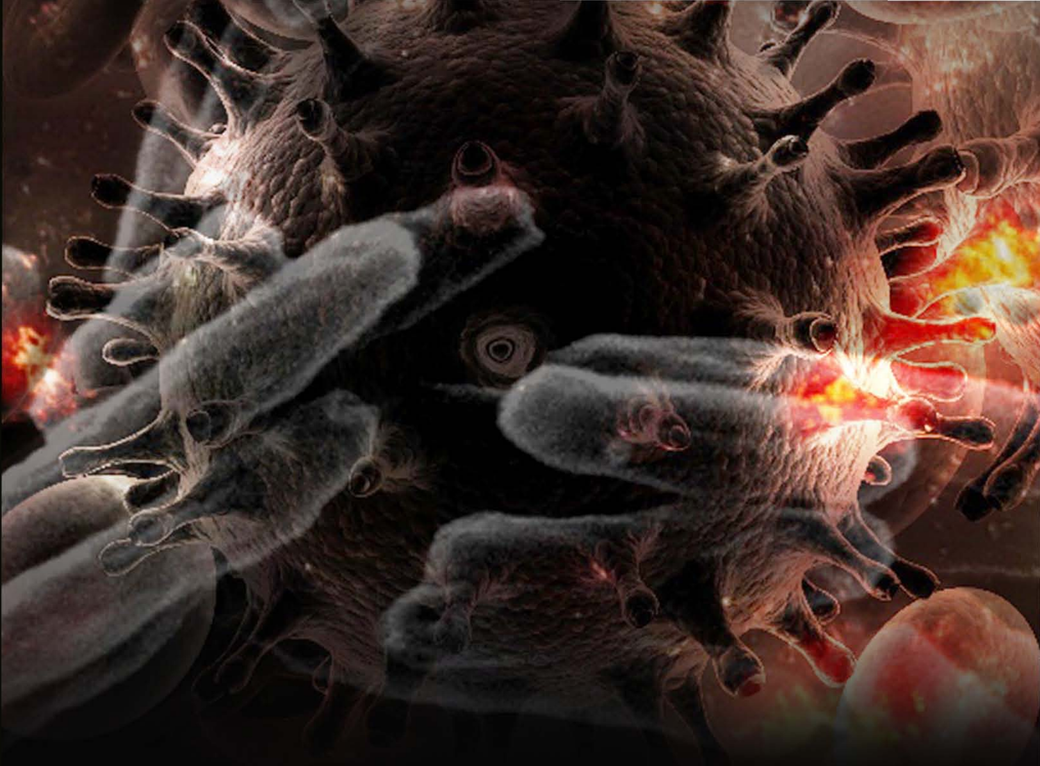

Tuberculosis

AND CO-INFECTION WITH

HIV-AIDS

Its History, Cause and Spread

Edited by Pranveer Singh

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Dedicated to my Parents (Mummy & Papa)
and Grandparents (Baba & Aazi)

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PREFACE

Epidemiology deals with the distribution pattern, cause, and effect of disease in a population. It analyzes the causative and risk factors for a disease over a period of time, so as to arrive at preventive measures and policy on the part of stakeholders. The science of epidemiology involves the collection, design, and analysis of statistical data, and some mathematical modeling to draw meaningful interpretation. Major sub-disciplines of epidemiology include forensic epidemiology, occupational epidemiology, investigation of the root causes of disease, outbreak, surveillance, screening, and biomonitoring.

Tuberculosis is a highly contagious disease. It is usually communicated from person to person, via droplets containing infectious particles. Tuberculosis infection is airborne, and follows person to person communication. Drug-resistant tuberculosis (DR-TB) is posing a serious threat to tuberculosis control measures in India. Tuberculosis coupled with co-infections, like HIV, in an immune-compromised individual, complicates the pathological state of the infected person. Tuberculosis is difficult to diagnose in an individual with HIV by conventional diagnostics, and cannot be cured by conventional therapy. This may pose challenges for the control of TB and HIV epidemics.

Madhya Pradesh is home to one of the largest tribal populations in India. Tribal people, owing to lack of awareness, lack of education and poor socio-economic conditions, fall prey to diseases like tuberculosis, which is a poor man's disease. Tribal people resort to 'black magic', witchcraft, or sometimes traditional medicine, but avoid modern allopathic treatment, which further aggravates the disease. This also makes the affected person a potential reservoir, transmitting the disease to others. Therefore, it is very important to carry out an epidemiological survey to address the issues involved with respect to tuberculosis in particular, which has now switched to drug resistant TB (MDR/XDR-TB) and HIV-AIDS co-associated with TB.

Current epidemiological study has focused on the selected regions of Madhya Pradesh, based on previous records of TB and HIV prevalence,

and looks into socio-economic, historical factors, as well as clinical factors, responsible for the emergence, prevalence and control of TB. The strategy involves meta-analysis and statistical analysis of previously available and current data, laboratory analysis, and mathematical modeling to study its projection and control. The main objectives include; studying the historical background and overview of tuberculosis in the identified regions of Madhya Pradesh; studying the efficacy of modem TB diagnostics coupled with herbal treatment protocols under traditional healers by tribes within the study area; studying the epidemiology of tuberculosis, including rate of incidence, prevalence, and spread; and study of the epidemiology of HIV and associated co-infection, particularly tuberculosis, including distribution and gender-based relationships. Results have clearly shown the efficacy of combined treatment which includes modem diagnostics, coupled with treatment using herbal medicine. Trends and seasonality analysis have established a season-dependent peak and fall, and a definite trend of tuberculosis infection in Madhya Pradesh. The epidemiology of HIV+TB has established gender-based prevalence, and also distribution across different blocks and sub-divisions of the Anuppur district of Madhya Pradesh.

LIST OF ABBREVIATIONS

AFB	Acid Fast Bacilli
AIDS	Acquired Immuno Deficiency Syndrome
ANC	Ante Natal Care
ART	Antiretroviral Therapy
BAL	Broncho Alveolar Lavage
BCG	Bacilli Chalmette Guerin
BMHRC	Bhubaneswar and Bhopal Memorial Hospital and Research Center
BRAC	Bangladesh Rural Advancement Corporation
BSI	Botanical Survey of India
CDC	Center for Disease Control and Prevention
C&DST	Culture and Drug Susceptibility Testing
CIMAP	Central Institute of Medicinal and Aromatic Plants
CP	Continuation Phase
CSF	Cerebrospinal Fluid
CT	Compound Tomography
DMC	Designated Microscopy Centers
DR	Drug-Resistant
DR-TB	Drug Resistant Tuberculosis
DST	Drug Susceptibility Testing
DOTS	Directly Observed Treatment Short-Course
DOTS	Direct Observed Treatment Strategy
EPTB	Extra Pulmonary Tuberculosis
EQA	External Quality Assessment
XDR	Extensively Drug Resistant
FIND	Foundation for Innovative New Diagnosis
HAART	Highly Active Antiretroviral Therapy
HDI	Human Development Index
HIS	Health Information System
HIV	Human Immune Virus
ICMR	Indian Council of Medical Research
INH	Isoniazid
IUATLD	International Union against Tuberculosis and Lung Disease
IVDU	Intravenous Drug Users
LCC	Leprosy Coordinating Committee

LPA	Line Probe Assay
LTBI	Latent TB Infection
LTB	Latent Tuberculosis
MCI	Medical Council of India
MDR-TB	Multi-Drug Resistant Tuberculosis
MOTC	Medical Officer TB Control
MOTT	Mycobacteria Other Than Tuberculosis
NGOs	Non-Governmental Organization
NTM	Non Tuberculosis Mycobacterium
NTP	National Tuberculosis Programme
NTI	National Tuberculosis Institute
NRLS	National Reference Laboratories
NIRT	National Institute for Research of Tuberculosis
NITRD	National Institute of Tuberculosis and Respiratory Disease
OI	Opportunistic Infection
PMDRT	Programmatic Management on Drug-Resistant Tuberculosis
PTB	Pulmonary Tuberculosis
PWB	Patient Wise Box
RMP	Rifampicin
RNRC	Regional Medical Research Center
RNTCP	Revised National Tuberculosis Control Programme
RRDR	Rifampin Resistance Determining Region
SCC	Short Course Chemotherapy
SNP	Single Nucleotide Polymorphism
STS	Senior Tuberculosis Supervisor
TB	Tuberculosis
TDR	Tropical Disease Research
TFRI	Tropical Forest Research Institute
TST	Tuberculin Skin Test
TU	Tuberculosis Units
UMTS	Union Mission Tuberculosis Sanatorium
UNAIDS	United Nations Acquired Immunodeficiency Syndrome
WHO	World Health Organization
WHA	World Health Assembly

CHAPTER I

EPIDEMIOLOGY OF TUBERCULOSIS: HIV-AIDS CO-INFECTION

PRANVEER SINGH, RAMSEVAK KACHHI, VIKAS KUMAR SAKET

Abstract

Tuberculosis caused by the bacterium *Mycobacterium tuberculosis* is a highly contagious disease. It usually communicates from person to person, via droplets containing infectious particles. Tuberculosis has threatened the human race since its inception, due to its social and economic aspects, in addition to its medical and physiological morbidity. Tuberculosis has become more pathogenic and less curable, in its drug-resistant TB (DR-TB) forms, and with the co-associated infection of HIV-AIDS. Tuberculosis usually affects the lungs, but other organs may also be affected. Where the lungs are the site of infection, tuberculosis is known as pulmonary tuberculosis (PTB), however, if other parts (brain, bones, glands, etc.) are affected, it is known as extrapulmonary tuberculosis (EPTB). Countries with the highest burden of tuberculosis and HIV invariably have higher numbers of tuberculosis cases, as observed in the Asian and African continents. India shares 20 percent of the global tuberculosis burden, with the highest number of tuberculosis cases for an individual country. Madhya Pradesh, housing the largest tribal population, is particularly vulnerable to the combined threat of HIV+TB, due to the lack of education, awareness, and other socio-economic factors. India, with its improvised initiatives of RNTCP and DOTs, has considerably limited the prevalence of HIV and TB. The need of the hour is an indomitable political will to develop health policies which are largely based on empirical data and technical know-how for positive intervention.

