“TO STUDY SERUM VITAMIN D LEVELS IN PATIENTS WITH PULMONARY TUBERCULOSIS – A ONE YEAR HOSPITAL BASED CROSS SECTIONAL STUDY”

REG NO. BG0111003

Dissertation

Submitted to the
KLE University, Belgaum, Karnataka

In Partial Fulfillment
of the requirements for the degree of

M. D.
in
GENERAL MEDICINE

DEPARTMENT OF MEDICINE,
JAWAHARLAL NEHRU MEDICAL COLLEGE,
BELGAUM, KARNATAKA

APRIL - 2014

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ENDORSEMENT

This is to certify that the dissertation entitled **"TO STUDY SERUM VITAMIN D LEVELS IN PATIENTS WITH PULMONARY TUBERCULOSIS – A ONE YEAR HOSPITAL BASED CROSS SECTIONAL STUDY"** is a bonafide research work done by **THE CANDIDATE REG NO. BG0111003**.

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LIST OF ABBREVIATIONS USED

25(OH)D	-	25-hydroxyvitamin D
AFB	-	Acid fast bacilli
AIDS	-	Acquired immunodeficiency syndrome
BACTEC	-	Bactenicin
BC	-	Before Christ
BCG	-	Bacillus Calmette-Guerin
CO2	-	Carbon dioxide
CR	-	Complement receptor
cum	-	Cummulative
CXR	-	Chest X-ray
DOTS	-	Directly observed treatment short course
DTH	-	Delayed type hypersensitivity
ELISA	-	Enzyme-linked immunosorbent assay
FEV_1	-	Forced expiratory volume in 1 s
gm	-	gram
HIV	-	Human immunodeficiency virus
IFN-g	-	interferon-gamma
LAM	-	Lipoarabinomannan
LD	-	linkage disequilibrium
LTBI	-	Latent Tuberculosis Infection
mg/dL	-	Milligram per decilitre
MGIT	-	Mycobacterial growth indicator tube
mRNA	-	Messenger ribonucleic acid

MTB	-	Mycobacterium tubercolosis
n	-	Total number
NHANES III	-	National Health and Nutrition Examination Survey III
NKT	-	Natural killer T
p	-	Probability value
PCP	-	Phenylcyclidine
PCR	-	Polymerase chain reaction
PI3K	-	Phosphatidylinositol-3-kinases
PTB	-	Pulmonary tuberculosis
RFLP	-	Restriction fragment length polymorphism
SD	-	Standard deviation
T→C	-	Thymine-to-cytosine
TB	-	Tuberculosis
Th	-	T-helper
TLR	-	Toll-like receptors
TST	-	tuberculin skin test
UK	-	United kingdom
UVB	-	Ultraviolet B
VDD	-	Vitamin D deficiency
VDR	-	Vitamin D receptors
VitD	-	Vitamin D
WBC	-	White blood cells
WHO	-	World health organization

ABSTRACT

Background and objectives

The present study was undertaken to estimate the levels of vitamin D in patients with pulmonary tuberculosis and to estimate vitamin D levels with different degrees of sputum positivity of pulmonary tuberculosis.

Methodology

This one year cross sectional study was carried out on a total of 50 patients diagnosed with pulmonary tuberculosis based on WHO criteria from January 2012 to December 2012 in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum.

Results

Of the 50 patients, 74% of patients were males and 26% were females and the male to female ratio was 2.8:1. The commonest age group was 31 to 45 years (36%) and the mean age among males and females was 39.5 ± 17.93 and 45.3 ± 14.09 years respectively. The vitamin D levels were low in most (66%) of the patients while 12% of patients had insufficient vitamin D levels and 22% of the patients had vitamin D levels within the normal range. The mean vitamin D levels were 17.05 ± 10.04. The sputum smear for AFB was negative in 26% of patients,1+ in 22%, 2+ in 32% and 3+ in 20% of the patients respectively. Of the 10 patients with 3+ AFB, 70% of patients had vitamin D levels of ≤ 20 while 30% of patients had vitamin D levels ≥ 30. The mean vitamin D levels in patients with negative AFB findings were significantly high (25.10 ± 7.83) compared to those with 1+ (14.77 ± 5.65), 2+ (13.39 ± 9.51) and 3+ (14.94 ± 12.34). Also the

mean vitamin D levels in patients aged ≤ 30 years were significantly high (26.86 ± 10.08) compared to those who were aged 31 to 45 (17.16 ± 9.60) and more than 60 years (10.61 ± 4.95).

Conclusion and interpretation

Vitamin D deficiency was observed in majority of the patients with pulmonary tuberculosis and there was a strong association between the vitamin D levels with increasing AFB grades and age.

Keywords

Acid fast bacilli; Pulmonary tuberculosis; Sputum smear; Vitamin D;

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Chapter 1

Introduction

INTRODUCTION

Tuberculosis, or TB, is an infectious bacterial disease caused by Mycobacterium tuberculosis, which most commonly affects the lungs. It is transmitted from person to person via droplets from the throat and lungs of people with the active respiratory disease.[1] It typically affects the lungs (pulmonary TB) but can affect other sites as well (extra pulmonary TB).[2]

Recent statistics by World Health Organization have reported TB as second only to HIV/AIDS as the greatest killer worldwide due to a single infectious agent. In 2011, 8.7 million people fell ill with TB and 1.4 million died from TB. Over 95% of TB deaths occur in low- and middle-income countries, and it is among the top three causes of death for women aged 15 to 44. It is a leading killer of people living with HIV causing one quarter of all deaths.[1]

Geographically, the burden of TB is highest in Asia and Africa. India and China together account for almost 40% of the world's TB cases. About 60% of cases are in the South-East Asia and Western Pacific regions. The African Region has 24% of the world's cases, and the highest rates of cases and deaths per capita.[3]

The estimated number of people falling ill with tuberculosis each year is declining, although very slowly, which means that the world is on track to achieve the Millennium Development Goal to reverse the spread of TB by 2015. The TB death rate dropped 41% between 1990 and 2011. An estimated 20 million lives saved through use of DOTS and the Stop TB Strategy recommended by WHO.[1]

Tuberculosis mostly affects young adults, in their most productive years. However, all age groups are at risk. Over 95% of cases and deaths are in developing countries.[1]

Patients with active pulmonary TB may be asymptomatic, have mild or progressive dry cough, or present with multiple symptoms, including fever, fatigue, weight loss, night sweats, and a cough that produces bloody sputum.[4]

Common symptoms of active lung TB are cough with sputum and blood at times, chest pains, weakness, weight loss, fever and night sweats. When a person develops active TB (disease), the symptoms (cough, fever, night sweats, weight loss etc.) may be mild for many months. This can lead to delays in seeking care, and results in transmission of the bacteria to others. People ill with TB can infect up to 10-15 other people through close contact over the course of a year. Without proper treatment up to two thirds of people ill with TB will die.[1]

Many countries still rely on a long-used method called sputum smear microscopy to diagnose TB. Trained laboratory technicians look at sputum samples under a microscope to see if TB bacteria are present. With three such tests, diagnosis can be made within a day, but this test does not detect numerous cases of less infectious forms of TB.[1]

Recently, research has found that vitamin D may play a role in multiple chronic diseases such as cancer, autoimmune diseases, infections, and cardiovascular disorders.[5] Vitamin D may also have a role in several diseases involving the respiratory system. Higher vitamin D concentrations, assessed by 25-hydroxyvitamin D [25(OH)D], have been associated with better lung function

as measured by forced expiratory volume in 1 s (FEV_1) in a large cross-sectional study of the U.S. population in the NHANES III.[5]

Several lines of evidence suggest a link between vitamin D and TB.[6-9] The active form of vitamin D (1,25(OH)2D3) has been shown to inhibit growth of Mycobacterium tuberculosis (*M. tuberculosis*) through stimulating cell-mediated immunity and activating monocytes.[6]

Vitamin D is a seco-steroid hormone important in bone mineralization and calcium homeostasis. Vitamin D modulates monocyte-macrophage activity in the body and plays a role in human innate immunity to certain infectious agents. This role may be important in the body's defence against tuberculosis, in which attack of macrophages is a key step in pathogenesis. Vitamin D acts by binding to nuclei receptors on target cells. Therefore both low levels of the vitamin and abnormalities in receptor structure and function may result in impairments in host immunity to the tubercle bacillus.[5]

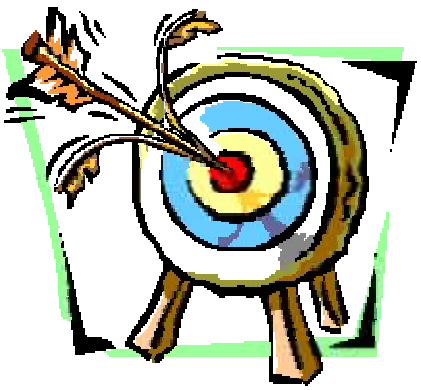
Although the precise connection between vitamin D status and lung function is unclear at this point, the mechanism by which vitamin D improves lung function may be through its action on regulating inflammation,[10-12] inducing antimicrobial peptides,[13] and/or its action on muscle.[14,15]

While the association between vitamin D and TB has been studied in many Asian communities in Western countries, few studies have been done in Asian populations especially in India. Also, no such study was undertaken in our hospital settings in the past. Hence the present study was undertaken to estimate the levels of vitamin D in patients with pulmonary tuberculosis and to estimate

vitamin D levels with different degrees of sputum positivity of pulmonary tuberculosis which would help in the management and prevention of pulmonary tuberculosis.

Chapter 2

Objectives



OBJECTIVES

The objectives of the study were;

- To estimate the levels of vitamin D in patients with pulmonary tuberculosis.
- To estimate vitamin D levels with different degrees of sputum positivity of pulmonary tuberculosis.

Chapter 3

Review of Literature

REVIEW OF LITERATURE

History of tuberculosis

Tuberculosis (TB) has a long history, documented in Egypt as early as 5,000 years ago. Its causative agent, *Mycobacterium tuberculosis*, may have killed more persons than any other microbial pathogen.[16]

The term “phthisis” (meaning consumption) appeared first in Greek literature. Around 460 BC, Hippocrates identified phthisis as the most widespread disease of the times and noted that it was almost always fatal.[17]

The TB epidemic in Europe, known as the “Great White Plague”, started in 17th century and continued for next 200 years. Death from TB was considered inevitable and, by 1650, TB was the leading cause of mortality. The high population density and poor sanitary conditions of the enlarging cities of Europe and North America at that time, provided the necessary environment, for the spread of this airborne pathogen.[18]

Precise pathological and anatomical descriptions of the disease began to appear in 17th century. Sylvius was the first to identify tubercles in the lungs and other areas of consumptive patients. He also described progression of the lesions to ulcers and cavities.[19] In 1720, English physician Benjamin Marten was the first to conjecture that TB could be caused by “minute living creatures". He further stated, that consumption may be caught by a sound person by lying in the same bed, eating and drinking or by talking to one another.[20]

The first real breakthrough came when German bacteriologist, Robert Koch isolated the infectious agent known as "tuberkle bazillus" in 1882. He was later awarded the Nobel prize for physiology and medicine in 1905. The scientific brilliance of Koch's discovery was indeed a huge milestone, and along with it came the certainity that the fight against humanity's deadliest enemy is now possible.[21]

Epidemiology of tuberculosis

TB occurs in every part of the world. About one third of the world's population is infected with tuberculosis. It is a treatable and curable disease that kills almost 2 million people every year. It is second only to HIV/AIDS as the greatest killer worldwide due to a single infectious agent.[22]

Effective drugs to treat and cure the disease have been available for more than 50 years, yet every 15 seconds, someone in the world dies from TB. Even more alarming: a person is newly infected with *M. tuberculosis* every second of every day. Left untreated, a person with active TB will infect an average of 10 to 15 other people every year.[20] The number of new cases that occur each year also continues to grow.

Global TB disease burden

Following neglect of disease during the 1980s[23,24] TB control has been high on international agenda since 1990s. In 1993, World Health Organization (WHO) declared TB a global public health emergency.

WHO has published a global report on TB every year since 1997. The 2011 edition of WHOs annual global TB report is 16th in the series.[2]

- Global incidence estimated at 8.8 million cases.
- Most of them occurred in Asia(59%) and Africa(26%)
- The 22 high tuberculosis burden countries that have been given highest priority at the global level since 2000 accounted for 81% of all estimated cases worldwide.
- The five countries with the largest number of incident cases in 2010 were India(2.0 –2.5 million), China(0.9 –1.2 million), South Africa, Indonesia and Pakistan.
- India and China combined accounted for 38% of all cases.
- Estimated 12.0 million prevalent cases of TB
- Of the 8.8 million incident cases, 1.0 – 1.2 million (12–14%) were among people living with HIV.
- The proportion of TB cases co-infected with HIV is highest in countries in the African Region(accounted for 82%)
- Approximately 1.4 million deaths (1.1 million cases in HIV negative cases of TB and an additional 0.3 million in HIV positive cases of TB)

<u>Other notable findings</u> [22]

- Tuberculosis mostly affects young adults, in their most productive years. However, all age groups are at risk.
- Over 95% of cases and deaths are in developing countries.
- It is among the top three causes of death for women aged 15 to 44.

- About half a million children (0-14 years) fell ill with TB, and 64 000(a range of 58 000 to 71 000) children died from the disease in 2010.
- In 2009, there were about 10 million orphan children as a result of TB deaths among parents.
- People infected with TB have a lifetime risk of TB of 10%.
- Persons with compromised immune systems, such as people living with HIV, malnutrition or diabetes, or people who use tobacco, have a much higher risk.
- At least one-third of the 34 million people living with HIV worldwide are infected with TB bacteria, although not yet ill with active TB. People living with HIV and infected with TB are 21 to 34 times more likely to develop active TB disease than people without HIV.

