consolidation
2	Mariappan	36	M	New	Negative		216	Bilateral upper zone infiltrates
3	Murugan	42	M	1	Negative		23	Right midzone consolidation
4	Meena	30	F	New	Negative		172	Bilateral lower zone infiltrates
5	Mariappan	36	M	New	Positive	2+	105	Right lowerzone consolidation
6	Murugan	40	M	New	Positive	Scanty/6	50	Miliary
7	Velthai	37	F	1	Negative		40	Bilateral diffuse infiltrates
8	Manikandan	34	M	New	Positive	1+	295	left upper zone infiltrates
9	Marimuthu	41	M	New	Positive	scanty/9	271	Bilateral upper zone infiltrates
10	Shanmuganathan	42	M	New	Negative		39	Bilateral mid and lower zone infiltrates
11	Subramani	38	M	New	Negative		99	Bilateral lower zone infiltrates
12	Jeyendrasankar	36	M	New	Negative		116	Left mid zone consolidation
13	Anthony	33	M	8	Positive	3+	69	Right lowerzone cavity
14	Murugesan	40	M	New	Positive	Scanty/5	98	Left lower zone infiltrates
15	Kathar mydeen	41	M	6 months	Positive	1+	133	Right midzone consolidation
16	Kamaraj	49	M	New	Negative		166	Bilateral mid and lower zone infiltrates
17	Muthu	36	M	9	Positive	3+	193	Right upper zone infiltrates
18	Muthu	27	M	4	Positive	1+	304	Bilateral upper zone infiltrates
19	Madasamy	45	M	New	Positive	Scanty/5	313	Right upper zone cavity
20	Murugalakshmi	31	F	New	Negative		66	Bilateral diffuse infiltrates

21	Thangamalai	35	M	New	Negative		49	Bilateral mid and lower zone infiltrates
22	Sheikh davood	45	M	New	Negative		195	Left lower lobe consolidation
23	Ranganathan	40	M	2	Negative		297	Bilateral upper zone infiltrates
24	Manikandan	65	M	New	Negative		234	Bilateral upper zone infiltrates
25	Rathakrishnan	39	M	3	Negative		240	Right upper zone infiltrates
26	Muruganantha n	33	M	6 months	Negative		121	Bilateral lower zone infiltrates
27	Ramalingam	47	M	2	Positive	2+	248	Right upper zone cavity
28	Mumtaj	48	F	New	Positive	2+	96	Right lower lobe consolidation
29	Paramasivan	48	M	5	Positive	Scanty/7	261	Left upper zone infiltrates
30	Manikandan	37	M	6 months	Positive	2+	339	Right upper lobe consolidation
31	Subramanian	33	M	New	Negative		33	Bilateral diffuse infiltrates
32	Ulagammal	55	F	New	Positive	3+	411	Bilateral upper zone infiltrates
33	Maniraj	31	M	New	Negative		103	Bilateral mid and lower zone infiltrates
34	Muthu	48	M	2 Y	Positive	3+	179	Left mid zone cavity
35	Raju	35	M	2 Y	Positive	Scanty/3	173	Right midzone consolidation
36	Kumar	32	M	2 Y	Positive	3+	334	Right upper zone cavity
37	Murugan	40	M	New	Negative		72	Bilateral mid and lower zone infiltrates
38	Petchiammal	30	F	2 Y	Positive	Scanty/12	126	Right mid and lower zone infiltrates
39	Punniabharathi	30	M	6 Y	Negative		47	Left mid and lower zone infiltrates
40	Ponnarasi	39	F	7 Y	Positive	2+	310	Left upper zone infiltrates
41	Vasantha	28	F	5 Y	Negative		152	Left mid zone infiltrates
42	Gomathy	37	M	New	Negative		33	Miliary
43	Ramalakshmi	45	F	7 Y	Positive	1+	261	Bilateral upper zone cavity

44	Sankar	37	M	6 Y	Negative		121	Bilateral mid and lower zone infiltrates
45	Kanagaraj	60	M	5 Y	Positive	2+	310	Right upper zone infiltrates
46	Jeyakumar	35	M	1 Y	Negative		18	Left mid zone consolidation
47	Maniyammal	29	F	7 Y	Positive	3+	330	Right upper zone infiltrates
48	Petchiammal	50	F	New	Negative		133	Bilateral lower zone infiltrates
49	Manikam	45	M	New	Negative		61	Miliary
50	Raveendran	43	M	New	Positive	3+	393	Left upper zone infiltrates