1.1 Background

Tuberculosis is thought to be the oldest disease of man, believed to be as old as the history of humankind (Rosenblatt 1973, Dye et al. 2008). Since the beginning of human life, tuberculosis continues to be the major cause of human morbidity and mortality (Agrawal et al. 2012). There are innumerable diseases which have surfaced alongside tuberculosis in humans, but over the course of time, these have either been completely eradicated, or tamed. However, tuberculosis has continued to be a great killer since the beginning of human civilization (Wirth et al. 2008). The impact of tuberculosis has encompassed the social, as well as the economic domains of human life, since time immemorial.

Vedas (*vide infra*) references this disease, referring to it as an ancient scourge, and 'rajayakshma' (meaning 'wasting disease'). Hippocrates (460-377 BC) has named this disease as 'phthisis', which, in Greek, means 'to consume/to spit and to waste away' (Garrison 1921, Flick 1925, Webb 1936, Canci et al. 1996, Daniel 2006). Around 460 BC, tuberculosis was also associated with the term 'consumption', meaning 'wasting of the body' due to pulmonary tuberculosis (PTB). Hebrew references this disease as 'schachepheth', meaning 'waste away' which can be found in the Bible (Rosenblatt 1973, Daniel 2011). However, Prof. J.L. Schonlein coined the term 'tuberculosis' for the first time (Rosenblatt 1973). The word 'tuberculosis' has its origin in the Latin word 'tubercula', which refers to 'a small lump'. Since the time of J.L. Schonlein, who gave a name to the disease, numerous names have been employed by various workers to refer to tuberculosis (Flick 1925, Dubos et al.1952, Waksman 1964, Gutierrez et al. 2005). Oliver Wendell Holmes called it 'white plague' (Dubos et al. 1952).

Tuberculosis, in its acute and progressive stage, was referred to as 'galloping consumption' (Grigg 1958, Rubin 1995). Pulmonary TB (PTB), or tuberculosis of the lungs, has been referred to as *Tube pulmonali*, while abdominal tuberculosis is referred to as *Tabesmesenterica* (Nuland 1988). Tuberculosis cervical lymphadenitis is referred to as 'scrofula', 'king's evil', and 'struma', while the term *Lupus vulgaris* is reserved for cutaneous TB, (Zink et al. 2001).

1.2 Tuberculosis in Ancient Times

It is generally believed that TB first originated in cattle, before humans contracted the disease via zoonosis (Pearce-Duvet 2006). TB has references in Vedas, where it is referred to as 'Rajayakshma'.

Muncamitvahavisajivanayakam
Agnatayakshmadutarajayakshma (RV, X, 116, 11)

In Krishna *Yajurveda Samhita,* there is a reference to the way Soma (Moon) had contracted 'Yakshma' (Samhita et al. 1973). Later, 'Yakshma' has evolved into 'Rajayakshma' owing to the royal (Kingship and ruler) status of Soma. In Sanskrit also, this disease is referred to as 'Rajayakshma', 'Ksayah', and 'Sosa' (*Vagabhata, Ast-s and Ast-hrd, Nidan V, 1-2)* and *(CharakaSamhita, Chikitsasthanam VII, 11)* (Debnath et al.1998).

Pathological changes of TB were first reported, and described in holistic detail, in the skeletal remains of Neolithic man. Empirical evidence for TB lesions of bone was reported in Egyptian mummies from 3400 B.C. (Meachen 1936, Cave 1939, Nerlich et al. 1997). Causative bacterium for TB, the *Mycobacterium tuberculosis* has been demonstrated in the mummy of a five-year-old child (Zimmerman 1979, Crubezy et al. 1998). Ancient Chinese literature has referred to this disease as 'lung cough' or 'lung fever', giving a remote reference to the disease as tuberculosis. A reference for TB can also be found in the code of Hammurabi of the Babylonian era.

Greek academicians Homer (800 B.C), Hippocrates, Aristotle (384-322 B.C), Plato (430-347 B.C), Galen (129-199), and Vegetius (420), have extensively referred to a disease similar to tuberculosis, which was referred to as 'consumption' (Kapur et al. 1994). AI Razi (850-953) and IbnSina (980-1037), the famous Arabic physicians, have correlated lung activities with skin ulcers (Morse et al. 1964). Literature of the Middle Ages is replete with evidence of the healing touch of monarchs to cure 'scrofula' (King's Evil). King Charles II was claimed to have cured an astounding number of patients of 'scrofula' through his divine healing touch around 1629 BC (Wilson 1990, Evans 1994). London used to provide death certificates for the disease, referring to it as 'consumption', suggesting tuberculosis. In ancient times, people were divided about the contagious nature of TB, and there was a school of thought that was vehemently opposed to the idea of TB being contagious. The Republic of Lucca is credited with pioneering the legislation aimed at controlling TB.

This has given a fillip to the slew of such legislation in countries like Italy and Spain (Dubos and Dubos 1952, Formicola et al. 1987, Canci et al.1996, Rothschild 2003).

Benjamin Marten, an English physician, provided an elaborate hypothesis about TB in his seminal study *A New Theory of Consumption.* He opined that certain species of 'animalcule', or microscopically small creatures, cause tuberculosis. He further suggested that once these organisms gain entry to the body, they can generate lesions and symptoms of the disease (Webb 1936, Mensforth et al. 1978, Rubin 1995, Brosch et al. 2002).

1.3 Tuberculosis in Arts and Literature

'Youth grows pale, and specter thin, and dies'
John Keats, *Ode to a Nightingale*

TB has been extensively described in literature and works of fiction. The immortal dramatist William Shakespeare described a 'consumptive lover' in his play *Much Ado about Nothing* and 'scrofula' in *Macbeth* (Webb 1936, Vickers 2007). Charles Dickens has also described the pains of Little Blossom in *David Copperfield*, while Thomas Mann, in *The Magic Mountain* gives a detailed sketch of a TB sanatorium (Webb 1936, Saville et al. 2002). Characters like Little Eva in Harriet Beecher's Stowe's *Uncle Tom's Cabin,* Milly Theale in Henry James' *The Wings of the Dove,* and Marguerite Gautier in Alexander Dumas' *La Dame Aux Camellias* are also described as having suffered from tuberculosis (Webb 1936, Lintz 2005).

Tuberculosis is a democratic disease, and it can happen to anyone, irrespective of class, caste, or status. However, by and large, it is a 'poor man's disease'. Innumerable personalities, statesmen, writers, poets, performers, and artists have been known to contract tuberculosis (Webb 1936, Moore 1993, Rubin 1995, Gaskell 2009). Tuberculosis consumed the families of Ralph Waldo Emerson and Henry David Thoreau. Many famous Indians, like mathematician Shrinivasa Ramanujan, writer Munshi Premchand, and Kamla Nehru, have also died of tuberculosis. The list is not inclusive, as many more Indians have succumbed to tuberculosis (Webb 1936, Mohan et al. 2009, Daniel 2011).

1.4 Tuberculosis at a Glance

Tuberculosis, since its inception, has refused to die, and relentlessly threatens the human race, owing to its social and economic impact. This is

in addition to its medical and physiological morbidity. Even in the current scenario, when humans have perceptibly conquered different aspects of life through scientific prowess, they have not been able to halt the aggressive and progressive march of tuberculosis. In fact, tuberculosis has become more pathogenic and incurable, in its new form as drug-resistant TB (DR-TB). Its new-found company in the human immunodeficiency virus (HIV), leading to acquired immunodeficiency syndrome (AIDS), has made it one of the worst of the scourges which have the potential of wiping out the human race, if allowed to progress unchecked (Getahun et al. 2010). To some extent, the developed world has claimed to control the disease by improving the human development index (HDI), but developing countries, which house the majority of the population, continue to grapple with the brutal onslaught of tuberculosis. Realizing its killing potential, in 1991, the World Health Assembly (WHA) passed a resolution recognizing TB as a major global public health problem, and set out twin targets for a national tuberculosis program, the first being the detection of 70 new smear-positive patients, and the second, providing a conclusive cure for 85 percent of similar cases, so as to rejuvenate global TB programmes (Walker et al. 1981, World Health Organization 1991, Whalen et al. 1995). Following this, the World Health Organization (WHO) declared TB a 'global emergency' in 1993, citing its lethal potential (Grange et al. 2001). The directly observed treatment strategy (DOTS) was unveiled in 1994, and has become the globally recommended strategy for tuberculosis control, adopted all over the world and establishing itself as a standard protocol for tuberculosis cure (Bayer et al.1995, Sharma et al. 2009).

1.4.1 Tuberculosis Epidemiology

Tuberculosis is a highly contagious disease. It usually communicates from person to person via droplets containing infectious particles. Tuberculosis infection is air-borne and follows person-to-person communication. The tendency to contract the disease is directly correlated with the index case, the bacillary burden in sputum, and frequency of cough in the index case (Comstock et al. 1974). Social factors include overcrowding, lower socio-economic status, and unhygienic, unsanitary living conditions which all play a role in acquiring the infection. It is caused by the bacterium *Mycobacterium tuberculosis* (Boire et al. 2013). Tuberculosis usually infects and affects the lungs, but other organs may also be affected. Where lungs are the site of infection, tuberculosis is referred to as pulmonary tuberculosis (PTB), however, if other parts (brain, bones, glands, etc.) are affected, then it is called extrapulmonary tuberculosis (EPTB) (Sakula

et.al. 1982, Saket et al. 2017). In India, the mortality caused by tuberculosis is higher than that of any other infectious disease. World Health Organization (WHO) TB statistics for India estimated there were 2.2 million cases for India, out of a global incidence of 9.6 million in 2015 alone (WHO 2015). According to an estimate, of the total tuberculosis cases of around 14 million, 3.5 million are sputum positive. One million sputum positive cases are added every year (Mishra and Mulani 2013).