TB Burden in India

Though India is the second-most populous country in the world, India has more new TB cases annually than any other country.[25] In 2010 of estimated 8.8 million cases India had 2.0-2.5 million cases accounting for one quarter (26%) of cases worldwide.[2] It is estimated that about 40% of Indian population is infected with TB bacillus.[22]

Estimates of TB burden: WHO global report - 2011 [Rates/ 1 Lakh population][2]

Variables	World	India
Incidence	128	185
Prevalence (Including HIV)	178	256
Mortality (Including HIV)	15	26

Natural history of tuberculosis

Tuberculosis can affect any organ in the body. Pulmonary tuberculosis is the most frequent site of involvement; extrapulmonary tuberculosis is less frequent.[26]

TB is a disease with an inter-human transmission. Tuberculous bacilli are spread out by infected patients (Flugge's droplets - small aerodynamic particles) coughing, sneezing, or speaking, and they can be inhaled by another individual in close contact. The inhalation of these sprays, presents a risk of infection. These particles can also remain in the air and play the role of reservoir.[27]

Once the infectious particles are inhaled, only those with two or three bacilli can reach the bronchic cells, the largest ones are stopped upstream and eliminated.[28] The success of such infection and the development of the pulmonary form of TB depend on four successive stages: bacilli phagocytosis, intracellular multiplication, the stationary stage, and the pulmonary form of TB.[27]

(i) **Bacilli phagocytosis**

This step takes place in first week following inhalation, and it depends on two main factors: the bacillus virulence and the bactericidic activity of the macrophage. In general, the phagocytosed bacteria are destroyed by the alveolar macrophages and the infection is stopped at this stage, otherwise they begin an intracellular cycle of multiplication.[29]

(ii) **Intracellular multiplication**

This second stage occurs between the 7th and the 21st day. It corresponds to intracellular bacilli multiplication in the macrophage alveoli and is also called the symbiotic stage. They are released after cellular lysis, and can thus infect other circulating macrophages and continue their multiplication. At the end of this stage, a huge number of macrophages and bacilli are concentrated at the level of early pulmonary lesions.[29]

(iii) **Stationary stage**:

Following the induction of the immune response of the host, particularly cell-mediated immunity bacterial growth becomes stationary.[30] This is the third stage of the infection called *primary infection*. Because of a delayed-type hypersensitivity, the macrophages in which bacilli multiply are destroyed. Bacterial toxins and cellular products are released, and this leads to the formation of solid caseous necrosis,[31] where a pseudo-equilibrium settles between inactivated and mature macrophages. At this stage, either the number of infected cells in the caseous center decreases if the released bacilli are phagocyted by the

mature macrophages or it increases if the bacilli multiply in the inactivated macrophages. Thus, the progression of the disease depends on which macrophage type prevails.[32] At this stage, bacilli may become dormant and never induce TB at all, which is referred to as a *latent infection* that is detected only by a *positive tuberculin skin test*; or the latent organisms can eventually begin to grow, with resultant clinical disease, known as TB reactivation.

(iv) **Pulmonary form of TB (PTB):**

When the equilibrium between the inactivated and mature macrophages is broken, the infection reaches the last stage, the disease, PTB. This step is characterized by the liquefaction of the caseous center, leading to the formation of a cavity detected by pulmonary radiography. The liquefied material present in this cavity constitutes an excellent growth media for the bacteria, and macrophages do not survive in this environment. At this stage of the disease, the person becomes contagious by releasing the bacilli into the air. Furthermore, without treatment, this individual can develop a chronic TB, presumably leading to death.[27]

These different stages can evolve into different outcomes: spontaneous healing, acute tuberculosis, latent infection, and reactivation or re-infection.[27]

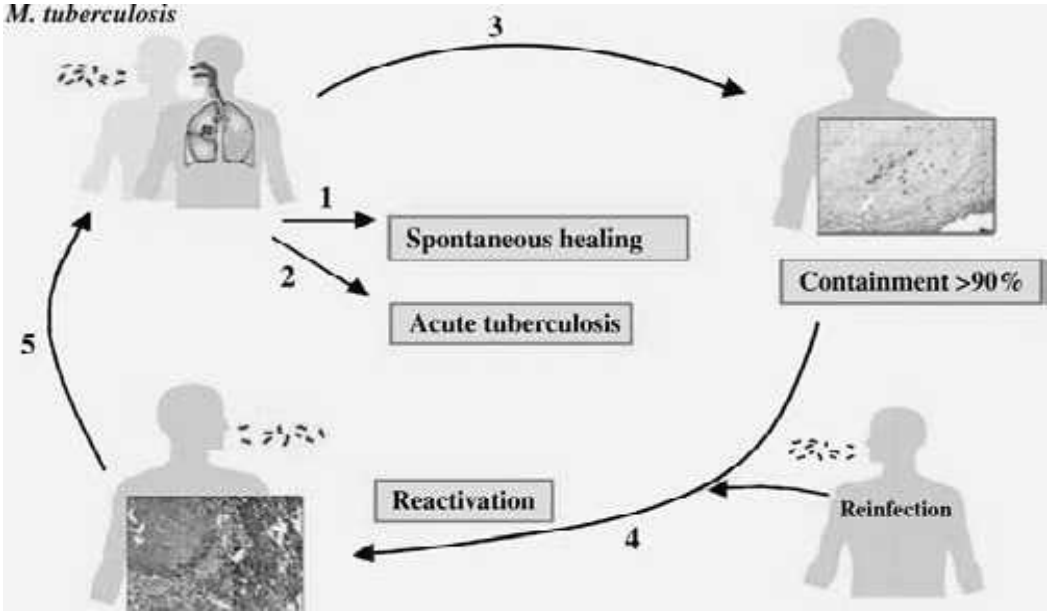


Figure 1. Mycobacterium tuberculosis enters the host within inhaled droplets. Different outcomes are possible (1) Immediate eradication of MTB by the pulmonary immune system (2) Infection transforms into active tuberculosis (3) Infection does not transform into disease because MTB is contained inside granulomas (4) After a latency phase, MTB can become active after either an endogenous reactivation or an exogenous re-infection or both (5) At this stage, there is dissemination and transmission of MTB.[33]

Factors that increase the likelihood of becoming infected with tuberculosis [26]

a) Increases in the intensity and/or duration of exposure.

b) Overcrowding, in buildings that are poorly ventilated.

c) Conditions leading to immune deficiency such as HIV infection, malnutrition, diabetes, or long-term treatment with corticosteroids or immunosuppressive medications.

Virulence Mechanisms

The basis for *M. tuberculosis* virulence is largely unknown. Cell wall components such as LAM (Lipoarabinomannan) have been implicated in binding to alveolar macrophages, utilizing surface fibronectin, mannose, or complement receptors (CR1, CR3) Once inside, multiple factors contribute to survival and continued multiplication. A number of genes have been identified that are linked to virulence by enhancing survival in the macrophage or by influencing the physical and chemical conditions(low pH, high lactic acid, high CO2) present in developing lesions, but their function remains unknown. Mycolic acids, sulfolipids, LAM, and proteins have been shown to disrupt phagosome–lysosome interactions and interfere with oxidative killing. LAM has also been shown to modulate cytokine production and down-regulate other aspects of T-cell function including antigen presentation.[34]

IMMUNITY

Humans generally have a high innate immunity to development of disease. This was tragically illustrated in the Lubeck disaster of 1926 where infants were administered *M. tuberculosis* instead of an intended vaccine strain. Despite the large dose, only 76 of 249 died and most of the others developed only minor lesions.[34]

Approximately 10% of immunocompetent persons infected with *M. tuberculosis* will develop active disease any time in their life. There is epidemiologic and historic evidence for differences in the immunity.[34]

DTH to tuberculoprotein and CMI to *M. tuberculosis* develop 2 to 6 weeks after primary infection. The subsequent course of the infection depends on the balance between these two defensive mechanisms.[34]

DTH, through the mediation of natural killer cells, destroys the inactivated macrophages as well as the surrounding tissues, releasing still viable mycobacteria into an area of necrosis unsuitable for bacterial multiplication.[34]

CMI develops when competent T lymphocytes recognize mycobacterial antigen complexes on the surface of *M. tuberculosis*– containing macrophages. In the presence of macrophage-produced interleukin-1, the activated lymphocytes respond to the presented antigens with the elaboration of several cytokines. Some of these proteins attract circulating monocytes. Others, including interferon and possibly tumor necrosis factor, activate local tissue macrophages and the recruited monocytes to enhanced destruction of ingested mycobacteria, resulting in a slowing or discontinuation of intracellular bacterial growth. Nitrous oxide or other reactive nitrogen intermediates probably mediate the destruction of the mycobacteria. Another cytokine, interleukin- 2, induces clonal expansion of the activated lymphocytes, thus amplifying the host's immunologic response. Still others stimulate accumulation of fibroblasts and deposition of collagen, which help wall off the area of infection and prevent further dissemination.[34]

Acquired immunity is cell mediated but incomplete. Both helper–inducer (CD4+) and cytotoxic (CD8+) T lymphocytes are involved. Two to three weeks after infection, macrophages are activated at the site of infection by a network of pro- and anti-inflammatory cytokines and chemokines from antigen-stimulated

CD4+ T lymphocytes, macrophages, and dendritic cells. This interaction between *M. tuberculosis* and the host is what eventually limits its multiplication and spread. Cytotoxic T cells release bacilli from inactivated phagocytic cells and allow them to be ingested and handled by the activated macrophages. The concomitant DTH to tuberculoprotein plays an important part in immunity to reinfection by mobilizing immune cells and macrophages to the site of deposition of tubercle bacilli. In the past, it was believed that re-infection from external sources was extremely rare, but it is now clear that loss of hypersensitivity and CMI can occur over time and that re-infection can develop into clinical tuberculosis.[34]

The role of DTH in immunity of established tuberculosis is complex, because high degrees of sensitivity can precipitate caseous necrosis and lead to spread of the disease. The importance of CMI and hypersensitivity in modulating the course of tuberculosis is, perhaps, most dramatically illustrated in patients with AIDS. Those with minimal impairment of cellular immune responses develop typical tubercles containing relatively few bacilli. Those with advanced impairment demonstrate abundant acid-fast bacilli without epithelioid cell accumulation or associated tissue necrosis.[34]

Clinical and subclinical infection

The term “TB infection’’ refers to a positive TB skin test with no evidence of active disease; this state is also called latent infection . “TB disease’’ refers to cases that have a positive acid-fast smear or culture for MTB or radiographic and clinical presentation of TB.[32] Only some people develop active

TB disease after infection, almost all TB infections are asymptomatic and remain latent.[35]

Active disease

A patient with PTB presents with the symptoms of chronic or persistent cough and sputum production. If the disease is at an advanced stage, the sputum will contain blood, and the patient will also have lack of appetite, weight loss, fever, night sweats etc.[27]

Patients with PTB are classified in different categories because a specific treatment is needed for each category. The main categories are as follows:[27]

New case: TB in a patient who either has never received anti-tuberculous treatment or started a treatment for less than 1 month.

Relapse: TB already treated and declared cured after sufficient treatment time, which has become active again.

Chronic TB: A case of relapse from which the microscopic exam of expectoration remains positive after a second complete treatment.

Primary resistance case: This characterizes the bacilli that are resistant to treatments, although patients have never been treated by anti-tuberculous drugs

Multi-resistance case: MTB resistant at least to both major anti-tuberculous drugs (isoniazid and rifampicin)

Latent infection

Latent TB is the product of a complex set of interactions between MTB and the host immune response. The bacilli remain dormant until the host defenses are impaired by a disorder such as HIV infection. MTB subsequently reactivate to cause active disease. The latent state of infection is a major obstacle for eradicating TB. In latent TB, the host immune response is capable of controlling the infection but fails to eradicate the pathogen. One-third of the world population is estimated to be infected with the pathogen in the latent stage.[27]

MTB and HIV

For many people, TB is the first sign of immune dysfunction associated with HIV infection, and active TB is an AIDS-defining illness. In some parts of the world, TB is the leading cause of death of people infected with HIV.[27]

In case of HIV co-infection, the risk of developing active TB disease after TB infection, or following an apparent cure of several years, increases considerably from 5% to 10% over a person's lifetime to 7% to 10% per year.[27]

It can occur early in HIV disease when CD4 cell counts are relatively high, in the 300–400 range. In early HIV infection, TB usually infects and affects only the lungs. As CD4 cell counts drop, however, TB is more likely to arise with atypical pulmonary manifestations and extra-pulmonary disease.[36] Thus smear-negative pulmonary and extra-pulmonary TB disease is on rise. HIV-positive patients with smear-negative pulmonary TB have worse treatment outcomes and higher mortality than HIV-positive patients with smear-positive pulmonary TB.[37]

In the long term, only effective control of the HIV epidemic will reverse the associated increase in TB incidence. In the meantime, interventions to reduce HIV-related TB morbidity and mortality need to be implemented.[37]

Manifestations

Primary Tuberculosis

Primary tuberculosis is either asymptomatic or manifest only by fever and malaise. Radiographs may show infiltrates in the mid-zones of the lung and enlarged draining lymph nodes in the area around the hilum. When these lymph nodes fibrose and sometimes calcify, they produce a characteristic picture (Ghon complex) on radiograph. In approximately 5% of patients, the primary disease is not controlled and merges into the reactivation type of tuberculosis, or it disseminates to many organs to produce active miliary tuberculosis. The latter may result from a necrotic tubercle eroding into a small blood vessel.[34]

PATHOPHYSIOLOGY[38]

Tuberculosis is a constitutional disease caused by infection with Mycobacterium tuberculosis, characterized by the production of tubercles in the internal organs, especially in the lungs, where it constitutes the most common variety of pulmonary phthisis.

Etiology[38]

Tuberculosis is one of the oldest known diseases known to affect humans caused by bacteria mycobacterium tuberculosis (M. tuberculosis)**,** characterized

by the production of tubercles in the internal organs, especially in the lungs, where it constitutes the most common variety of pulmonary phthisis (consumption). If properly treated it is curable in virtually all the cases, if untreated the disease may be fatal within five years in more than half the cases. Pulmonary tuberculosis is the one involving the lung with progressive wasting of the body.

Mycobacterium belongs to the family Mycobacteriaceae and order Actinomycetales. They are rod shaped, non-spore forming thin aerobic bacteria measuring 0.5 µm by 3 µm. They do not stain by gram method of stain, faintly gram positive, but once stained cannot be decolorized by acid alcohol hence classified as acid fast bacilli-acid fastness is due to the organisms' high content of mycolic acids, long chain cross linked fatty acids and other cell wall lipids.

The interaction of M. tuberculosis with human host begins when droplet nuclei (less than 10 µm in diameter) which are aerosolized by coughing, sneezing or speaking from an infectious patient are inhaled. While the majority of the inhaled bacilli are trapped in the upper airways and expelled by ciliated mucosal cells, a fraction reaches the alveoli.

Risk Factors for active tuberculosis among persons who have been infected with tubercle bacilli[38]

Risk Factor	Relative risk / Odds
Recent Infection (less than one year)	12.9
Fibrotic lesion spontaneously healed	2 – 30
Comorbidity	
HIV Infection	100
Silicosis	30
Chronic renal failure / Hemodialysis	10 – 25
Diabetes	2 – 4
Intravenous drug use	2 – 4
Immunosuppressant treatment	10
Gastrectomy	2 – 5
Jejunoleal bypass	30 – 60
Post transplantation period	20 – 70
Malnutrition and underweight	2

The risk of developing the disease after being infected depends on factors like individual's innate susceptibility to the disease and level of cell mediated immunity. Clinical illness directly following infection is classified as primary tuberculosis. Among the infected individuals the incidence is highest during late adolescence and early adulthood peaks between 25 to 34 years of age. In children

and in persons with impaired immunity (for example, malnutrition or HIV infection) the disease may progress rapidly to clinical illness.

In the initial stage of host bacterium interaction, either the host macrophages contain bacillary multiplication by producing proteolytic enzymes and cytokines or the bacilli begin to multiply. If the bacilli multiply, their growth quickly kills the macrophages which lyse. The balance between the bactericidal activity of the macrophage and the number and virulence of the bacilli determines the events following phagocytosis.

After about two to four weeks after infection, two additional host responses to M. tuberculosis develop a tissue damaging response and a macrophage activating response.

The tissue damaging response is result of delayed type hypersensitivity (DTH) reaction to various bacillary antigens; it destroys non activated macrophages. The macrophage activating response is a well mediated phenomenon resulting in activation of macrophages that ingest and kill the bacilli.

With the development of specific immunity the accumulation of large number of activated macrophages at the primary sites, granulomatous lesions are formed. These lesions are comprised of lymphocytes, epitheloid cells and giant cells. Initially the newly developed tissue damaging response is the only event capable of limiting mycobacterial activity. This not only destroys the macrophages but also produces early solid necrosis in the centre of the tubercle of

these lesions, some may heal by fibrous and calcification while others may undergo further evolution cell mediated immunity is critical at this early stage.

In the majority of infected individuals, local macrophages are activated when bacillary antigens processed by macrophages stimulate T lymphocytes to release a variety of lymphokines.

In a minority of cases the macrophage activating response is weak and mycobacterial growth can be inhibited only by intensified DTH reaction which leads to tissue destruction. Bronchial walls and blood vessels may be invaded and destroyed leading to spread into the airways and discrimination through blood vessels, hematogenous dissemination may results leading to military tuberculosis or tuberculous meningitis.

Classification of tuberculosis[38]

1. Pulmonary tuberculosis
    a. Primary disease
    b. Post primary (Secondary)
2. Extrapulmonary tuberculosis
3. HIV associated tuberculosis

Pulmonary tuberculosis

Primary disease

Results from an initial infection with tubercle bacilli. Common in persons with impaired immunity (for example, malnutrition and HIV infection) and may

progress rapidly to clinical illness. The lesion forming after the infection is usually peripheral accompanied by hilar or paratracheal lymphadenopathy called Ghon's lesion.

Post Primary (secondary)

Results from endogenous reactivation of latent infection, localized to apical and posterior segments of lung. May lead to cavitation or become fibrotic and undergo calcification and may undergo spontaneous remission or proceed along a chronic debilitating course.

Extra-pulmonary tuberculosis

Commonly involve the lymphnodes, pleura, genitourinary tract, bones and joints, meninges, peritoneum, pericardium. It occurs due to hematogenous spread of infection.

Human immunodeficiency virus associated tuberculosis

Tuberculosis is an important opportunistic disease among HIV infected persons, worldwide. A person with skin test documented M. tuberculosis infection who acquires HIV infection has three to fifteen percent annual risk of developing active tuberculosis.

Tuberculosis can appear at any stage of HIV infection and its presentation varies with the stage. When cell mediated immunity is only partially compromised, pulmonary tuberculosis present as a typical pattern of upper lobe

infiltrates and cavitation, without significant lymphadenopathy or pleural effusion.

In late stages of HIV infection a primary tuberculosis like pattern with diffuse interstitial or military infiltrates, little or no cavitation and intrathoracic lymphadenopathy is more common.

Overall, sputum smears may be positive less frequently among tuberculosis patients with HIV infection than among those without; thus the diagnosis of tuberculosis may be unusually difficult, especially in view of the variety of HIV related pulmonary conditions mimicking tuberculosis.

Extrapulmonary tuberculosis is common among HIV infected patients. The most common forms are lymphatic disseminated pleural and pericardial.

The diagnosis may be difficult not only because of increased frequency of sputum negativity but because of atypical radiographic findings, a lack of classic granuloma formation and negative results in PPD skin tests.

Symptoms and signs of pulmonary tuberculosis[38]

In the early course of disease symptoms and signs are non specific and insidious, consisting of night sweats and fevers, weight loss, anorexia, general malaise and weakness.

Cough eventually develops initially, non productive but subsequently accompanied by production of purulent sputum. Blood streaking of sputum is frequently documented.

Massive hemoptysis may ensue. Pleuritic chest pain sometimes develops in patients with subpleural parenchymal lesion. It may also be result of muscle strain due to persistent coughing. Extensive disease may produce dyspnoea and acute respiratory distress syndrome.

Physical findings[38]

May have no abnormalities detectable by chest examination while others have detectable rales in the involved areas. Occasionally rhonchi due to partial bronchial obstruction and classic amphoric breath sounds in areas of large cavities are heard.

Systemic features include fever (low grade intermittent) and wasting, and in some cases pallor and finger clubbing develop.

Diagnosis

Sputum Smear Microscopy

Persons with cough for two weeks, or more, with or without other symptoms suggestive of TB, should be promptly identified as PTB suspects and steps taken to subject them to sputum smear microscopy for AFB, for diagnosis of TB. Two sputum specimens are collected over one, or two consecutive days. Of the two sputum specimens, one is collected on the spot and the other is an early morning specimen collected at home by the patient. Sputum smear examination is done by Ziehl-Nielson or auramine, rohdamine (fluorescent) staining which is the simplest test to perform.[39]

Slide reporting

The number of bacilli seen in a smear reflects disease severity. Therefore it is important to record the number of bacilli seen on each smear.[39]

Number of bacilli	Microscopy	Result reported
No	AFB per 100 oil immersion fields	0
1 – 9	AFB per 100 oil immersion fields	Scanty
10 – 99	AFB per 100 oil immersion fields	+ (1+)
1 – 10	AFB per oil immersion field	++ (2+)
> 10	AFB per oil immersion field	+++ (3+)

Categorizing the patients as smear positive or negative require results from more than one smear

Smear positive	Intermediate	Smear negative
Aleast 2 smears examined and both positive i.e. reported 1 – 9 per 100 fields, scanty or greater	Several possibilities a. Only one smear examined (whatever the grading) b. Three smear examined but only one reported In either of these situations either further sputum smear or chest X-ray are required before a patient can be classified	Atleast two smears reported negative

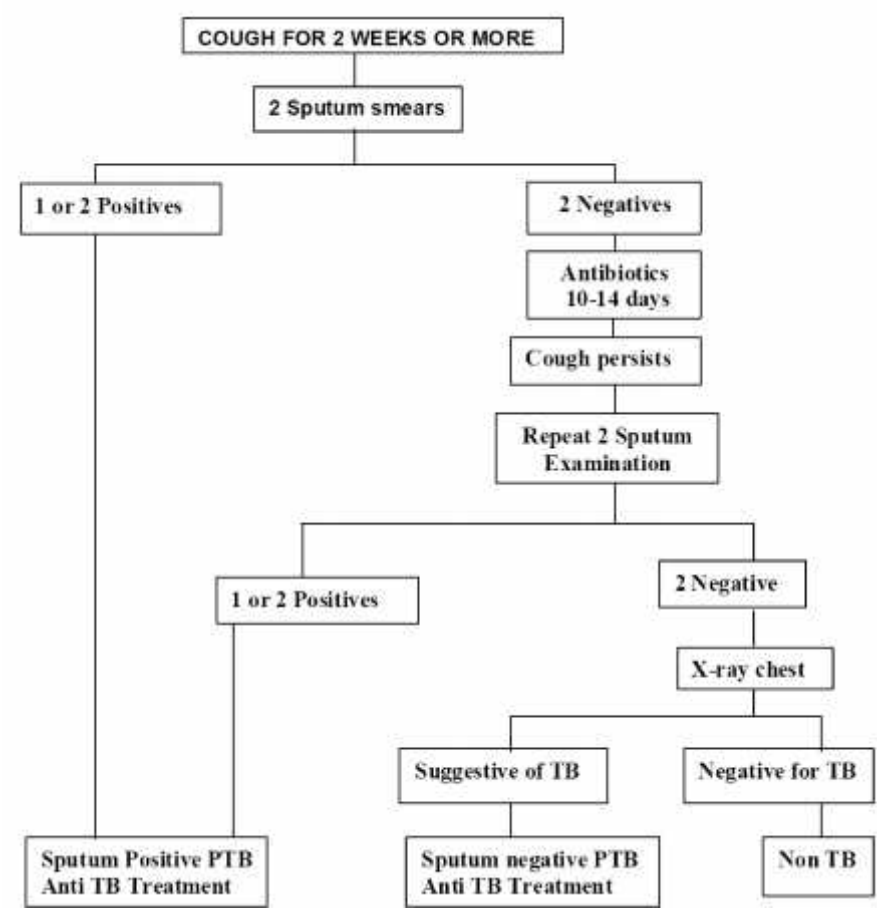


Figure 2. Diagnostic algorithm for pulmonary TB

Culture

Culture (on Lowenstein-Jensen medium) is much more sensitive but takes six to eight weeks and requires specialized laboratory facilities and trained staff. Automated liquid culture systems that detect bacterial carbon dioxide production or oxygen consumption with radiometric (BACTEC), fluorescent (MGIT), colorimetric (MB/BacT system) or pressure sensors (ESP culture system II) roughly halve the time to detection and are being evaluated for use in resource-poor settings.[40]

Molecular assay

Molecular assays have been developed for rapid detection of growth as well as species identification and drug susceptibility testing. The three most widely used assays, PCR (Roche Diagnostics), transcription mediated amplification (Genprobe) and strand displacement amplification (Becton Dickinson) have shown excellent sensitivity, specificity and speed.

Chest radiography

While radiography is a commonly performed investigation, it must be remembered that patients with HIV co-infection may not have typical radiographic features of PTB. While patients with higher CD4 cells (>350 cells/mm^3) have radiographic abnormalities similar to their HIV negative counterparts, patients with immunosuppression often have minimal or atypical findings.[41]

Diffuse pulmonary infiltrates/opacities are the dominant radiological presentation and cavitation is uncommon in HIV patients. Miliary pattern, mediastinal adenopathy and pleural effusion are more common and x-rays may be normal in 5 to 10% of HIV positive individuals.[42] Differential diagnoses include PCP, non-tuberculous mycobacteria, nocardiosis, fungal infections and non-infective complications like lymphocytic interstitial pneumonitis.

The table below shows so-called "classical" and "atypical" CXR patterns. The classical pattern is more common in HIV-negative patients, and the atypical pattern in HIV-positive patients.

Classical pattern	Atypical pattern
Upper lobe infiltrates	Interstitial infiltrates (especially lower zones)
Bilateral infiltrates	Intrathoracic lymphadenopathy
Cavitation	No cavitation
Pulmonary fibrosis and shrinkage calcification	No abnormalities

Tuberculin skin test (Mantoux test)

A positive tuberculin skin test can detect an immunological memory to previous or ongoing contact with MTB antigens. However, in HIV-1 infected patients with CD4+ T-cell counts of less than 200 cells/mm^3, the tuberculin skin test is usually non-reactive.[43] False positive results may be found in patients who were BCG-vaccinated or who had contact with non-tuberculous mycobacteria. The test should only be performed intradermally according to the method described by Mendel and Mantoux. The standardized dose that is recommended by the WHO and the IUATLD is 2 TU in 0.1 ml of PPD RT 23/Tween 80. In the United States and some other countries, 5 TU PPD-S is used, which is thought to be similar in strength, is in use. Following the intradermal inoculation of the injection, the diameter of the induration along the short axis of the lower arm is measured by the ball-point technique.[44]

In HIV-infected patients, an induration of > 5 mm is positive.108 In TB high prevalence countries like India, tuberculin skin testing has no value in TB diagnosis, as a positive test indicates prior infection only and false negativity (due to anergy or other unknown factors) is common. In the late stages of HIV, the

tuberculin test may be negative because of anergy but anergy testing with a panel of antigens (tetanus, mumps, Candida) is not recommended as the results are not reproducible.