1.5 The Mycobacteria

Mycobacterium is the main causative organism of tuberculosis. The generic name *Mycobacterium* was coined and introduced by Lehmann and Neumann in 1896 (Lehmann and Neumann 1896). The fungus (Myco)-like appearance of the bacterium has given it a name, *Mycobacterium*. Genus *Mycobacterium* is the only member in the family *Mycobacteriaceae* and the order *Actinomycetales*, with over 150 species of the genus (Runyon 1959, Central TB Division New Delhi 2008).

Mycobacteria are aerobic, non-motile bacteria-possessing capsules, but they do not form endospores. The cell wall is comparatively thicker, waxy, and rich in mycolic acid/mycolates. The cell wall is further supplemented with a hydrophobic mycolate layer and a peptidoglycan layer, held together by arabinogalactan. The optimum temperature for growth varies from 25°C to 50°C. *Mycbacteria* are classical acid fact organisms, taking Fite's stain, Ziehl-Neelsen stain, and Kinyoun stain (Figure 1) (Bhamidi 2009). *Mycobacterium* usually resists decolorization by a weak mineral acid after staining with one of the aryl-methane dyes, which constitutes very important characteristics of the bacterium. However, acid fastness is not unique to *Mycobacteria*, as *Nocardia* species and bacterial spores also display acid fast character. The genus *Mycobacterium* can better be defined based on the chemical structure of the mycolic acid and its antigenic structure (Runyon 1959, Grange et al. 1997, Chapman 1982, Cosma et al. 2003, Appelberg 2006).

The World Health organization (WHO) has classified *Mycobacterium tuberculosis* in risk group III, owing to its air-borne infectious route. The number of TB bacilli required to infect a person is known as the 'quantum of infectious dose' and is as low as 10 TB bacilli, indicating its infection potential. The infectious TB bacilli form aerosols and can circulate in the environment as moist droplets (Bhatia 2009).

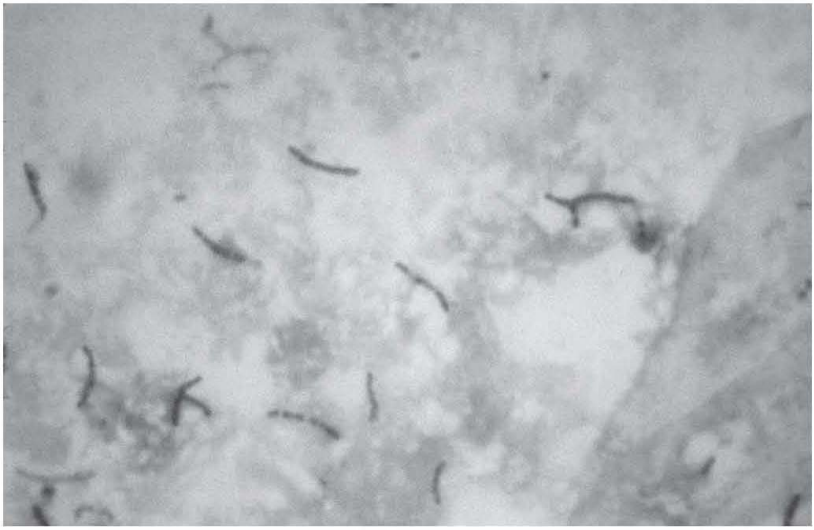

Fig. 1-1 Tuberculosis bacilli in Ziehl-Neelsen stain

1.5.1 Clinical and Taxonomic Classification

There may be anonymous, unclassified, tuberculoid, paratubercle or non-tuberculosis *Mycobacteria* (NTM), or *Mycobacteria* other than tuberculosis (MOTT). Runyon has classified these into several groups (Figure 2) (Runyon 1959, Marais et al. 2010). Clinical classification of *Mycobacterium* facilitates diagnosis and treatment. It could be classified into *Mycobacterium tuberculosis complex (MTBC)* which includes *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. microti* and *M. leprae*, which causes Hansen's disease, or leprosy. *Non-tuberculous Mycobacterium* (NTM) can cause pulmonary disease which resembles tuberculosis, lymphadenitis, skin disease, or disseminated disease (Figure 2, Table 1) (McCann et al. 2009).

Fig. 1-2 Schematics of classification of *Mycobacterium Tuberculosis*

Table 1-1 Types of tuberculosis, affected organs and causative organisms

S.No.	Type of Tuberculosis	Affected organ	Causative organism
1.	Pulmonary tuberculosis	Lungs	*Mycobacterium tuberculosis*
2.	Lower lung field tuberculosis	Lungs	*Mycobacterium tuberculosis*
3.	Endo-bronchial tuberculosis	Tracheobronchial tree	Mycobacterium tuberculosis
4.	Pleural effusion tuberculosis	Pleural cavity	Mycobacterium tuberculosis
5.	Silico-tuberculosis	Upper mid and lower lobe of lung	Mycobacterium tuberculosis
6.	Abdominal tuberculosis	Gut, peritoneum, abdominal lymph nodes, and rarely liver, spleen and pancreas	Mycobacterium tuberculosis
7.	Neurological tuberculosis	Central nervous system, meninges, sylvian fissures, basal cisterns, brainstem, and cerebellum, spinal cord, nerve roots	*Mycobacterium tuberculosis*
8.	Musculo-skeletal tuberculosis	Spine, hip, knee, intervertebral disks in the dorsolumbar regions, cervical vertebrae, craniovertebral junction, sacrum, sacroiliac joints, Ribs, pelvic bones, small bones of foot, long bones, sternoclavicular joint, sternum and bursae	*Mycobacterium tuberculosis* complex (MTBC)

9.	Cardiac tuberculosis	Endocardium	*Mycobacterium tuberculosis*
10.	Cutaneous tuberculosis	Skin	*Mycobacterium tuberculosis, Mycobacterrium bovis*
11.	Lymph node tuberculosis	superficial lymph nodes, posterior and anterior cervical chains or the suprascapular fossae but others like submandibular, periauricular, inguinal and axillary groups	*Mycobacterium tuberculosis*
12.	●cular tuberculosis	Eyes	*Mycobacterium tuberculosis*
13.	Mammary tuberculosis	Breast	*Mycobacterium tuberculosis*
14.	Genitourinary tuberculosis	Kidney and genital organs	*Mycobacterium tuberculosis*
15.	Tuberculosis in pregnancy	Lungs, bones, kidneys, uterus, spine, nervous system and brain	*Mycobacterium tuberculosis*
16.	Disseminated and miliary tuberculosis	Lungs, liver and spleen	*Mycobacterium tuberculosis*
17.	Drug-Resistant tuberculosis	Lungs and other parts of body	*Mycobacterium tuberculosis*

1.6 Types of Tuberculosis

The type of tuberculosis depends on the organ affected, such as pulmonary tuberculosis, lower lung field tuberculosis, endo-bronchial tuberculosis, pleural effusion tuberculosis, silico-tuberculosis, abdominal tuberculosis, neurological tuberculosis, heart tuberculosis, musculo-skeletal tuberculosis, cutaneous tuberculosis, lymph node tuberculosis, ocular tuberculosis, breast tuberculosis, tuberculosis in pregnancy, female genital tuberculosis, genitourinary tuberculosis, tuberculosis in chronic renal failure, disseminated

and miliary tuberculosis, tuberculosis and human immunodeficiency virus infection, tuberculosis in children, and drug-resistant tuberculosis (Table 1) (Rylance et al. 2010). The main types can be classified as pulmonary tuberculosis (PTB) and extra-pulmonary tuberculosis (PTB).

1.6.1 Pulmonary Tuberculosis (PTB)

Pulmonary tuberculosis (PTB) is a chronic and highly contagious disease caused by the *Mycobacterium tuberculosis*. *Mycobacterium africanum* and *Mycobacterium bovis* are the other *mycobacteria* which can lead to PTB. Patients having PTB with cavity lesions act as a reservoir of infection. These patients are sputum smear-positive. Coughing from an infected person produces tiny infectious droplets. Usually, a single cough produces 3000 droplets nuclei which can persist in the air for a long period of time (Rouillon et al. 1976). These infectious nuclei can survive in the dark for long periods. However, proper ventilation removes these infectious nuclei. Direct exposure to sunlight quickly kills the bacilli. There are several factors that increase the risk of exposure to the individual. The two most important factors which increase the exposure risk and disease communication, are the concentration of droplets nuclei in the contaminated air, and the quantum of time over which air is inhaled. However, the possibility of transmission of infection from a sputum smear-negative person is considerably lower. Infection with the *Mycobacterium bovis* is quite rare in the Indian scenario, due to their habit of boiling milk before use (Keers 1978).

The lung is an important site of infection, and infectious droplets gain access to the lung through inhalation of air contaminated with the *Mycobacterium* droplets nuclei which have been coughed up by an infected person. An infected person is sputum smear-positive who has either not received the treatment, or has left the treatment midway through the disease. The initial contact with the bacilli produces few observable signs or symptoms (Fregnan and Smith 1962). This is followed by a localized infection in the peripheral part of the lung. However, four to six weeks later, tuberculin hypersensitivity, coupled with mild fever and other morbidity, sets in. In most of the patients, the process is restrained by local and systemic defenses. However, the rupture that the sub-pleural primary pulmonary tuberculosis focuses on the pleural cavity may result in the development of TB pleurisy with effusion (Mohan et al. 1995).

Pulmonary tuberculosis (TB) can be further divided, into primary TB, and post-primary tuberculosis (TB). The main bases of classification of the two types of PTB are the nature of the lesion, the site of involvement, lymphadenopathy, and pleural involvement. However, the classification is not accurate, as there is substantial overlap in the symptomatic findings reported in both forms of tuberculosis (Schaaf et al.1995).