Induced sputum/gastric lavage

Inhalation of nebulised hypertonic saline or salbutamol 2 mg given three times daily for a week are used in order to induce sputum production. Gastric lavage can be performed in patients unable to expectorate. The acidic gastric aspirate should be buffered in phosphate solution prior to transportation to the laboratory. These specimens are tested for AFB by smear and culture.[39]

VITAMIN D

Historical perspective

The discovery of vitamin D began with the recognition of rickets as childhood bone disease by Francis Glisson in 1650. The association between rickets and a lack of sunlight exposure was reported by Sniadecki in 1822. By the mid-19th century, cod liver oil has been established as an effective treatment for rickets.[45]

Mellanby emphasized the role of the vitamin D as the preventing agent of rickets in 1919;[46] while the first demonstration of its anti-rachitic properties was given by Elmer McCollum in 1922.[47]

By the 1930s, the use of cod liver oil in the treatment and prevention of rickets became common place. The bridging of the knowledge that

photosynthesized vitamin D and vitamin D in cod liver oil were similar and was responsible for the eventual conquest of rickets. In the same way, the utilization of vitamin D for treatment of tuberculosis was common in the pre-antibiotic era.[48]

In the mid-19th century, cod liver oil was reported by Williams to provide improvement to patients with pulmonary tuberculosis[49] and it was subsequently found to contain high quantities of vitamin D3. The isolation of vitamin D3 from cod liver oil in order to treat tuberculosis in the 1930s led to its widespread use in TB treatment and prevention, until the introduction of antibacterial chemotherapy in the 1950s.

Vitamin D is a seco-steroid hormone important in bone mineralization and calcium homeostasis. Recently, research has found that vitamin D may play a role in multiple chronic diseases such as cancer, autoimmune diseases, infections, and cardiovascular disorders.[5]

Physiology of vitamin D

Cholecalciferol (vitamin D_3) is synthesized upon exposure of the skin to UVB (290–315 nm), resulting in the conversion of endogenous 7-dehydrocholesterol to previtamin D_3, which isomerizes to vitamin D_3. After entering the circulation, it is transported by the vitamin D binding protein or albumin. Vitamin D_3 is hydroxylated in the liver by 25-hydroxylase to its major circulating metabolite, $25(OH)D_3$, which is converted to the biologically active form of vitamin D, 1,25 dihydroxyvitamin D_3 [$1,25(OH)_2D_3$], in the kidney and other tissues by the 1α-hydroxylase.[5]

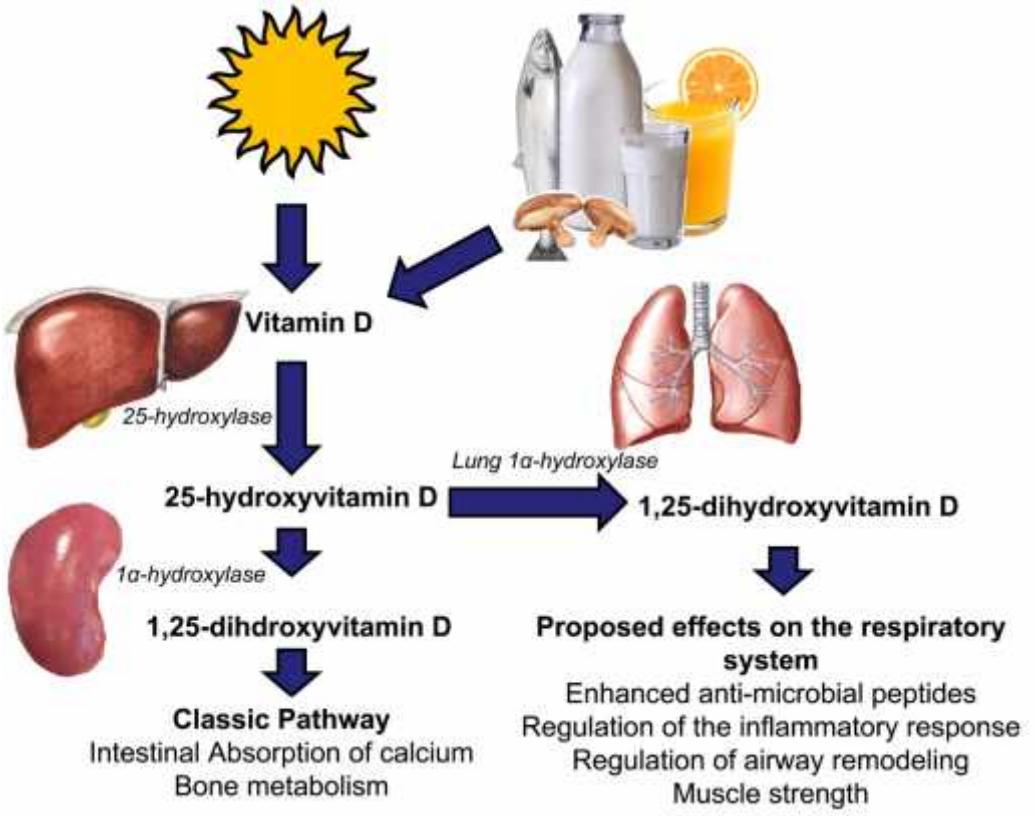


Figure 3. Vitamin D is produced in skin upon exposure to UVB radiation from the sun or from limited dietary sources such as fish and irradiated mushrooms and fortified foods such as milk and orange juice. Vitamin D (D_3 from skin only and D_2 or D_3 from dietary sources) enters the circulation and is hydroxylated in the 25- position by the 25-hydroxylase to form its major circulating form, 25(OH)D, which has a circulating half-life of 3 wk. The 25(OH)D then circulates to the kidney and is hydroxylated in the 1-position by the 1α-hydroxylase to form the hormonal form of vitamin D, $1,25(OH)_2D$. Other tissues such as the epithelial lining of the lung and immune cells in the lung also possess the 1α-hydroxylase to produce local concentrations of $1,25(OH)_2D$. Proposed extrarenal effects of $1,25(OH)_2D$ produced by epithelial and lung cells include increasing antimicrobial peptide production, regulation of the inflammatory response, and airway remodeling. Vitamin D may also play a role in respiratory muscle function.[5]

The prevalence of vitamin D deficiency has been increasing in the general population in recent decades. The majority of circulating 25(OH)D is derived from sun exposure, with a limited dietary contribution. The increased prevalence of vitamin D deficiency is attributed to sun avoidance, indoor lifestyle, use of sunscreen, and decreased intake of vitamin D-containing foods. Because vitamin D is sequestered in adipose tissue, the increasing prevalence of obesity also increases the prevalence of vitamin D deficiency.[5]

Worldwide vitamin D deficiency

It is now widely recognized that vitamin D deficiency (VDD) is one of the most common conditions in the world. It has been estimated that upward of 50% of both children and adults living in the United States, Canada, Mexico, Europe, Asia, New Zealand, and Australia have vitamin D deficiency.[50]

A recent national survey in the United States demonstrated that the prevalence of vitamin D insufficiency doubled in the last ten years: more than 90% of pigmented populations (Blacks, Hispanics, and Asians) suffer from vitamin D insufficiency, as well as nearly three fourths of the white population.[51]

The reason why both Hispanics and Blacks are at much higher risk for VDD is mainly related to their skin pigmentation that gives them a natural sun protection factor, thereby reducing by 50% to 90% skin's efficiency in producing vitamin D3. When black American men and women were exposed to simulated sunlight, they were unable to raise their blood levels of vitamin D3, whereas white adults who received the same amount of simulated sunlight raised their blood levels of vitamin D3 by almost 50 fold.[52]

High prevalence of hypovitaminosis D among black Americans has been found mainly in women.[53]

A clinical research that investigated vitamin D metabolism showed that people from India residing in southern United States have reductions in serum vitamin D and 25(OH)D and are at risk of developing VDD, rickets and osteolmacia.[54]

People in poor countries often have low vitamin D levels. In addition, darkly pigmented skin reduces the amount of UV light available in the skin for production of vitamin D. A comparative study carried out in Ethiopia has demonstrated that both ordinary Ethiopians and full term pregnant Ethiopian women living in Addis Ababa have a significantly decreased vitamin D status compared with pregnant Norwegian women living in Oslo.[55]

In the Indian sub-continent several studies assessed the vitamin D level in the population.

A study conducted in Northern Pakistan found 89% of adult patients with low vitamin D level.[56]

An investigation from India has drawn attention towards wide prevalence of VDD in all the country, in all age groups including newborns, children, pregnant women and adult males and females residing in rural and urban areas. In both areas, widely prevalent VDD is functionally relevant to skeletal health including osteomalacia and rickets.[57]

In 2005 a very high prevalence of hypovitaminosis D was observed in a research carried out among pregnant women and their newborn in northern India.[58]

In the Middle East and other Arab countries, the hypovitaminosis D is very frequent in children and adults. A cross-sectional study observed high prevalence of VDD in apparently healthy children living in Jeddah.[59] For cultural and religious reasons, the dress style of women outdoors prevents exposure of skin to sunlight.

A cross-sectional randomized study conducted in Saudi Arabia indicated that VDD among healthy Saudi women of 25-35 years was 30% and 55% in women of ≥ 50 years.[60]

In Iran high percentage of VDD was defined in a population study: in Teheran prevalence of severe, moderate and mild VDD was 9.5%, 57.6% and 14.2% respectively.[61] New lifestyles, with an increase in time spent in artificial environment (offices, houses, commercial centres), mainly in the hottest season, limit the physiological ability of human body to synthesize from precursor "active" form of vitamin D.

The prevalence of vitamin D deficiency increased significantly with age. Although vitamin D levels may differ by latitude and skin pigmentation worldwide, there is growing evidence that living in sunlit areas may not provide adequate amounts of vitamin D, especially in postmenopausal women and old people. A study in Brazil assesses that although severe vitamin D deficiency leading to rickets or osteomalacia is rare in the country, there is accumulating

evidence of the frequent occurrence of subclinical VDD, especially in elderly people.[62]

Hypovitaminosis D and main diseases

Much debate has taken place over the definition of vitamin D deficiency. Most agree that a 25(OH)D concentration ≤ 50 nmol/L, or 20 ng/mL, is an indication of vitamin D deficiency, whereas a 25(OH)D concentration of 51-74 nmol/L, or 21-29 ng/mL, is considered to indicate insufficiency; concentrations > 30 ng/mL are considered to be sufficient.[63]

VDD is associated with various chronic diseases: osteopathy, myopathy, infections, inflammatory disease, hypertension, and diabetes mellitus, and auto-immune diseases (systemic lupus erythematosus and rheumathoid arthritis) and is a risk factor for developing cardiovascular disease.[50]

Rickets, although rare, is still diagnosed in the United States. Infants who are recent immigrants or adopted from orphanages abroad are at risk for rickets. Vitamin D deficiency rickets is persistent in Canada, particularly among children who reside in the north and among infants with darker skin who are breastfed without appropriate vitamin D intake.[50]

Rickets is among the most frequent childhood disease in many developing countries. In the Indian subcontinent and Middle East rickets is directly caused by VDD.[50]

Moreover, a study in India suggests that rickets is common in malnourished children.[64]

Inadequate exposure to sunlight appears to be the main factor responsible for the high incidence of the disease. In addition, malnutrition may contribute to the development of rickets.[65]

The VDD is an important factor in the development of osteoporosis. An international epidemiological investigation of 18 countries at various latitudes conducted in 2004 and 2005 found a prevalence of vitamin D inadequacy among women with osteoporosis[66] and a study carried out in Italy assessed a high prevalence of osteoporosis in postmenopausal women due mainly to VDD.[64]

Vitamin D deficiency and tuberculosis

TB patients frequently have lower vitamin D levels than the general population. Scientific research is studying the effect of vitamin D on the course of the TB disease. A recent study in Spain indicated that a high proportions of contacts of TB patients had low serum 25(OH)D levels and suggested that sufficient 25(OH)D levels protect against tuberculin skin test (TST) conversion, therefore supporting the hypothesis that deficient vitamin D status is a TB risk factor.

One of the major causes for globally VDD is a lack of sun-induced vitamin D synthesis.

A survey in Germany among immigrant children and adolescents with a Turkish or Arab-Islamic origin found a high prevalence of vitamin D deficiency.[68]

VDD has clearly re-emerged as a problem also in the UK, especially in children, mostly from Asian or Black ethnic minority groups.[69] The observation that migrants have lower serum vitamin D than do their healthy matched controls have led authors to conclude that the fall in vitamin D levels associated with migration from sunshine-rich to sunshine-poor areas is the cause for consequent reduction in cellular immunity that may allow a previously quiescent tuberculous focus to break down.[70]

Martineau in 2011 observed that VDD was highly prevalent among black African adults living in Cape Town and is associated with susceptibility to active TB in both the absence and the presence of HIV infection, the association being stronger in HIV-infected people. Moreover, he pointed out that seasonal variations in vitamin D status and TB incidence among black Africans in Cape Town are causally related and suggested that all African immigrants should have their vitamin D levels checked, in order to restore the vitamin D deficiency.[71]

A cross sectional study was conducted among pulmonary tuberculosis (PTB) patients in Mwanza, Tanzania to identify the predictors of their vitamin D status. The authors deduced that serum 25(OH)D is a valid measure of vitamin D status during the acute phase response and that the lower concentrations in PTB+ patients may reflect increased utilization of vitamin D.[73]

A study by Gibney confirms the strong association between VDD and Latent Tuberculosis Infection (LTBI) in African immigrants in Melbourne.[73]

A further study, among sub-Saharan African immigrants attending the infectious diseases clinics in Melbourne, documents the frequency of latent or active TB infection and the relationship with vitamin D deficiency.[75]

In the Indian sub-continent, TB has historically been a public health concern, due to a high prevalence and incidence in the general population. In a cohort follow-up study from Pakistan, low vitamin D levels were associated with progression to active TB disease in healthy household contacts. The findings suggested also the higher susceptibility of women to the infection, because of their low socioeconomic status, poor nutrition, traditional/cultural traits, and little exposure to sunlight.[75]

In Southeast Asian countries, TB is also a major public health issue. A study among the Vietnamese population in Ho Chi Minh City suggests that low vitamin D status is an antecedent risk factor for TB.[76]

A review of several observational studies (UK, Indonesia, Kenya, Thailand, Hong Kong, India) found that patients with TB have, on average, lower serum levels of vitamin D than healthy controls matched on sex, age, ethnicity, diet and geographical location. Worries about VDD and the risk of TB may therefore not be limited to Arab countries, Afro-Asian indigenous and migrant communities, as half of people in Europe aged more than 60 years are vitamin D deficient and concerns have been expressed in the UK about increasing malnutrition in the elderly.[77]

Even before the discovery of the etiologic cause of tuberculosis by Robert Koch in 1903, vitamin D from cod liver oil and from exposure to sun or radiation was used to treat tuberculosis.[78]

Several recent studies in different populations have associated a deficiency in vitamin D with increased risk of tuberculosis.[77]

In a recent metaanalysis by Nnoaham and Clarke,[79] a pooled effect size of vitamin D was estimated to be 0.68 (95% CI 0.43–0.93), indicating that vitamin D levels were 0.68 SD lower in persons with tuberculosis than in controls. However, these findings cannot be considered conclusive since the association may be confounded by important variables, such as smoking and sunlight exposure, which were not accounted for in the analysis.

It is well-established that immune cells can produce the hormonally active metabolite of vitamin D. Macrophages and other immune cells can express 1a-hydoxylase, the enzyme that converts circulating 25-hydroxyvitamin D3 into 1,25-dihydroxyvitamin D3, the active form of vitamin D.7 Moreover, *M. tuberculosis* infection activates Toll-like receptors (TLR1/2) that mediate the activation of different cells in the innate immune system and their expression of cytokines and antimicrobial peptides.[78,80]

Recent observations indicate that activation of this cell surface receptor also upregulates expression of both the 1-a-hydroxylase enzyme and the vitamin D receptors (VDR) in monocytes and macrophages, leading to both increased levels of active vitamin D and increased potential binding of 1,25-dihydroxyvitamin D with the VDR.[78,80]

Although the biological mechanisms through which vitamin D modulates the immune system to fight *Mycobacterium* infection are still under study, two possible mechanisms have emerged as the most likely. For instance, 1,25-dihydroxyvitamin D3 appears to reduce the viability of *M. tuberculosis* by enhancing the fusion of the phagosome and lysosome in infected macrophages.[77]

The capacity of *Mycobacterium* infection to prevent macrophage maturation and formation of the phagolysosome is completely reversed in the presence of 1,25-dihydroxyvitamin D3.[77]

The pathways used to promote vitamin D-induced phagolysosome formation are independent of the classical interferon-gamma (IFN-g)-dependent macrophage activation and involve products of phosphatidylinositol-3-kinases (PI3K), which help to regulate the transport of endosomes to lysosomes.[77]

In addition, 1,25-dihydroxyvitamin D may enhance the production of LL-37, an antimicrobial peptide of the cathelicidin family. Antimicrobial peptides, such as defensins and cathelicidins, are involved as a first line of defense in the prevention of infections, including tuberculosis. Although cathelicidins are widely distributed in mammals, LL-37 is the only member of the cathelicidin family that has been identified in humans, where it is found in alveolar macrophages, lymphocytes, neutrophils, and epithelial cells. In addition to having direct bactericidal activity, LL-37 also modulates the immune response by attracting monocytes, T cells, and neutrophils to the site of infection.[78] The presence of 1,25-dihydroxyvitamin D3 in neutrophils and macrophages upregulates in a dose-dependent manner the *hCAP-18* gene that codes for LL-

37,7,24 which suggests that 1,25-dihydroxyvitamin D induction of LL-37 may play a role in host defense against TB infection.

Vitamin D role in the immune system and tuberculosis

Vitamin D, in its active form 1,25-hydroxyvitamin D, has a complex action on the immune system, by modulating and inhibiting its activity in different ways. In 2006, Liu and colleagues proved that *Mycobacterium tubercolosis* sensing by the Toll-like receptor 2/1 (TLR2/1) complex increases expression of vitamin D receptors (VDR) and CYP27B1 in monocytes [6]. The synthesis of 1,25-dihydroxy vitamin D promotes VDR-mediated transactivation of the antimicrobial peptide cathelicid in and killing of intracellular *Mycobacterium tubercolosis*.[77]

Cathelicidins have a direct antimicrobial function. In addition to anti-bacterial effects including membrane disruption, they have antiviral effect in the inhibition of herpes simplex viruses, adenovirus and retrovirus.[50]

A study showed that macrophages are most efficient in producing cathelicidin antimicrobial peptide LL-37 after infection with *M. tuberculosis*, suggesting that cathelicidin from macrophages may be an important participant in the innate immune response during early infection in humans.[81]

Liu (2006) demonstrated that transcriptional regulation of cathelicidin can be mediated by activation of 1,25-dihydroxivitamin D. Stimulation of TLR receptors in macrophages by microbial products results in increased conversion from the inactive 25-hydroxyvitamin D to the active 1,25-hydroxyvitamin D.[80]

According to Adams and colleagues, a consequence of TLR activation is the production of defensin-2 and of cathelicidin: these two antimicrobical peptides are strongly up-regulated by 1,25-hydroxyvitamin D.[82]

In fact, according to Liu (2006), serum from donors with insufficient levels of dihydrovitamin D supported a lower induction of cathelicidin in monocytes compared to serum of donors with sufficient vitamin D levels.[80]

A similar conclusion has been reported by the above study of Adams, using serum from patients with insufficient levels of vitamin D, before and after vitamin D supplementation. Those further experiments have confirmed earlier evidence: the 4 μg/ml concentrations of vitamin D metabolites have been able in a reproducible way to protect infected human macrophages and restrict mycobacterial growth *in vitro*.[83]

The crucial role played by vitamin D in the immune response to *M. tuberculosis* consists, as well as the production of the LL-37, in promoting phagolysosome formation.[50]

A research has documented that 1,25-dihydroxyvitamin D promotes autophagy in monocytes.[85] Autophagy and vitamin D3-mediated innate immunity have demonstrated to confer protection against infection with intracellular *Mycobacterium tuberculosis*. A study of Eun-Kyeong confirms the scientific findings that antimicrobial peptides play central roles in innate immunity to mycobacteria, including direct killing and indirect immune modulation. Of particular interest, human cathelicidin LL-37 has shown to be a key component linking vitamin D3-dependent immunity and autophagy.[85] The study indicated

further researches into how cathelicidin regulates innate immune responses in order to facilitate the development of combinatorial treatments involving antimicrobial peptides, nutritional deficit correction and conventional chemotherapy. This could represent a breakthrough in therapies for TB, especially for multidrug-resistant TB, which shows resistance to conventional antibiotics.

On the other hand, vitamin D binds the vitamin D receptors on natural killer T (NKT) cells and CD8aa. Both cells have an important role in regulating cytokine production and in protecting against the generation of autoimmunity.[50]

Researches have demonstrated that 1,25- dihydroxyvitamin D is a potent modulator of the T-cell phenotype: it inhibits the T-helper (Th) 1 T cells associated with cellular immune response while conversely enhancing humoral Th2 cells response.[86]

A recent finding indicates that a balance between pro-(Th1) and anti (Th2) inflammatory responses is optimal for control of TB, thus suggesting that the role of 1,25-dihydroxyvitamin D3 may have a relevant importance.[87]

Vitamin d receptor gene polymorphisms and tuberculosis

The vitamin D receptor (VDR) gene is found in the chromosomal 12q13 region. VDR gene polymorphisms are commonly found in many population groups, although the prevalence of certain VDR genotypes varies among different populations. Most of the gene polymorphisms studied are based only on restriction fragment length polymorphism (RFLP) analysis, which does not

identify the functional effects of these changes. Genetic alterations of the VDR gene may lead to defects in gene activation or to changes in the protein structure of the VDR, both of which could affect the cellular functions of 1,25-dihydroxyvitamin D3.[50]

Various VDR polymorphisms could also be linked to each other or to unidentified genes that are important determinants of disease risk. In the 3' end of the VDR gene, several polymorphisms (*BsmI, ApaI*, and *TaqI*) with strong linkage disequilibrium (LD) have been studied. Although these nucleotide changes in the VDR gene are predicted to be "silent" and to have no effect on the structure of the expressed VDR protein, they may be involved in regulating VDR gene expression, or they could potentially be linked with other truly functional nucleotide sequences in theVDR gene.[50]

It has been suggested that the mRNA coded from the *TaqI* t allele of the VDR gene is more stable than the mRNA from the T allele of the VDR gene.[50]

A non-silent VDR gene polymorphism is the *FokI* polymorphism, found in exon 2, which is at a translation initiation start site and is predicted to change the structure of the coded protein.A thymine-to-cytosine (T→C) change found in the F allele leads to an alternative translational start site and a VDR protein that is three amino acids shorter than that of the f variant. Although the difference between the two proteins is only three amino acids, it has been suggested that the more commonly observed shorter VDR protein is functionally more active.[50]

Several studies have examined *TaqI* and *FokI* VDR gene polymorphisms and their association with susceptibility to tuberculosis in different populations

with inconclusive findings.[77] An early study of this question by Bellamy et al.[88] found that the *TaqI* tt VDR genotype was associated with decreased risk of TB in The Gambia compared with the TT and Tt genotypes combined (OR = 0.53, 95% CI 0.31–0.88). In contrast, a study in India found that the tt *TaqI*VDR genotype was associated with increased TB susceptibility, at least in women.[89]

A recent study among native Paraguayans found that the *TaqI* t VDR allele protects against active disease, but not infection, while the *FokI* F VDR genotype was associated with decreased risk of TB (assessed by PPD status).[90]

Other studies found no significant association between the *TaqI* and *FokI* polymorphisms and risk of TB, but probably had limited statistical power due to small sample sizes.[77]

Vitamin D receptor gene polymorphisms and response to treatment of pulmonary tuberculosis

VDR polymorphisms may affect not only host susceptibility to tuberculosis, but also the response to treatment. Two studies have focused on the association of polymorphisms in the *FokI* and *TaqI* VDR genes[90] and TB susceptibility; one of them also evaluated the association with the *ApaI VDR* gene polymorphism.[92]

Roth et al.[92] studied the association between *FokI* and *TaqI* VDR gene polymorphisms and susceptibility to TB and sputum conversion time following treatment among inhabitants of an area in the outskirts of Lima, Peru, where the incidence of TB is very high.12 A case-control study design was used to evaluate

the association between VDR gene polymorphisms and susceptibility to TB. Study cases were 103 persons, aged 15–45 years, with confirmed TB (excluding HIV-positive patients and pregnant women) who were receiving directly observed therapy, as recommended by theWHO.[93] Two age- and sex-matched controls were chosen for each case, one with a positive tuberculin test (PPD) and the other with a negative test.

The prevalence of the *TaqI* t allele in the VDR gene was lower among the PPD-positive controls than among the PPD-negative controls and even lower among the TB cases, suggesting that the t allele was somehow protective against TB susceptibility. However, there was no significant difference in TB susceptibility between the *FokI*VDR genotypes ff versus FF (OR = 0.84, 95% CI 0.34–2.86) or Ff versus FF (OR = 0.64, 95% CI 0.25–1.62), or the *TaqI* VDR genotype Tt versus TT (OR = 0.61, 95% CI 0.28–1.29).[77]

Although the *FokI* VDR genotype had no apparent effect on susceptibility to TB, quite different effects were seen regarding the response to TB treatment. To evaluate this effect, the researchers[94] conducted a cohort study among 78 patients with confirmed pulmonary TB (positive sputum test and TB symptoms). Kaplan-Meier survival analysis for the total treatment-time follow-up period showed that patients with the VDR *FokI* FF genotype had significantly faster conversion times in the sputum cultures and in the auramine staining tests compared to those with the VDR *FokI* ff genotype, thus supporting the notion that the F allele, which results in the expression of a shorter VDR protein, somehow enhances the treatment response compared to the *FokI* f allele, which codes for a slightly longer VDR protein. With respect to the *TaqI*

polymorphisms, no study participants had the tt genotype. Among TB patients, the conversion of culture tests – but not the auramine staining tests – was significantly faster for those with the TTVDR genotype as compared to the Tt genotype. In conclusion, there was a beneficial effect on the host response to TB among participants with the TT or FFVDR genotypes, especially for culture conversion, as compared to the Tt and the ff VDR genotypes.[91]

Recently, Baab et al.[91] conducted a similar study among a population of women and men, aged 18–65 years, from Western Cape, South Africa, where the incidence of TB in 2003 was high (919/100,000). Study cases were 249 persons with newly diagnosed TB (excluding pregnant women and persons with HIV or other chronic diseases); controls were 352 persons with no previous history or symptoms of TB selected from clinics, households, and workplaces in the same geographical area. A cohort study was conducted among the cases of TB, starting from the day of diagnosis up to 12 months. Patients were required to provide sputum samples for Ziehl-Nielsen staining and *M. tuberculosis* culture on the day of diagnosis, the following 2 days, then weekly up to month 2, and then monthly until month 6. After that, two subsequent samples were collected at months 9 and 12. Conversion time (from positive to negative *M. tuberculosis* in sputum) was calculated in days from the day of diagnosis to an average between the dates of the last date of a positive result and the first of two successive negative results if less than 92 days had elapsed between the positive and negative results. Otherwise, the last positive day was used as the conversion day. Participants were categorized as “fast respondents” if their conversion time was before day 55 after diagnosis, and “slow respondents” otherwise.