1.6.2 Primary Tuberculosis

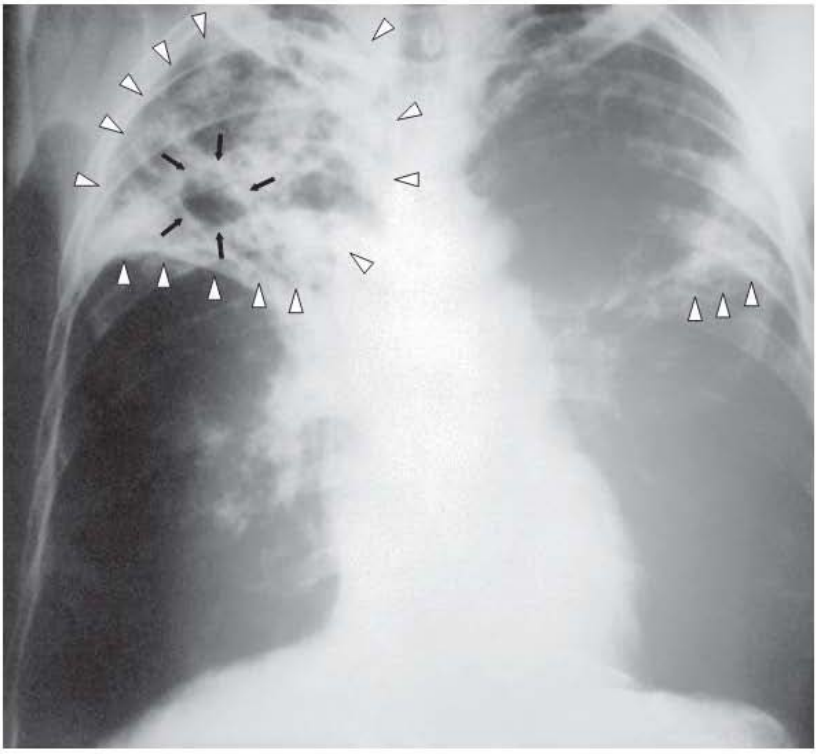

Fig. 1-3 Chest X-ray of a patient diagnosed with advanced stage of tuberculosis

Primary PTB comprises 23 to 34 percent of all reported adult cases of tuberculosis. Primary PTB incorporates lung parenchyma, lymph nodes, tracheobronchial tree, and pleura. However, the chest radiograph may appear normal in up to 15 percent of cases. The primary parenchymal lesion is visible in the area of consolidation (Volmink et al. 2003). The lesions may incorporate the whole lobe of the lung, primarily due to

endobronchial obstruction. Consolidation next to a fissure may show sharp margins (Figure 3). PTB is often indistinguishable from pneumonia as far as consolidation is concerned. However, the absence of universal toxicity, coupled with lymphadenopathy and/or failure to respond to conventional antibacterial therapy, may aid in diagnosing tuberculosis etiology (Frieden and Sbarbaro 2007).

1.6.3 Post-Primary Tuberculosis

The majority of post-primary PTB occurs due to a relapse of the infection acquired in an individual's past life. Occasionally, disease results from the initial infection by the bacterium in individuals vaccinated with bacilli chalmette guerin (BCG) (Anderson and Doherty 2005). Primary and post-primary PTB show substantial overlap on a chest radiograph. However, post-primary TB can be distinguished by the tendency for upper lobe involvement, proclivity for cavitation, and infrequent lymphadenopathy (Ducatti et al. 2006).

1.7 Drug Resistant Tuberculosis (DR-TB)

DR-TB is a serious threat to tuberculosis control programs. The first World Health Organization International Union against Tuberculosis and Lung Disease (WHO-IUATLD) anti-tuberculosis drug resistance surveillance was carried out in 1994, in around 35 countries, which reported the median prevalence of primary and acquired multi-drug resistance to be 1.4 percent, and 13 percent respectively (Abu-Raddad et al. 2009). The WHO-IUATLD has carried out drug surveillance for the second, third, and fourth time via global drug resistance surveillances in 1996-99, 1999-2002, and 2002-2007 respectively, in many more countries (WHO/IUATLD 1996). This drug resistance information was based on drug resistance from 114 countries, where no such survey had been carried out, and therefore no such information was previously available. The survey has estimated a staggering 489,139 cases of MDR-TB which emerged in 2006, of which China and India shared around 50 percent of the global DR-TB burden (Reid et al. 2006). Drug-resistant tuberculosis (DR-TB) is posing a serious threat to tuberculosis control measures in India. However, so far, no countrywide surge/prevalence data are available. Estimates of DR-TB in HIV-co-infected patients are likewise unknown. Undiagnosed and untreated DR-TB among HIV-infected patients contributes substantially to tuberculosis-related mortality and morbidity (Saket et al. 2017).

1.7.1 Multi Drug Resistant Tuberculosis (MDR-TB)

MDR-TB occurs when a patient develops resistance to, at least, isoniazid (INH) and rifampicin (RMP), the two most powerful first-line anti-tubercular drugs. Isolated cases that show multiple-resistance to any other combination of anti-TB drugs, but not to INH and RMP, cannot be considered as MDR-TB. MDR-TB usually develops when treatment is stopped midway, or the treatment regimen is not completed or followed properly (Ramchandran et al. 2009, 2010, Pai et al. 2010). In such cases, the level of anti-TB drug to kill 100 percent of the bacteria is low. To aggravate the issue further, tuberculosis usually occurs in immune-compromised high HIV-prevalence settings, where HIV/TB goes undiagnosed via conventional laboratory infrastructure, thereby affecting timely diagnosis and treatment. Also, circumstantial delays in current drug-susceptibility testing (DST) methods lead to clinical deterioration and subsequent transmission of MDR and XDR-TB. Inaccurate diagnosis in private clinics, and limited health service providers, such as under-qualified doctors, pose a serious threat to the control of tuberculosis (Murray et al. 1989, 1993, Saket et al. 2017).

A person infected with TB acts as a potential reservoir of infection, and can possibly infect 10-15 people annually. India, with its highest tuberculosis burden, accounts for about one-third of globally reported cases. About 40 percent of the Indian population is infected with tuberculosis bacilli, a majority of them having latent tuberculosis, and thus acting as a reservoir of infection for those coming into contact (National Framework for Joint HIV/TB Collaboration Activities 2009). Incomplete treatment, due to long periods of hospitalization, and the cost involved (300-700 Rs per day for up to 50 weeks), may lead to drug-resistant (DR) TB in its two most dangerous forms, such as multi-drug resistant TB (MDR-TB) or extensively-drug resistant TB (XDR-TB) (Styblo 1980).

An epidemiological survey was conducted among adults and children, at ART centers, between 2005 and 2013 in Greater Mumbai (Styblo 1989). All the suspected cases were subjected to smear microscopy, culture (phenotypic liquid culture MGIT) and drug-susceptibility-testing (DST) against first and second-line TB-drugs. This was done to determine DR-TB prevalence, and the pattern of resistance for new and previously treated, culture-positive TB-cases. The outcome of this retrospective, an observational study, has led to the categorization of the resistance pattern into four categories, viz. MDR-TB, pre-XDR-TB, XDR-TB, and XXDR-TB (Johnston et al. 2009, Boehme et al. 2010, Chen et al. 2011).

1.7.2 Issues Related to MDR-TB

MDR-TB is mostly an issue in developing countries, due to the prevailing socio-cultural and economic conditions. This situation exists in India as well. Epidemiological studies and surveys do not reflect the actual picture, or the status of the infection and prevalence of DR-TB, particularly MDR and XDR forms. The advent and circulation of DR-strains have crippled existing healthcare provision pertaining to tuberculosis. Further, the emergence of new DR-forms has outpaced the development of drugs and vaccines for them. The combined threat of new resistant forms has made tuberculosis, otherwise a curable infection, a formidable challenge for healthcare and health service providers. TB continues to pose a serious threat to the survival of the human race, despite positive intervention from healthcare providers. Tuberculosis continues to be a global challenge, despite the efforts of the World Health Organization, and centers for disease control and prevention (WHO/CDC) at the global level which aim to eradicate it. Incomplete treatment regimens, partially cured or relapsed cases, non-adherence, and poor drug availability, are the major causes of drug resistance (DR) cases. Re-infection in cases of tuberculosis, from a similar strain or new strain, is possible, as the first episode of infection does not lead to lifelong immunity (Velayati et al. 2009).

1.8 Geriatric Tuberculosis

Tuberculosis shows different epidemiological patterns in high and low incidence countries. For instance, young adults show high epidemic peaks, and the elderly show low epidemic peaks in high incidence countries. For low incidence countries, the TB burden increases with the advancing age of patients (Dutta and Stead 1992, Doherty et al. 2006). Also, older people are more prone to adverse reactions to anti-TB drugs (Davies 1994).

1.8.1 Types

In elderly patients, TB lymphadenitis, TB pleural effusion, TB of bones and joints, genitourinary TB, and TB meningitis are more common. Older people contract TB infection largely due to **exogenous infection,** or re-infection that is acquired from an external source, while **endogenous reactivation** is due to the quiescent lesions in pulmonary or extra-pulmonary regions. Endogenous reactivation is the predominant cause of TB in elderly people. This is particularly true when the rate of transmission is low in the community. In that case, increased TB cases in older age can

only be attributed to the reactivation of latent infection (Nagami and Yoshikawa 1984). However, when transmission is high in the community, then infection is largely due to exogenous reinfection. This might be true in developed countries which receive a huge influx of refugees and asylum seekers, making elder people with weakened immunity vulnerable to TB infection. This delays the transmission of TB from a community (Canetti et al. 1972). Elderly patients remain asymptomatic, due to lack of fever or a persistent cough and other systemic symptoms. This delays diagnosis and palliative therapy.

1.8.2 Diagnostic Limitations

Hematological TB parameters are found to be similar in young and elderly patients. Serum biochemistry is also similar in both, except for mild elevation of alkaline phosphatase and liver enzymes, which could be due to asymptomatic involvement of the liver by TB (Umeki 1989, Lee et al. 2005, Das et al. 2007, Gupta 2014). Although, the treatment regimen is similar for young adult and elderly patients, compliance is less from elderly people on account of forgetfulness, dementia, poor eyesight, and lack of motivation to go for treatment, on account of infirmity and old age. Therefore, medication under DOTS is highly recommended for elderly people (Yoshikava 1992, WHO 2003).

1.8.3 Treatment Challenges

Elderly patients having pneumonia and not responding to the antibiotics are the usual suspects for TB. Elderly patients may not cough up enough sputum for microscopic analysis. Therefore, if the clinical suspicion is high, and the sputum smear is negative (false negative), more invasive protocols such as laryngeal swab, fibreoptic bronchoscopy, and examination of various bronchoscopic secretions and gastric aspirate, may be examined for diagnostic purposes (Stead 1981, Chan et al. 1992). Similarly, a negative tuberculin skin test (TST) may not rule out a TB diagnosis. In such cases, efforts should be made to take microbiological or histopathological specimens, such as fine needle aspiration, cytology material from the lymph nodes, cold abscess, and other body fluids and secretions, depending upon the clinical situation, for diagnostic purposes (Stead 1981, Chan et al. 1992).

1.9 Pediatric TB

Tuberculosis in children is mostly an exogenous infection. It reflects the rate of ongoing transmission in the community, and indirectly provides an index of adult TB (Chintu 2007, Rekha and Swaminathan 2007). The epidemiology of paediatric TB essentially reflects the epidemiological pattern of adult TB. TB is one of the major causes of mortality in children in both developing and developed countries. Mortality occurs largely due to meningeal and miliary TB in early childhood (Datta and Swaminathan 2001). Paediatric TB adds 10 percent to the total TB burden. However, this may go up to 40 percent in high incidence communities (Donald 2002, Mandalakas and Starke 2005).

Paediatric TB infection is more prevalent in developing nations, with the annual risk ranging from 2 to 5 percent. India shows an annual risk of 1.5 percent of paediatric TB. Around 40 percent of children contract TB at less than one year of age. If left untreated, it may escalate into lymphadenopathy or segmental lesion (Miller and Seale 1963, Comstock et al. 1974, Enarson 1995, Munoz and Starke 2000, Chadha 2005, Chadha et al. 2005).

1.10 Molecular Diagnostics

The pace with which drug resistance is developing requires rapid development of new diagnostics to outpace it. This will complement tuberculosis control programs in an effective manner. The latest developments in phenotypic drug susceptibility testing incorporate *mycobacterium* growth indicators and phage-based assays. These methods can provide results on phenotypic resistance in 2 to 10 days. However, the culture of viable bacilli poses a serious health hazard to laboratory personnel, and therefore requires stringent biosafety measures. Numerous PCR-based molecular techniques have revolutionized the pace of detection of drug resistance, thus neutralizing the limitations associated with it (Boehme et al. 2010, Saket et al. 2017).

Non-synonymous single nucleotide polymorphism (SNPs), and the number conferring resistance, pose a challenge to the development of genotypic drug susceptibility-determining protocols (Migliori et al. 2012, Saket et al. 2017). These issues are partially addressed by analyzing the prominent non-synonymous single nucleotide polymorphism (SNPs), though with low sensitivity and specificity. However, lack of downstream processing, which could lead to the identification of non-synonymous single nucleotide polymorphism (SNPs) within the PCR amplified domain

(e.g., hybridization to immobilized oligonucleotides, microarray, dot blot hybridization, denaturing high-performance liquid chromatography, and DNA sequencing), has further acted as a severe roadblock (Saket et al. 2017). These steps are complicated to perform, and being multistep processes, could increase the chances of cross-contamination, resulting in inaccurate diagnosis (Jacobson et al. 2010). It has been shown that the analysis of conformational changes created by non-synonymous single nucleotide polymorphism (SNPs) in a heteroduplex could be used to determine rifampin susceptibility (Anh et al. 2000). Further, thermal denaturation profiles of heteroduplexes could also lead to the enhanced detection of non-synonymous single nucleotide polymorphism (SNPs) that leads to resistance in *M. tuberculosis*. Since the thermal denaturation profile of DNA is largely dependent on the nucleotide content of the fragment, any change in the sequence would alter the thermal denaturation profile. The altered thermal denaturation profile could be detected by analyzing the binding efficiency of fluorescent dye to DNA fragment at variable temperature. However, a transversion point mutation (A:T and G:C) cannot be detected by this protocol, as this mutation has very little effect on the overall thermal denaturation profile. This limits the efficiency of point mutation detection (transition and transversion) analyzing the thermal denaturation profiles of DNA duplexes having DNA fragments, with, and without, change into heteroduplex and homoduplex, respectively (Mohan et al. 2009, Jacobson et al. 2010).

Caminero et al. (2001) have devised a protocol for determining rifampin resistance by analyzing the unique thermal denaturation profile of the rifampin resistance-determining region (RRDR) of the *rpoB* gene. Mono-resistance to rifampin is a rarity, as it is mostly accompanied by isoniazid resistance, thereby providing the rifampin resistance profile as a possible biomarker for suspected MDR-TB and XDR-TB cases (Eisenach et al. 1988, Dale et al. 2001, Saket et al. 2017).

1.11 Treatment

Chemotherapy for TB was initiated with the discovery of streptomycin in 1994. However, this monotherapy soon led to TB bacilli developing resistance to streptomycin. Later, combined therapy involving streptomycin with para-aminosalicylic was found to be effective in drug-resistant cases. This was further improved by the advent of isoniazid, which formed the basis for primary chemotherapy in the 1950s and 1960s. Therefore, the standard regimen comprised of streptomycin, isoniazid and para-

aminosalicylic acid for a period of up to 18-24 months. Para-aminosalicylic could be substituted with ethambutol or thioacetazone, depending upon the availability and acceptability in the community. Drug interactions, particularly between isoniazid and rifampicin, should be taken into account before prescribing these drugs, as rifampicin enhances the metabolic degradation of drugs like ascorticosteroids, digoxin, oral anticoagulants, and hypoglycemic agents. Anti-TB drugs should be administered according to body weight. Pyridoxine should be given along with isoniazid, and streptomycin should be avoided in elderly patients (Yoshikava 1992, WHO 2003).

1.11.1 Intermittent Treatment

Studies have proved that thrice-a-week alternate day treatment provides an effective cure. This is because intermittent treatment allows an organism to enter the reproductive phase where bactericidal agents like isoniazid and rifampicin are more effective (Kasozi et al. 2015).

1.12 Tuberculosis (TB) Associated Co-infection

1.12.1 HIV-AIDS

Tuberculosis, though an ancient disease, continues to wreak havoc for the sustenance and existence of the human race. The effects are particularly visible in the resource-starved developing world. Tuberculosis, coupled with co-infections like HIV, in an immune-compromised individual, complicates the pathological state of the infected person (Corbett et al. 2003, 2010). This problem has now assumed pandemic proportions, both for the developing and the developed world, though the problem is more severe in developing countries. Tuberculosis is difficult to diagnose in HIV-status individuals by conventional diagnostics, and cannot be cured by conventional therapy. This may pose a challenge for the control of TB and HIV epidemics. HIV-related TB continues to spread, posing a challenge to the established TB control programmes, such as directly observed treatment strategy (DOTs) and revised national tuberculosis control programmes (RNTCPs) (Robertson et al. 2006). Infection with HIV weakens the immunity of the affected individual. In immune-compromised individuals, tuberculosis is the first opportunistic infection that sets in. Additionally, many other pathological and genetic morbidities, such as cancers, may be established.

1.12.2 HIV-AIDS Pandemic

Immune deficiency caused by HIV infection affects the natural history of TB infection. In this way, an HIV pandemic exerts an influence on tuberculosis (TB) epidemiology, as well as tuberculosis (TB) control measures. Countries with the highest burden of tuberculosis and HIV have an invariably higher number of tuberculosis cases, as observed in the Asian and African continents (Dhasmana et al. 2008). Current estimates by the World Health Organization (WHO) and the joint United Nations programme on HIV/AIDS (UNAIDS) have revised the number of people living with HIV/AIDS to 33.2 million. These estimates show a 16 percent downward trend from 39.5 million in 2006 (WHO 2005). These estimates include a major contribution from India. The revised figure for India estimates that 2.5 million people, i.e. 0.4 percent of the adult population in India, are HIV-seropositive. In the context of the global figure, these estimates represent less than half of the estimated figure of 5.7 million (Dhasmana et al. 2008).

1.12.3 The Burden of HIV-AIDS Co-infection in India

India shares 20 percent of the global tuberculosis (TB) burden, with the highest number of tuberculosis cases for any individual country. The major challenges which are faced in tuberculosis control are the emergence of drug resistance forms, HIV/AIDS co-infection, diabetes, malnutrition, smoking, etc. The 'Holy-Grail' of RNTCP for the year 2017 is to ensure universal access to quality TB diagnostics, and cures for all the forms of tuberculosis, including drug-resistant forms and HIV/AIDS co-infection, as well as relapsed or uncured cases. Under the aegis of LEPRA, a UK-based international charity which controls, manages and rehabilitates people affected from the disease, a programme called 'Dastak' meaning 'knock at the door' has been organized, to educate and inform at least one member in every family about tuberculosis. This program, launched by the 'Buniyad' project in Madhya Pradesh, sensitized over 54,810 households in 185 villages on the occasion of World Tuberculosis (TB) Day. Under this program, people were made aware about the relevant aspects of tuberculosis, such as its genesis, cause, spread, pathogenesis, prevention, precaution, and cure, etc., with the tagline 'Stop TB in my lifetime: Zero TB death and a world free of TB'. A number of such programmes have been organized by the state and district authorities, with stakeholders, to spread the word about tuberculosis which continues to be a major global health problem. According to an estimate from the WHO (2006), about 2

million people die of tuberculosis annually, while around 9 million develop the disease each year (Zwarenstein et al. 1998, WHO 2011).