For the case-control study, no significant associations were observed between the individual VDR genotypes and the presence or absence of pulmonary TB. Moreover, after adjusting for age and gender, no statistically significant differences were observed between the diplotype frequencies in cases and controls. However, for the response-to-treatment cohort study, Babb et al.[92] found a faster smear conversion time for the VDR genotypes *ApaI* AA and *TaqI* TT and Tt as compared to the *ApaI* aa and *TaqI* tt VDR genotypes, respectively. No statistically significant difference in the culture conversion time was observed between genotypes. For the categorization between "fast respondents" and "slow respondents", there was a significant trend to a faster smear conversion in those with a VDR *FokI* f allele and for a faster culture conversion in patients with the *ApaI* A allele. In summary, Babb et al. found no significant association between the *ApaI, TaqI* and *FokI* polymorphisms and susceptibility to TB. However, *ApaI* AA and *TaqI* Tt and TT genotypes may contribute to a faster response to treatment.

The association between VDR polymorphisms and response to TB treatment remains inconclusive, and larger studies are needed. In the study among Peruvians, the *FokI* F and *TaqI* T alleles were associated with a faster response to TB treatment.9 In the South African study, there was also a faster response to TB treatment among people with the *TaqI* T and the *ApaI* A alleles, whereas no definitive association was observed for the *FokI* genotypes.[91]

Selection of the optimalmethod to evaluate response to treatment (auramine staining or culture of sputum) is of substantial importance given the differences found among the studies. Roth et al.[91] found significant associations

for the *TaqI* genotypes among the TB cultures – not the stained samples – suggesting that VDR genotypes are potentially associated with mycobacterial viability, rather than with the quantity of expectorated microorganisms.[92] A low number of viable bacteria can give positive results in the culture, but can be negative in the auramine staining test.

Chapter 4

Methodology

METHODOLOGY

This study was done in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum on patients with pulmonary tuberculosis.

Study design

The study design was a one year hospital based cross sectional study.

Study period and duration

The present study was done from January 2012 to December 2012 for the period of one year.

Place

The present study was conducted at Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum a tertiary care teaching hospital attached to Jawaharlal Nehru Medical College, Belgaum.

Source of Data

Patients presenting with pulmonary tuberculosis at the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum during the study period were included in the study.

Sample size

A total of 50 patients with pulmonary tuberculosis were selected for the study.

Sampling procedure

The sample size was calculated using the following formula as below.

$n = 4 p q / d^2$

Where, p = Prevalence (50%)

q = 100 – p = 50%

d = Absolute error considered as 15%

Hence,

n = 4 x 50 x 50 / 225

n = 44.44 ≈ 50

Hence a sample size of 50 was considered.

Selection criteria

Inclusion

- All cases diagnosed with pulmonary tuberculosis based on WHO criteria that is;
    - Smear-positive pulmonary TB case: Defined as one with;
        - Two or more initial sputum smear examinations positive for AFB, **or;**
        - One sputum smear examination positive for AFB plus radiographic abnormalities consistent with active PTB as determined by a clinician, or;
        - One sputum smear positive for AFB plus sputum culture-positive for M. tuberculosis.

- Smear-negative PTB cases should either:
  - Have sputum that is smear-negative but culture-positive for M. tuberculosis or;
  - Meet the following diagnostic criteria:
    - Decision by a clinician to treat with a full course of anti-TB therapy; and
    - Radiographic abnormalities consistent with active pulmonary TB and either laboratory or strong clinical evidence of HIV infection or if HIV-negative (or unknown HIV status living in an area of low HIV prevalence), no improvement in response to a course of broad-spectrum antibiotics (excluding anti-TB drugs and fluoroquinolones and aminoglycosides).

Exclusion

- Liver failure.
- Renal failure.
- Diabetes mellitus.
- Malabsorption syndromes.

Ethical clearance

Prior to the commencement, the ethical clearance for the study was obtained from Institutional Ethics Committee, Jawaharlal Nehru Medical College, Belgaum.

Informed Consent

The patients fulfilling selection criteria were screened for eligibility based on selection criteria and selected patients were explained about the nature of the study and interventions being done. Those willing to participate were enrolled in the study after obtaining a written informed consent (Annexure I).

Method of collection of data

Demographic data such as age and sex were recorded. Patients were interviewed regarding the history of other comorbid conditions and complaints. A thorough physical examination for the presence clinical signs and symptoms was done followed by systemic examination. These findings were recorded on a predesigned and pretested proforma (Annexure II).

Investigations

The selected patients underwent the following investigations.

- Complete blood count.
- Chest x-ray
- Sputum for AFB
- Liver Function Tests
- Renal Function Tests
- Serum 25-hydroxy Vitamin D (using ELISA method)
- Random blood sugar

Serum 25-hydroxy vitamin D[95]

The estimation of Vitamin D was done using Siemens, ADVIA Centraur VitD assay, a one pass 18 minute antibody competitive immunoassay.

Outcome variables

Serum 25-hydroxy vitamin D

The serum 25-hydroxy vitamin D levels were tested and interpreted as;

Normal – ≥ 30 mg/dL

Insufficient – 21 to 29 mg/dL

Deficient – ≤ 20 mg/dL

Statistical analysis

The data obtained was coded and entered into Microsoft Excel Worksheet (Annexure III). The categorical data was expressed as rates, ratios and proportions and comparison was done using chi-square test. The continuous data was expressed as mean ± standard deviation (SD) and comparison was done by two sample 't' test with unequal variance. A probability value ('p' value) of less than or equal to 0.05 was considered as statistically significant.

Chapter 5

Results

RESULTS

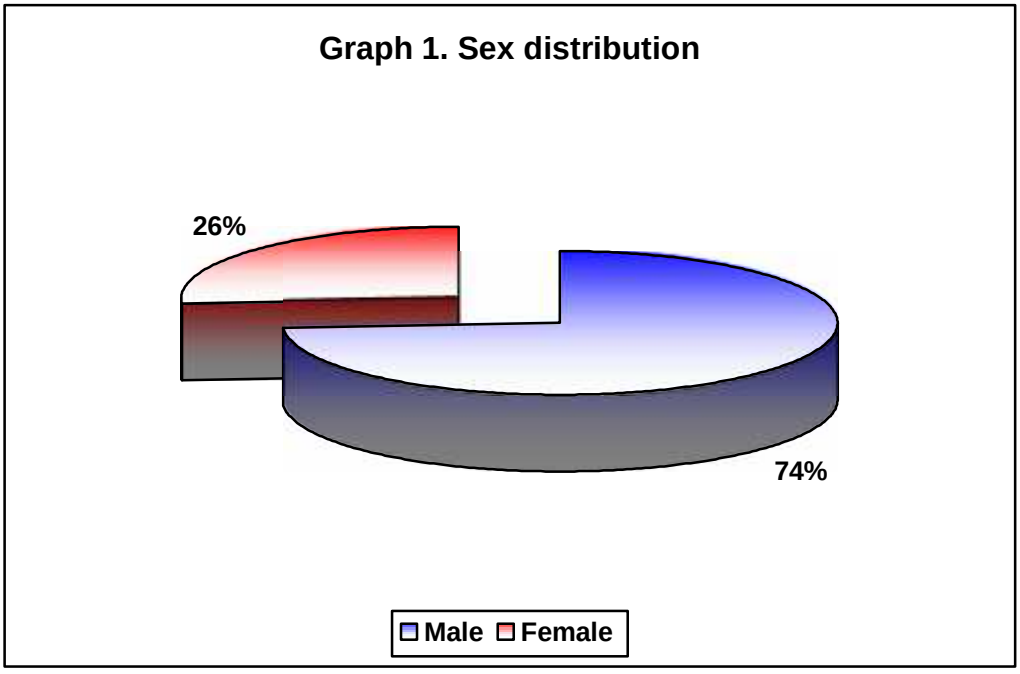
This one year hospital based cross sectional study was done in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum.

A total of 50 patients diagnosed with pulmonary tuberculosis based on WHO criteria from January 2012 to December 2012 were studied.

The data is analysed and the observations are tabulated as below.

Table 1. Sex distribution

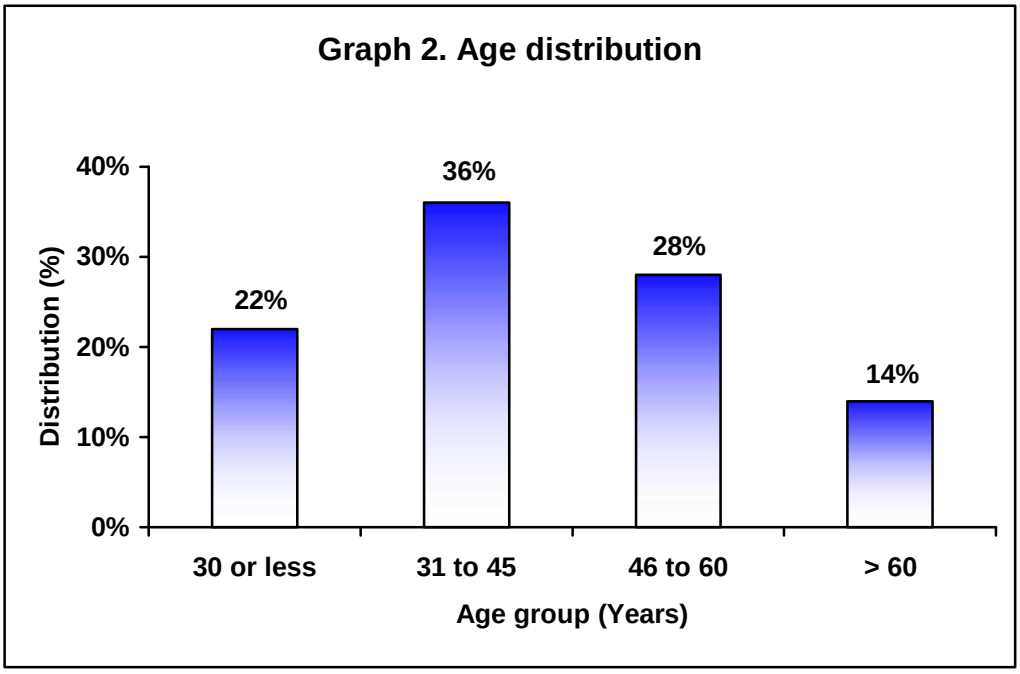
Sex	Distribution (n=50)	
	Number	**Percentage**
Male	37	74.00
Female	13	26.00
Total	**50**	**100.00**



In the present study 74% of patients were males and 26% were females. The male to female ratio was 2.8:1.

Table 2. Age distribution

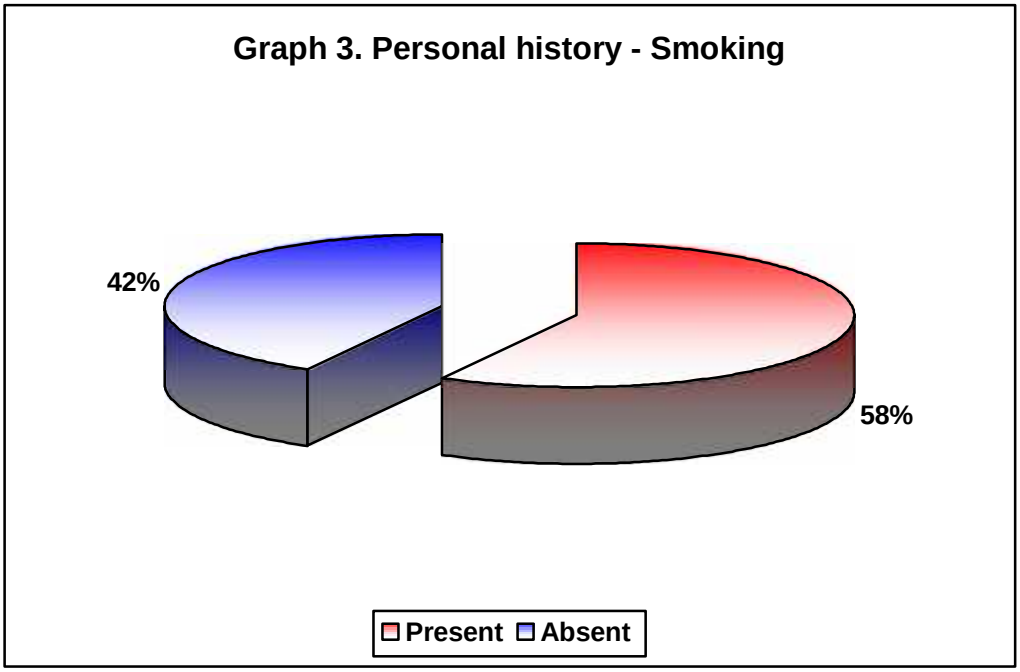
Age group (Years)	Distribution (n=50)	
	Number	Percentage
30 or less	11	22.00
31 to 45	18	36.00
46 to 60	14	28.00
> 60	7	14.00
Total	**50**	**100.00**



In this study 31 to 45 years was the commonest age group which comprised 36% of patients followed by 46 to 60 years with 28% of patients. However, 22% and 14% of patients were aged ≤ 30 years and > 60 years respectively. The mean age among males was 39.5 ± 17.93 years while in females it was 45.3 ± 14.09 years.