1.13 Trends in Global Tuberculosis

The WHO's 2008 global data on tuberculosis epidemics is based on estimates from around 202 countries, which constitute 99.9 percent of the global population. This report provides detailed and in-depth insight into the current status and the future trends of tuberculosis epidemics. In 2006, the WHO's global tuberculosis report estimated 9.2 million new tuberculosis cases, at the rate of 139 cases per 100,000 population, of which 4.1 million, at the rate of 62 per 100,000, were new sputum smear-positive cases (Central TB division 2008, WHO 2013). The good news was that the rate of tuberculosis prevalence had followed a downward spiral, which was positive and relieving for the stakeholders, as well as the healthcare providers. The year 2006 witnessed a falling trend in global tuberculosis, which appeared to have levelled off for the first time since 1993 (WHO 2015). The total number of new tuberculosis cases registered shows an increasing trend, because of a continuing increase in the caseloads in the African, Eastern Mediterranean and south-east Asian regions. The majority of the cases (55 percent) were reported from Asia, particularly south-east Asia and the western Pacific regions. The second-largest figure (31 percent) was reported from the sub-Saharan African regions. Based on the number and frequency (incidence), 22 countries were given special attention. This inflated number might be due to HIV/AIDS co-infection. According to an estimate, in 2006 alone, 17 million people died of tuberculosis, of which 231,000 were co-infected with HIV (Zaki 1971, WHO 2008, Ricks et al. 2009, Zumla et al. 2014).

Mycobacterium tuberculosis infects and affects one-third of the global population through tuberculosis infection. According to 2006 estimates, of the 9.2 million new reported cases of tuberculosis, 7 million were co-infected with HIV, while in the same year, the number of tuberculosis deaths was reported to be 1.7 million, of which 230,000 were co-infected with HIV. Twenty percent of these deaths could have been avoided. Globally, 95 percent of tuberculosis cases and 98 percent of tuberculosis deaths occur in developing countries. Also, 75 percent of tuberculosis cases occur in the economically productive age group, i.e. 15-40 years in developing countries (Bonnet et al. 2006).

1.14 The National Pattern of Tuberculosis: Indian Case Scenario

India, being the second-most populous country, adds 25 percent of tuberculosis cases to the global pool annually. In 2012 alone, out of 8.6 million tuberculosis cases, 2.3 million (25 percent) cases were reported to have occurred in India. The tuberculosis control programmes in India, such as DOTs and RNTCPs are in full swing, which has led to a 42 percent reduction in tuberculosis-related deaths in 2012, relative to 1990 levels, and a 51 percent reduction in tuberculosis prevalence in 2012, as compared to 1990 levels (Ministry of Health and Family Welfare 2002).

1.14.1 National Tuberculosis Programme (NTP)

A contagious disease like TB needs to have trend analysis data in order to achieve a picture of prevalence and case incidence over long periods. However, health information systems (HIS) in developing countries are plagued by inefficiency and lack of expertise in providing meaningful data. The Indian Council of Medical Research (ICMR) has conducted a survey for the period 1955-58, to devise a concrete strategy, in the form of a 'TB Action plan', under the Tribal Action Plan 2005. Data that came out of this survey has led to the formulation of the National Tuberculosis Programme (NTP) for TB diagnosis, cure, and control. For instance, the comparative distribution of healthcare services in rural and peripheral areas was decided by the relative distribution of disease across the population. The relative stress on planned efforts towards making tuberculosis services available in rural areas was based on the observed distribution of both the population and the disease (Chakraborty 1997, 2004, Mishra et al. 2014). Also, a strategy was devised to offer a simplified diagnostic protocol for sputum-smear-positive cases for adults. Based on the overall requirements specific to the area, a strategy could be designed to create a program which will be tailor-made to suit the health needs of the region (Nakielna et al. 1975, Yatin et al. 2000, Agarwal et al. 2005).

1.14.2 Limitations and Challenges

Following its launch, the NTP has made significant contributions in improving the health index of tuberculosis in India, for over a decade. However, the actual estimates about the number of people cured, and to what extent, are not known. Similarly, the actual reduction in the number

of tuberculosis cases is also unknown (Vijay et al. 2004). As a result of the continued shortfall in the cure-rate over the year, the tuberculosis situation in India was interpreted to be showing signs of an epidemic of left–over disease, i.e. particularly treated cases with extended life-spans, increasing the problem in terms of prevalence of disease, even though a reduction in the incidence of cases or in transmission of infection had been expected. This situation had developed over the few decades prior to the 1990s in India, notwithstanding the World Health Organization (WHO) observation in a 1974 document, that, "An effective national TB programme can be delivered under any situation, provided planning and application are guided by a clear understanding of the epidemiological, technical, operational, economic and social aspect" (Gupta 2014). In its quest to reduce the tuberculosis burden and completely eradicate tuberculosis, India has kept pace with the global trend in reducing mortality and morbidity with respect to the development and improvisation of diagnostics and treatment, and also the effective management of cases. The key to this effective strategy could be a massive cut in observed cases, as well as a spike in the successful cure rate. This way the number of tuberculosis cases can be cut down to its minimum level. A decadal reduction of 1 to 2 percent in the number of tuberculosis cases could well help in achieving the target of a tuberculosis-free world. The government of India, in consultation with the WHO and the World Bank reviewed the performance of the NTP, and came up with the RNTCP. This program was introduced in a phased manner, and is covering the entire country (Sharma et al. 2009, World Health Organization 2011, Sachdeva et al. 2012).

1.14.3 The Unchecked Death-March of Tuberculosis

Tuberculosis, despite advancements in effective and accurate diagnostics and therapy, control, and preventive measures, continues to be a threat to the human race, and a major cause of mortality and morbidity in humans. The failure of control measures in halting the death-march of tuberculosis is largely due to the host and environment, as much as to the microbe itself. Increasing incidence of the Human Immunodeficiency Virus infection (HIV) in conjunction with poverty, illiteracy, smoking, malnutrition, political uncertainty, and war-induced migration, constitutes an ideal environment for the survival and spread of tuberculosis (Dewan et al. 2010, Agrawal et al. 2012). The need of the hour is an indomitable political will to develop health policy that is largely based on empirical data and technical know-how for positive intervention. Evidence-based public health practice requires both the political will to develop data-

driven health policy, and the technical capacity to collect sound data. However, these essential parameters are lacking in the hardest hit regions of the country (Lal et al. 2011).

1.15 Madhya Pradesh, India: State-Level Pattern of Tuberculosis

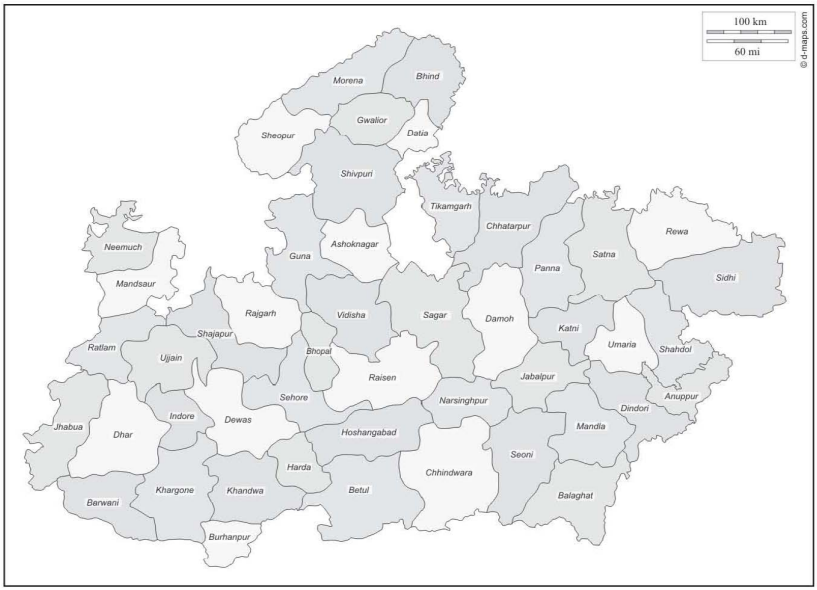

Fig. 1-4 Map of Madhya Pradesh showing different districts

Madhya Pradesh (MP), the second-largest state in India, was founded on November 1 1956. Geographically, Madhya Pradesh lies between 21'6' to 26'30' north latitude and 74'9' to 81'48' east longitude. The total geographical area is 3,08,245 square kilometers, which is about 9.38 percent of the total geographical area of the country (Madhya Pradesh 2014). Madhya Pradesh is surrounded by Uttar Pradesh in the north, Chhattisgarh in the north-east, Rajasthan and Gujarat to the west, and Maharashtra to the south. The total boundary line of the state is 605 kilometers from north to south, and 870 kilometers from east to west. Madhya Pradesh comprises 51 districts and 272 tehsils (Figure 4). Out of 313 sub-divisions, 89 are tribal sub-divisions. Anuppur, Dindori, Mandala, and Jhabua districts are dominated by tribal populations (Rao et al. 2010).

1.15.1 Tuberculosis: A Notified Disease

The Madhya Pradesh government has already declared tuberculosis as a notified disease, due to its alarming rise. The State has nearly 90,000 patients, with the addition of 17 MDR-TB cases per month. It has a poor showing in almost all the reported parameters related to tuberculosis, and is much above the national average. For example, the Saharia tribe alone shows a very high prevalence of 1,270 reported cases per lakh population, against the national average of 216 cases per lakh. Indore has reported 200 MDR-TB cases, while the state capital (Bhopal) has 37 active cases. Keeping these vital statistics under consideration, Madhya Pradesh has revised its strategy, particularly in the selected tribal regions, to address the specific healthcare needs of the tribal population. At the community level, the infection load, along with disease prevalence, is the result of the complex interplay of the epidemiological process. There is a need to undertake an exhaustive comparative analysis of TB epidemiology between western countries and the Indian scenario, to develop and elucidate the underlying principles and concepts (Chakma et al. 1996). Tuberculosis manifests itself in low nutrition, poor hygiene and improper conditions of sanitation. Madhya Pradesh, housing the largest tribal population, is particularly vulnerable to the combined threat of tuberculosis (TB), MDR/XDR-TB and HIV, which are cause and effect to each other.

1.15.2 Anuppur – Amarkantak (A Tribal Population-Dominated Region)

The Amarkantak region lies in the Anuppur district, covering 4 blocks, namely, Pushparajgarh, Jaitahari, Kotma, and Anuppur. The Pushparajgarh block in Anuppur is predominantly a tribal block, with more than 95 percent of population being from the scheduled tribe (ST), followed by scheduled caste (SC) populations (Saxena 1986, 2012). Amarkantak forms a border with Anuppur and Dindori in Madhya Pradesh, and Bilaspur in Chhattisgarh. These districts are dominated by Gond, Baiga, Panika, Kol, Pradhan, Kumhar and Saharia tribes (Mishra 1956).

These tribes live in jungles, called 'Van grams', to reinstate their ownership right to the forest produce. These Van grams are not well connected to the cities by commutable roads. Owing to abject poverty, illiteracy, and lack of awareness, these tribes resort to 'hokum' or occult practices, and sometimes traditional healers, to get relief from various diseases (Jain 1990). Due to extreme poverty and unhygienic, unsanitary,

cramped, dark, and dingy living conditions, these people are easy prey to 'poor man's' diseases, such as tuberculosis. This kind of living condition creates an ideal situation for tuberculosis bacilli, *Mycobacterium tuberculosis*, to grow and spread.

1.16 Overall Challenges

1.16.1 Diagnostical Perspective

Tuberculosis is one of the major causes of mortality and morbidity in humans. Around 30 percent of the global population is believed to be infected with tuberculosis (active or latent). This is a cause of concern, as the quantum of this number acts as a reservoir to spread the infection to others, thus creating a chain of infection (Corbett et al. 2003, 2010, WHO 1994, 2005). Like any other disease, tuberculosis management and control requires rapid and accurate diagnosis to start corrective treatment. This is important, as conventional diagnostic measures, like sputum smear microscopy and chest X-ray radiography, may not work in the case of drug-resistant TB or TB with an HIV-AIDS background (Foulds and Brien 1998, Perkins and Small 2006, Perkins 2006). Increased incidence of TB epidemics has sent alarming signals globally, and efforts from both government and non-governmental organizations are being made to come up with improved therapy and diagnostics (Doherty and Rook 2006, Perkins et al. 2006, Spigelman and Gillespie 2006).

The chest radiograph can raise the suspicion of TB in an elderly person. Since it is only a two-dimensional shadow, it cannot be considered as a confirmation of the diagnosis. This holds also true for computed tomography scans (CT) scans. All elderly patients with pneumonia, who do not respond to antibiotics, should be investigated for TB (Chan et al. 1992). Microscopy of a smear prepared by the Ziehl–Neelsen method, or fluorescence staining and detecting the acid-fast bacilli, facilitates immediate diagnosis. However, elderly people have insufficient strength to expectorate and may not be able to provide an adequate sputum sample for testing. If the clinical suspicion is high, and the sputum smear is negative, more invasive methods, such as a laryngeal swab, fibreoptic bronchoscopy, and examination of various bronchoscopic secretions and gastric aspirate, can be undertaken. Sputum examination is more likely to give positive results in the elderly with reactivation TB, as compared to primary TB. In the progressive primary disease, where the number of bacilli is usually small, their demonstration is usually difficult (Stead 1981).

The value of the tuberculin skin test (TST) for the diagnosis of active TB in the elderly is limited. In a patient suspected to have TB, a strongly positive TST increases the likelihood of diagnosis. However, a negative TST does not rule out the diagnosis of TB. In patients with extra-pulmonary TB, efforts should be directed to obtain appropriate specimens for microbiological and/or histopathological diagnosis, and these include fine needle aspiration cytology material from lymph nodes, cold abscess, and other body fluids and secretions, depending upon the clinical situation. Tuberculosis in children can be diagnosed by laboratory tests and tuberculin skin tests (TST).

Laboratory tests: The diagnostic tests for pulmonary TB can be broadly divided into two categories: [i] demonstration/isolation of *Mycobacterium tuberculosis* or one of its components; and [ii] demonstration of the host's response to exposure to *Mycobacterium tuberculosis*.

Tuberculin skin test (TST): The tuberculin skin test is most widely available, and commonly used, for establishing the diagnosis of TB infection in children.

1.16.2 Diagnosis of Tuberculosis

A person with a persistent cough (> 3 weeks), and associated fever, is a usual suspect for tuberculosis. Microscopic analysis of sputum smear forms the preliminary, and most fundamental, diagnostic for pulmonary tuberculosis (PTB). At least three sputum samples are collected, observed, and analyzed microscopically, for tuberculosis bacilli. Diagnosis of tuberculosis via sputum smear analysis is usually available in a resource-limited set up of a peripheral laboratory. In addition, the government has ensured the establishment of 'designated microscopy centers' (DMC) per one lakh population all over the country, through the RNTCP (Saket et al. 2017).

Tuberculosis diagnostics looks for *Mycobacterium tuberculosis*, the causative organism for tuberculosis. Diagnosis can be done through the microscopic analysis of sputum smear to detect *M. tuberculosis*, or through liquid culture (growing *M. tuberculosis* on LJ media or other compatible media for determining drug sensitivity). However, microscopic analysis of sputum smear forms the most basic diagnostic test in high-burden resource-limited setups. Sputum smear analysis is not very accurate, or confirmatory in nature, particularly for patients with drug-resistant tuberculosis (DR-TB) or those having HIV-AIDS co-infection.

Therefore, sputum analysis must be confirmed via other diagnostic protocols, like chest X-rays etc. The management of tuberculosis relies heavily on timely and accurate diagnosis (Saket et al. 2017).

A chest X-ray usually complements the sputum analysis for tuberculosis, but alone it is not very reliable, as other chest diseases may also give the impression of tuberculosis (Figure 3). Therefore, unless confirmed by sputum analysis, patients with abnormal chest radiographs should not start the treatment. Microscopic analysis of sputum smear is confimatory in nature, and also provides the degree of infectivity, thereby facilitating the monitoring of the response of patients to anti-TB drugs.

Smear-positive pulmonary cases: Patients with at least two sputum samples which are positive for acid-fast bacilli (AFB) via microscopic analysis of sputum smear, fall into this category. Patients with only one sputum positive sample, but who are confirmed through chest X-ray also fall under this category.

Smear-Negative pulmonary cases: Patients with at least three sputum samples negative for AFB via microscopic analysis of sputum smear, fall into this category. Patients with radiographic abnormalities, history/clinical evidence consistent with active pulmonary TB, and a confirmed diagnosis by a physician based on observable symptoms, fall into this category (Dutt and Stead 1994).

Extra-pulmonary (EPTB) cases: This form of TB manifests in other parts of the body like pleura, lymph nodes, bones and joints, the genito-urinary tract, and the central nervous system, instead of the lungs. Diagnosis is not straightforward, as in the case of pulmonary TB, and involves complicated and specialized diagnostic procedures such as 'fine-needle aspiration cytology (FNAC)' or biopsy. Extra-pulmonary TB is not contagious, and cannot be spread from an infected person to a healthy person (Bhatia 2009).

1.17 Pathological Perspective

Tuberculosis (TB) has continued to rattle the human population since time immemorial. Since inception tuberculosis is associated with irresolvable myths, and social stigma, due to associated morbidities like scourge, phthisis, struma, phyma, hectic fever, and consumption, it has been aptly referred to as 'white death'/'white plague' or 'Men of Death' (as suggested by John Bunyan, an evangelist). The etiology, pathogenesis, clinical features,

and treatment, of TB have been the subject of controversy and myths for centuries. The biggest limitation in TB cure and management is delayed diagnosis, and therefore, delayed treatment, which adds to the complications, in the form of DR-TB or relapse cases. This is further complicated by the emergence of the Human Immunodeficiency Virus (HIV), leading to the Acquired Immunodeficiency Syndrome (AIDS) pandemic. The AIDS-TB combination has a devastating effect on human life, as it becomes an incurable infection. Further, DR-TB and TB with HIV-coinfection do not respond to standard diagnostic protocols and therapy. There is a huge number of tuberculosis cases that go undetected/untreated due to lack of awareness or abject poverty. Such people act as hosts, or reservoirs, of infection, spreading the disease in an uncontrolled manner. This creates an insurmountable barrier to healthcare professionals for tuberculosis control (Sharma and Mohan 2003).

The life cycle and pathology of tuberculosis displays basic similarities with most other infectious diseases. Tuberculosis is essentially the result of interaction between the TB bacilli and the immunity of the host. Depending upon the relative defense of the host, and the virulence or disease-causing ability of the bacteria, interaction varies, from being simple to complex, and shortlived to a lifelong and chronic condition. Hosts with compromised defenses may fall easy prey to the disease. Tuberculosis can be latent when it persists for longer without visible or identifiable symptoms. Tuberculosis affects almost every organ of the body, but usually, the lungs are the primary site of infection leading to pulmonary TB (PTB), while tuberculosis to the other parts of the body constitutes extrapulmonary TB (EPTB). Discoveries of *Mycobacterium tuberculosis* by Robert Koch, in 1882, leading to the detection of TB bacilli in sputum, forms the most common diagnostic test for tuberculosis. However, it has some severe limitations, such as (i) low clinical sensitivity (ii) failure to diagnose tuberculosis in HIV co-infection background, and (iii) a lack of designated microscopy centers (DMC) in peripheral laboratories. The limitation of sputum smear analysis for tuberculosis detection is largely circumvented by the emergence of Nucleic acid-based molecular tests (DNA/RNA) and culture-based assays, which facilitate rapid and accurate detection of DR-TB, or TB associated with HIV-AIDS (Saket et al. 2017).