Table 3. Personal history – Smoking

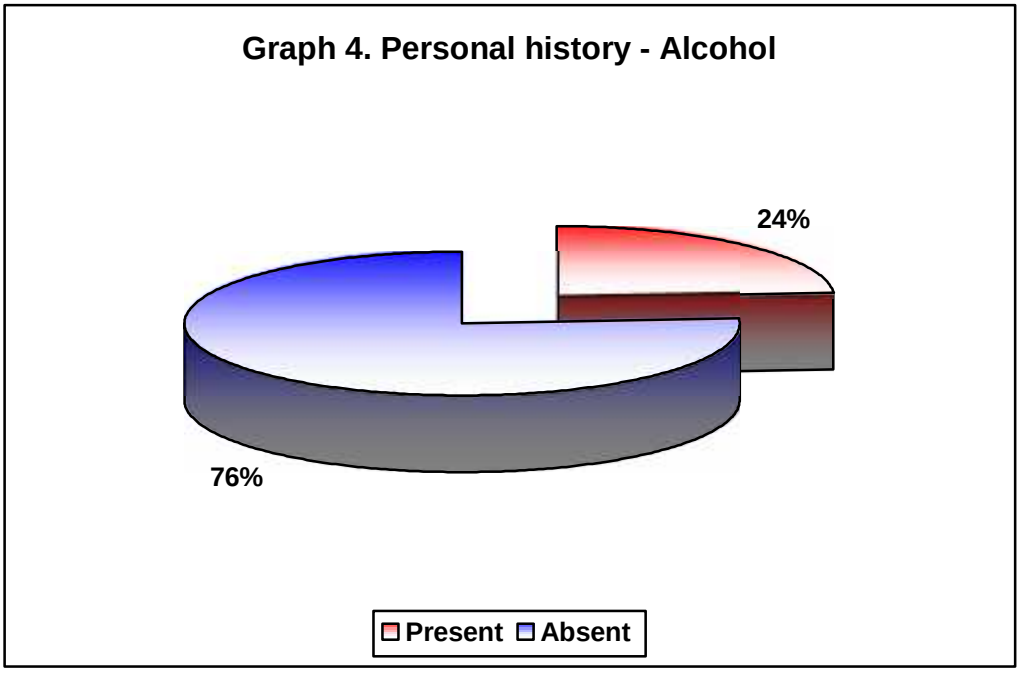
History	Distribution (n=50)	
	Number	**Percentage**
Present	29	58.00
Absent	21	42.00
Total	**50**	**100.00**



In the present study history of smoking was present in 58% of patients while 42% of patients were non smokers.

Table 4. Personal history – Alcohol

History	Distribution (n=50)	
	Number	Percentage
Present	12	24.00
Absent	38	76.00
Total	**50**	**100.00**

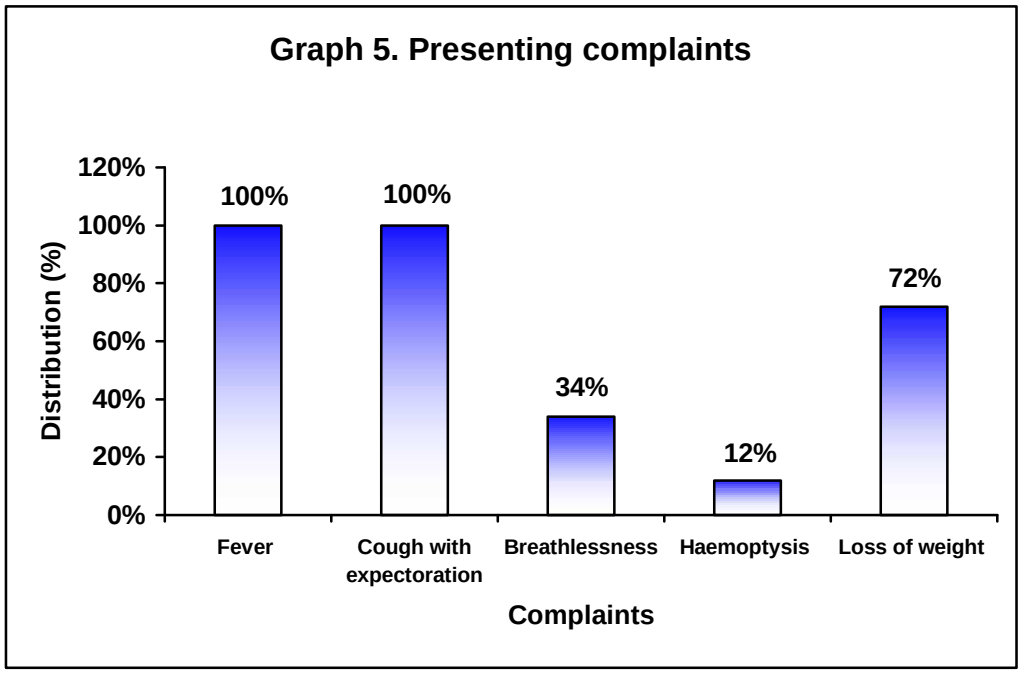


In this study, history of alcohol consumption was reported by 24% of the patients.

Table 5. Presenting complaints

Complaints	Distribution (n=50)	
	Number	Percentage
Fever	50	100.00
Cough with expectoration	50	100.00
Breathlessness	17	34.00
Haemoptysis	6	12.00
Loss of weight	36	72.00

Multiple findings present hence total not shown



In the present study all the patients (100%) presented with fever and cough with expectoration while loss of weight was noted in 72%, breathlessness in 34% and haemoptysis in 12% of patients.

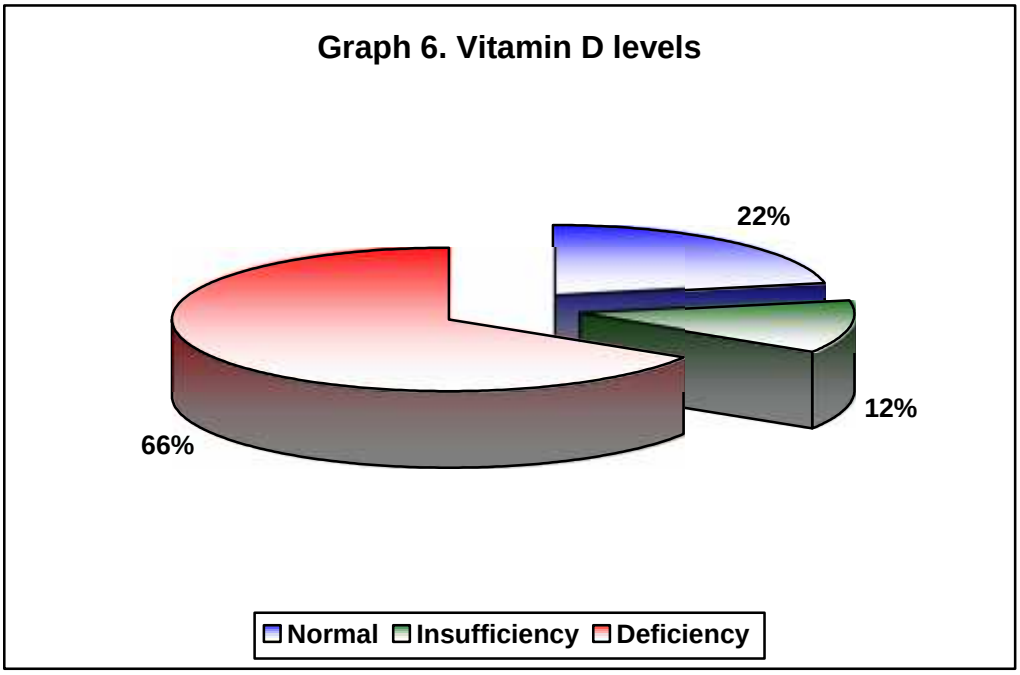
Table 6. Investigations

Variables	Distribution (n=50)	
	Mean	**S.D.**
Haemoglobin (gm%)	10.5	2.15
Total WBC (/cum)	9891.2	4011.14
Platelet count (Lakhs/cum)	3.20	1.55

The mean haemoglobin, total WBC count and platelet count are as shown in table 6.

Table 7. Vitamin D levels

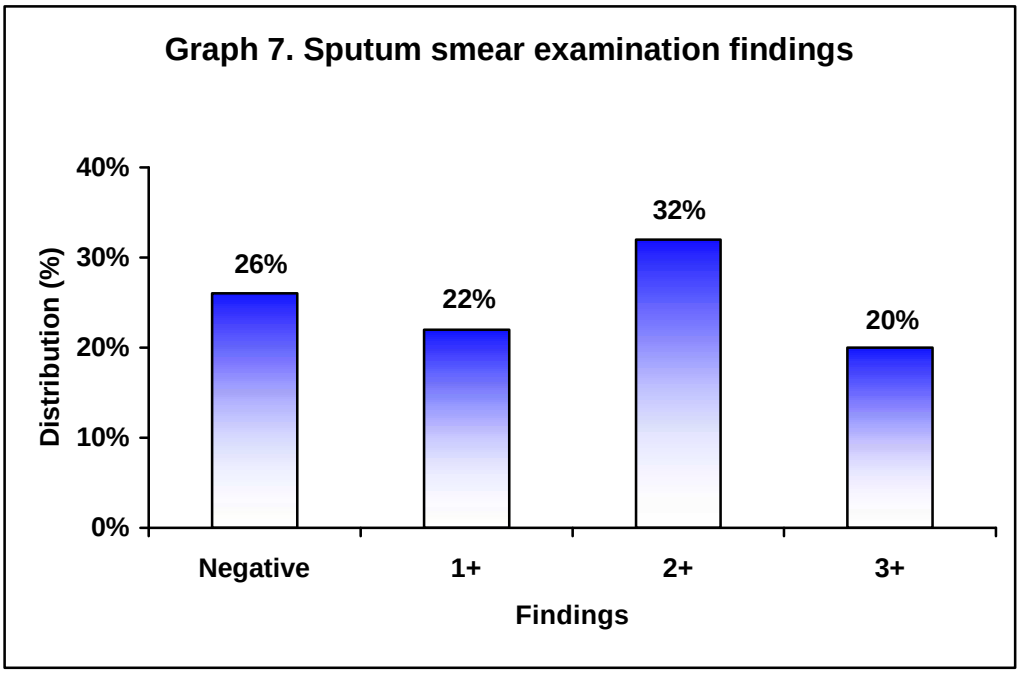
Vitamin D	Distribution (n=50)	
	Number	**Percentage**
Normal (≥30)	11	22.00
Insufficiency (21-29)	6	12.00
Deficiency (≤ 20)	33	66.00
Total	**50**	**100.00**



In this study majority (66%) of the patients had vitamin D deficiency while 12% of patients had insufficient vitamin D levels. The mean vitamin D levels were 17.05 ± 10.04.

Table 8. Sputum smear examination findings

Findings	Distribution (n=50)	
	Number	**Percentage**
Negative	13	26.00
1+	11	22.00
2+	16	32.00
3+	10	20.00
Total	**50**	**100.00**



In the present study sputum smear examination was negative in 26% of patients. In the remaining, 2+, 1+ and 3+ findings were noted in 32%, 22% and 20% respectively.

Table 9. X ray findings

Findings	Right side (n=50)		Left side (n=50)	
	Number	**Percentage**	**Number**	**Percentage**
Infiltration	22	44.00	28	56.00
Cavitation	18	36.00	13	26.00

In this study chest X-ray findings revealed infiltration at right side in 44% and left side in 56% of the patients. The cavitation was noted on right side in 36% of the patients and in 26% of patients it was present on left side.

Table 10. Association of sex with vitamin D levels

Sex	Vitamin D levels						Total (n=100)	
	0 to 20		20.1 to 29.9		30 or more			
	No	%	No	%	No	%	No	%
Male	24	64.86	4	10.81	9	24.32	37	75.68
Female	9	69.23	2	15.38	2	15.38	13	100.00
Total	**33**	**66.00**	**6**	**12.00**	**11**	**22.00**	**50**	**100.00**

p = 0.796

In the present study of the 33 patients had vitamin D deficiency, 24 (64.86%) out of 37 were males and 9 (69.23%) out of 13 were females. No statistically significant difference was observed between sex and vitamin D levels (p=0.796).

Table 11. Association of vitamin D levels with AFB findings

AFB findings	Vitamin D levels						Total (n=100)	
	0 to 20		20.1 to 29.9		30 or more			
	No	%	No	%	No	%	No	%
Negative	4	30.77	4	30.77	5	38.46	13	100.00
1+	10	90.91	0	0.00	1	9.09	11	100.00
2+	12	75.00	2	12.50	2	12.50	16	100.00
3+	7	70.00	0	0.00	3	30.00	10	70.00
Total	**33**	**66.00**	**6**	**12.00**	**11**	**22.00**	**50**	**100.00**

p = 0.033

In the present study of the 10 patients with 3+ AFB, 70% of patients had vitamin D levels of ≤ 20 while 30% of patients had vitamin D levels ≥ 30. Similarly of the 16 patients with 2+ AFB, majority (75%) of patients had vitamin D levels of ≤ 20. In 11 patients with 1+ AFB findings, 9.09% of the patients had had vitamin D levels ≥ 30 and 90.91% had ≤ 20. This difference was statistically significant (p=0.033).

Table 12. Sex wise mean vitamin D levels

Sex	Distribution (n=50)	
	Mean	**SD**
Male	17.40	10.52
Female	16.05	8.83

p=0.657

In the present study mean vitamin D levels among males were slightly high (17.40 ± 10.52) compared to females (16.05 ± 8.83). However this difference was statistically not significant.

Table 13. Age wise mean vitamin D levels

Age group (Years)	Distribution (n=50)	
	Mean	**SD**
30 or less	26.86	10.08
31 to 45	17.16	9.60
46 to 60	12.42	6.56
> 60	10.61	4.95

F=7.691 **p<0.001**

In this study the mean vitamin D levels in patients aged ≤ 30 years were significantly high (26.86 ± 10.08) compared to those who were aged 31 to 45 (17.16 ± 9.60) and more than 60 years (10.61 ± 4.95) ($p<0.001$).

Table 14. Mean vitamin D levels in different sputum smear findings sample 1

Sputum smear findings	Distribution (n=50)	
	Mean	**SD**
Negative	25.10	7.83
1+	14.77	5.65
2+	13.39	9.51
3+	14.94	12.34

F=4.691 **p = 0.006**

In this study the mean vitamin D levels in patients with negative AFB findings were significantly high (25.10 ± 7.83) compared to those with 1+ (14.77 ± 5.65), 2+ (13.39 ± 9.51) and 3+ (14.94 ± 12.34) ($p<0.006$).

Chapter 6

Discussion

DISCUSSION

Simple and inexpensive strategies to improve outcomes from tuberculosis (TB) are highly sought-after. Adjunctive immunotherapies have been acknowledged as a priority TB research area, and interest in the use of supplemental nutritional agents for TB has been longstanding. Vitamin D, a micronutrient derived from dermal ultraviolet B (UVB) radiation and some dietary sources, and L-arginine, a conditionally-essential amino acid, have antimycobacterial properties directly or via downstream mediators, rendering them suitable as candidate adjunctive immunotherapies in TB.[96]

The present study was undertaken to estimate the levels of vitamin D in patients with pulmonary tuberculosis and to estimate vitamin D levels with different degrees of sputum positivity of pulmonary tuberculosis.

The present one year hospital based cross sectional study was done on a total of 50 patients diagnosed with pulmonary tuberculosis based on WHO criteria from January 2012 to December 2012 in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum.

In the present study vitamin D levels were low in most (66%) of the patients while 12% of patients had insufficient vitamin D levels and 22% of the patients had vitamin D levels within the normal range. The mean vitamin D levels were 17.05 ± 10.04. Summing up the patients with vitamin D deficiency and insufficiency 78% of the patients had low vitamin D levels and the mean vitamin D levels were much below than the normal reference range. A review to explore the association between low serum vitamin D and risk of active

tuberculosis in humans found that, patients with tuberculosis have, on average, lower serum levels of vitamin D than healthy controls matched on sex, age, ethnicity, diet and geographical location. Similarly five other studies found lower levels of serum vitamin D in tuberculosis patients compared to controls.[79]

A study[97] from India to look for evidence of vitamin D deficiency in patients with active tuberculosis concluded that, vitamin D deficiency exists in patients with tuberculosis and it is possibly a cause rather than effect of the disease; deficiency is due to decreased dietary intake. Vitamin D deficiency can occur without any symptoms. If symptoms are present, it indicates severe deficiency.

Another study[98] from Kerala, India showed that, Vitamin D deficiency was present in all patients with active Tuberculosis. Deficiency was noted in 90% of the apparently healthy controls even though the level was higher as compared to the patients with TB. The population studied was a representative sample of Kerala and presumably whole of India, and the results indicate the possibility of widespread deficiency in the apparently healthy population in India in spite of adequate sunlight exposure. Considering the role of Vitamin D in Cell Mediated Immunity and the protective effect of it in several other diseases, the article highlights the importance of improving the nutrition and if required even supplementing Vitamin D by food fortification, rather than hoping to get vitamin D from sunlight exposure alone.

In the present study 32%, 22% and 20% of the patients had 2+, 1+ and 3+ AFB findings respectively. Of the 10 patients with 3+ AFB, 70% of patients had

vitamin D levels of ≤ 20 while 30% of patients had vitamin D levels ≥ 30. Also, of the 16 patients with 2+ AFB, 75% of patients had vitamin D levels of ≤ 20. This difference was statistically significant (p=0.033). The mean vitamin D levels in patients with negative AFB findings were significantly high (25.10 ± 7.83) compared to those with 1+ (14.77 ± 5.65), 2+ (13.39 ± 9.51) and 3+ (14.94 ± 12.34) (p<0.006). These findings strongly suggest there is strong positive association exists between the AFB grades and vitamin D levels and as the grades increase vitamin D levels significantly decrease. However these findings could not be compared with the other studies due to the scarcity of data.

In the present study 74% of patients were males and 26% were females. The male to female ratio was 2.8:1. In the present study of the 33 patients with vitamin D deficiency, 24 (64.86%) were males and 9 (69.23%) were females. No statistically significant difference was observed between sex and vitamin D levels (p=0.796). In the present study mean vitamin D levels among males were slightly high (17.40 ± 10.52) compared to females (16.05 ± 8.83). However this difference was statistically not significant. The sex-specific association between vitamin D and TB is a notable finding, which deserves some remarks. A study found the association between vitamin D status and the risk of TB in men, not in women.[76] This finding was actually consistent with a previous study in West African population, in which the vitamin D - TB association was only observed in men, not in women.[101] The underlying reason for this sex-specific association is not clear. However, men in general have higher risk of tuberculosis than women, and this has been attributed to the sex-related differences in social economic and health care access which predispose men to a greater exposure to

TB than women. Recently, it has been proposed that estrogen is a potential mediator that could account for the lower risk of TB in women; however, this hypothesis remains to be tested in empirical studies.[76]

In this study 31 to 45 years was the commonest age group which comprised of 36% patients followed by 46 to 60 years with 28% of patients. The mean age among males was 39.5 ± 17.93 years while in females it was 45.3 ± 14.09 years. The mean vitamin D levels in patients aged ≤ 30 years were significantly high compared to patients aged between 31 to 45 (26.86 ± 10.08 vs 17.16 ± 9.60) and more than 60 years ((26.86 ± 10.08 vs 10.61 ± 4.95) ($p<0.001$). However these findings could not be compared with the findings in the literature due to the scarcity of data and most of the available literature is based on age and sex matched data.

Overall, vitamin D deficiency was observed in majority of the patients with active tuberculosis. There is definite relationship between vitamin D deficiency and tuberculosis. The limitations of the study were the small sample size and that the study did not consider the history of other comorbid condition such as metabolic disorders. Further studies with large sample considering the other comorbid condition would explore the precise role of vitamin D in the pathogenesis of pulmonary tuberculosis.

Chapter 7

Conclusion

CONCLUSION

The present study showed that, summing up the patients with vitamin D deficiency and insufficiency 78% of the patients had low vitamin D levels. The mean vitamin D levels were 17.05 ± 10.04 suggestive of lower than the normal reference range.

There was strong positive association between the AFB grades and vitamin D levels and as the grades increase vitamin D levels significantly decreased. Further positive association was observed between age and vitamin D levels suggestive of significantly lower vitamin D levels with increasing age.

Chapter 8

Summary

SUMMARY

The present study was undertaken to estimate the levels of vitamin D in patients with pulmonary tuberculosis and to estimate vitamin D levels with different degrees of sputum positivity of pulmonary tuberculosis.

This one year cross sectional study was carried out on a total of 50 patients diagnosed with pulmonary tuberculosis based on WHO criteria from January 2012 to December 2012 in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum.

Of the 50 patients, 74% of patients were males and 26% were females and the male to female ratio was 2.8:1. The commonest age group was 31 to 45 years(36%). The mean age among males and females was 39.5 ± 17.93 and 45.3 ± 14.09 years respectively. The vitamin D levels were low in most (66%) of the patients while 12% of patients had insufficient vitamin D levels and 22% of the patients had vitamin D levels within the normal range. The mean vitamin D levels were 17.05 ± 10.04.

The sputum smear for AFB was negative in 26% of patients,1+ in 22%, 2+ in 32% and 3+ in 20% of the patients respectively.

Of the 10 patients with 3+ AFB, 70% of patients had vitamin D levels of ≤ 20 while 30% of patients had vitamin D levels ≥ 30. Similarly of the 16 patients with 2+ AFB, majority (75%) of patients had vitamin D levels of ≤ 20. In 11 patients with 1+ AFB, 9.09% of the patients had vitamin D levels ≥ 30 and 90.91% had ≤ 20. This difference was statistically significant.

In this study the mean vitamin D levels in patients with negative AFB findings were significantly high (25.10 ± 7.83) compared to those with 1+ (14.77 ± 5.65), 2+ (13.39 ± 9.51) and 3+ (14.94 ± 12.34). This difference was statistically significant.

The mean vitamin D levels in patients aged ≤ 30 years were significantly high (26.86 ± 10.08) compared to those who were aged 31 to 45 (17.16 ± 9.60) and more than 60 years (10.61 ± 4.95).

Overall, vitamin D deficiency was observed in majority of the patients with pulmonary tuberculosis. There is a strong association between the vitamin D levels with increasing AFB grades and age.

Chapter 9

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Annexures

Annexure I

ANNEXURE I – CONSENT FORM

"CORRELATION OF SERUM VITAMIN D LEVELS WITH SPUTUM POSITIVITY IN PATIENTS WITH PULMONARY TUBERCULOSIS – A ONE YEAR CROSS SECTIONAL STUDY."

Objective and purpose of the study:

This research is intended to estimate the Serum Vitamin D levels in patients with Pulmonary Tuberculosis in relation to sputum positivity. The principal investigator of the study is Dr. **** ****, under the guidance of Dr. ***** *******

Procedure:

If you agree to be part of the research study you will be asked the relevant history and will be subjected to relevant clinical examination and investigations. You will also have to give blood sample and get a chest x ray done for the same study.

Risk and Benefits:

The only risk and possible discomfort you might get is while taking blood from your arm for the investigations. It may cause swelling, pain, redness, bruising or infection (rarely happens) at the site from where the blood is drawn.

You may also face some radiation hazards while getting an x ray done.

Alternatives

Taking part in this study is voluntary. You may choose not to take part in this study, or if you decide to take part you can later change my mind and withdraw from the study. Your decision will not change the present or future

health care or other services that you receive. The study doctor or sponsorer may stop your participation in this study any time. If you choose not to take part in the study you will receive the standard treatment for patients with your condition.

VOLUNTARY PARTICIPATION/ WITHDRAWAL:

Your participation in this study is entirely voluntary and you may withdraw from the study at any time.

Privacy and Confidentiality

All information collected about you during the course of this study will be kept confidential to the extent permitted by law. The code numbers will identify you in this research record. Information from this study may be published but your identity will be confidential in any publication.

Institution / Sponsor's policy

Does not apply to this research

Financial incentives for participation

You will not be paid / offered any gifts /incentives for participating in the study.

Authorization to publish the results

The results of the study would be forwarded to the KLE University, Belgaum as part of requirement towards the completion of MD degree, review and publishing.

If you have any questions about my rights as a participant you may call:

1. Dr. **** ******,
   Investigator,
   PG in General Medicine,
   JNMC,
   Phone No.: **** ******

2. Dr. ***** ******
   Associate Professor,
   Dept of General Medicine,
   JNMC,

3. Dr. **** *******,
Professor & HOD,
Department of Medicine,
JNMC,
Phone No: ***** ****
Extn: **** ****

4. Dr. ***** ****,
Chairman,
J.N.M.C Ethical Committee for
Human Research,
Phone number: **** ****.
Extn: ****

CONSENT

I voluntarily agree to take part in this study by signing on the line below. I may withdraw at any time. I am not giving up any of my legal rights by signing this form. My signature below indicated that I have read this entire consent form or it has been read to me, and has been explained to me in my vernacular language and had all my questions answered. I will be given a copy of this consent form.

Signature /Left Thumb print of the Participant or legally authorized representative.

Participant's Name/ Signature/ Left Thumb	: …….
Impression of the participant's	: …….
Name of the legally authorized representative/ Guardian	: …….
Signature/ Left Thumb Impression.	: …….
Witness's Name	: …….
Signature/ Left Thumb Impression.	:…….
Investigators name and Signature	: …….
Date and Place	: …….

Dr. ***** ****
Asso. Professor,
Dept. of Medicine, J. N. Medical College,
K.L.E. University, Belgaum 10.
Ph.No. **** ******
Ext. ****

Dr. ***** ******
Post-Graduate,
Department of Medicine,
J.N. Medical College,
Belgaum
Ph.No. *******
Ext. ****

Annexures

Annexure II

ANNEXURE II – PROFORMA

Patient Name: I.P number:

Age: Sex:

Address: Occupation:

Date of admission: Date of discharge:

SYMPTOMS :

1. Fever Yes/No
2. Cough with expectoration Yes/No
3. Breathlessness Yes/No
4. Hemoptysis Yes/No
5. Loss of Weight Yes/No

PAST HISTORY:

1.Diabetes Mellitus Yes/No

2.Renal Failure Yes/No

3.Liver Disease Yes/No

TREATMENT HISTORY:

Antitubercular therapy Yes/No

PERSONAL HISTORY:

Habits: h/o smoking Yes/No

H/o Alcohol consumption Yes/No

PHYSICAL EXAMINATION:

GENERAL CONDITION:

Pallor: Yes/No

Icterus: Yes/No

Lymphadenopathy: Yes/No

Cyanosis: Yes/No

Clubbing: Yes/No

Edema: Yes/No

VITALS:

Temperature:

Pulse:

Respiratory rate:

Blood pressure:

SYSTEMIC EXAMINATION:

R. S.:

Inspection:

Palpation:

Percussion:

Auscultation:

C.V.S.:

P.A.:

C.N. S:

LABORATORY INVESTIGATIONS:

- Sputum for AFB
- CBC
- Liver Function Tests
- Renal Function Tests
- Chest x-ray
- Random blood sugar
- Serum Vitamin D

DIAGNOSIS:

Annexures

Annexure III

ANNEXURE III – KEY TO MASTER CHART

-	-	Negative
+	-	Positive
BB	-	Bilateral breathing
F	-	Female
gm%	-	Gram percent
GPE	-	General physical examination
LC	-	Left side crepts
M	-	Male
mg/dL	-	Milligram per deciliter
mm	-	Millimeter
N	-	No
RC	-	Right side crepts
Y	-	Yes