1.17.1 Clinical Specimens: Collection and Transportation

For pulmonary TB (PTB), a clinical specimen is sputum. For extrapulmonary TB (EPTB), where organs other than the lungs are involved, the specimen

is usually collected from the affected organ. For instance, in the case of renal TB, urine is the clinical specimen, while for TB meningitis, cerebrospinalfluid (CSF) is the clinical specimen. Lesions showing cassation have abundant TB bacilli (Saket et al. 2017).

1.17.2 Sputum

Sputum, which is the clinical specimen of choice for PTB, is coughed up, or collected in a sterile container to avoid the risk of contamination from environmental *Mycobacterium*. A container for sputum collection should be wide-mouthed, to facilitate coughing without risking contamination from outside, should have a minimum volume of 25ml, must be transparent enough to observe the quantity and quality of sputum without opening it, must have provision for sealing to avoid leakage during transportation, be rigid enough to avoid breakage, and should have provision for labeling and identification. Keeping these criteria into account, a glass container, preferably a capped bottle with a volume of around 25ml could be ideal. These containers could be reused following proper cleaning and sterilization. A tuberculosis cure relies heavily on the quality and quantity of the specimen collected for precise diagnosis and proper treatment. Therefore, precautions and proper care must be taken to collect a specimen for timely diagnosis and treatment (Saket et al. 2017).

1.17.3 Sputum Collection (Pulmonary Clinical Specimen)

Sputum should be collected in the early morning, on an empty stomach, as food particles mixed with sputum might obstruct the microscopic observation of sputum smear. Ideally, three sputum samples are collected. First, immediately after the patient's visit, secondly, early morning sputum, and thirdly, at a random time. However, sputum collection may be a limitation for infants, children, and the elderly.

1.17.4 Extra-Pulmonary Clinical Specimens

Fibreoptic bronchoscopy - This sample is required from smear-negative TB patients who cannot cough enough sputum. This includes bronchial washings, brushings, bronchoalveolar lavage (BAL) fluid and, trans-bronchial lung biopsy.

Gastric Lavage: This type of sample is required for PTB diagnosis in infants and children. It should be taken in the early morning, after a

minimum of eight hours of fasting.

Urine: A few milliliters of urine (early morning sample) for three consecutive days are required for diagnostic purposes. The sample should be analyzed as early as possible.

Cerebrospinal fluid (CSF): CSF is required for EPTB. It is usually collected (5-10ml) in a sterile vial for culture.

Serous fluids: This includes pleural, pericardial, synovial and ascetic fluid for the culture, usually required for the diagnosis of EPTB.

Tissue: This type of clinical sample includes tissue biopsy of lymph nodes, liver, bronchial secretions, bone-marrow aspirates, and pus. The sample is collected in a sterile vial for laboratory diagnosis.

1.18 Drug Susceptibility Testing (DST)

The national health laboratory service provides the facility for drug susceptibility testing (DST). The protocol involves the indirect proportion method using Middlebrook medium. This medium is supplemented with the critical concentrations of 0.2g/ml isoniazid and 30g/ml rifampin (Dalovisio et al. 1996). This is followed by DNA sequencing and high resolution thermal melt analysis (HRTM). Discrepant phenotypes are further subjected to rifampin drug susceptibility testing in mycobacterial growth indicator tubes (Becton Dickinson) (Lipsky et al. 1984).

1.19 Preparation of Crude DNA Templates

Decontaminated sputum specimens are cultured at 37°C in Bactec 12B medium (Becton Dickinson) for 7 days in the Bactec 460 system, followed by pelleting the bacteria by centrifugation, suspended in 100 Bactec 12B medium, and boiled to generate a crude-DNA template (Mathew et al. 1995).

1.20 Tuberculosis Treatment Protocol

The chief criteria related to the successful management of DR-TB are the judicious use of first and second line anti-tubercular drugs, personalized treatment based on the drug-susceptibility test (DST), which includes first and second-line anti-TB drugs, and also the treatment history of the patient (Saket et al. 2017).

1.21 Allopathic Treatment: History of Tuberculosis Treatment and Control in India

The control measures adopted by stakeholders in India are as old as the disease itself. Tilaunia, in Rajasthan, pioneered the concept of the sanatorium as early as 1906, followed by other sanatoria in Almora and Pendra Road in Central Provinces, Madhya Pradesh, in 1908. In 1909, the Harding Sanatorium at Dharampur, Shimla was the first sanatorium opened without the support of Christian missionary organizations. The Lady Linlithgow Sanatorium opened in Kasauli in 1941. In 1915, the Union Mission Tuberculosis Sanatorium (UMTS) was created in Arogyavaram, Madanapalle, in the Chittoor district of Andhra Pradesh. This was subsequently converted to the Aroyavaram medical center, and is actively providing tuberculosis medicare even now. Among global organizations, India joined the International Union Against Tuberculosis in 1929. The Tuberculosis Association of India came into existence in 1939, with financial support from Vicereine Lady Linlithgow and King George V. By 1940, the government of India had teamed up with the Tuberculosis Association of India to establish the New Delhi tuberculosis center as a model clinic, which was subsequently upgraded to a TB training and demonstration center. Later, the tuberculosis chemotherapy center in Chennai, and the National Tuberculosis Institute (NTI), in Bengaluru came into existence, to upgrade and modernize tuberculosis treatment in India (Sikand et al. 1956, Agrawal et al. 2005, Shrivastava et al. 2008).

The National Tuberculosis Institute: The government of India, under the aegis of the directorate general of health services (DGHS), and the ministry of health and family welfare, established the National Tuberculosis Institute (NTI) in 1959, in Bangalore. Pandit Jawaharlal Nehru, the first Prime Minister of India, dedicated it to the nation by formally inaugurating it, in 1960. The NTI later evolved into a premier WHO collaboration in June 1985 (Mohan and Sharma 2009). Since then, the NTI has played a key role in organizing and imparting training and tuberculosis management-related activities for medical and paramedical staff, in line with WHO guidelines related to DOTS. The NTI also disburses funds related to tuberculosis epidemiology research, and monitors tuberculosis management programs in India (Mohan and Sharma 2009).

1.22 Allopathic Treatment of TB and RNTCP

The RNTCP has mandated three-tier laboratory facilities for sputum smear microscopy, including national reference laboratories (NRL), intermediate reference laboratories (IRL), and designated microscopy centers (DMC) for providing economical and cost-effective diagnostic services. To ensure international quality and standards, the program provides world-class equipment and reagents to the nationwide network of laboratories. The RNTCP has devised a consistent internal review system to monitor the efficiency of sputum microscopy. This includes the mechanism for external quality assessment (EQA), and senior tuberculosis (TB) laboratory supervisors (STLSs), for evaluating tuberculosis diagnostics at intermediate and national reference laboratories at state and national levels. The RNTCP also offers a certification procedure for culture and drug susceptibility testing (C&DST), and line probe assay (LPA), ensuring quality as per WHO and global laboratory initiative recommendations (Jain et al. 2010).

Isoniazid (H), Rifampicin (R), Pyrazinamide (Z), Ethambutol, (E) and Streptomycin (S) are used for tuberculosis treatment. Usually, these drugs are given three times per week. New tuberculosis patients are subjected to six months of treatment, including two months of intensive phase (IP) and four months of continuation phase (CP). Retreatment or relapse cases receive eight months of treatment, with three months of IP and five months of CP. Re-treatment of TB cases involves eight months of treatment with three months of IP, two-month HRZES, one month (HRZE), and five months of CP (HRE). Drugs are supplied in an individual patient-friendly box (PWB), which contains the entire course of treatment for each patient (Chavez et al. 2004).

The PWB comes with a color code, indicating red for category I and blue for category II. In each PWB, there are two pouches; one for the intensive phase (A), and one for the continuation phase (B). All doses of the intensive phase (IP), and at least the first dose of each week of the continuation phase (CP), are given under direct observation by a DOT provider. Follow up sputum smear examinations are done at the end of the intensive phase (IP), two months into the continuation phase (CP), and at the end of the treatment. If the smear is positive at the end of the intensive phase (IP), the same drugs are given for one more month, and then the CP is started. The treatment outcome is determined according to the results of the follow-up smear examinations done during the treatment.

For pediatric tuberculosis patients, a separate PWB has been developed under the RNTCP programme. Asymptomatic, but smear-positive, children under six years are given chemoprophylaxis with isoniazid (10 mg/kg body weight), administered daily for a period of six months. In the year 2013, the RNTCP put 14,16,014 patients on treatment via quality-assured laboratory services. The RNTCP has established a nationwide laboratory network of over 13,000 DMCs, which are supervised by the IRLs at the state level and the NRLs, and a central TB division at national level. The RNTCP aims to consolidate its laboratory network and organize a defined hierarchy for conducting sputum microscopy tests with external quality assessment (EQA).

National Reference Laboratories: Under the programme, the six NRLs include: the National Institute for Research of Tuberculosis (NIRT) in Chennai; the National Tuberculosis Institute (NTI) in Bangalore; the National Institute of Tuberculosis & Respiratory Diseases (NITRD) in Delhi; the National Japanese Leprosy Mission for Asia (JALMA); the Institute of Leprosy and other Mycobacterial Diseases in Agra; The Regional Medical Research Centre (RMRC) in Bhubaneswar; and the Bhopal Memorial Hospital and Research Centre (BMHRC) in Bhopal. The NRLs work closely with the IRLs, supervise their activities, and also undertake periodic training of the staff, with respect to EQA and C&DST. Three microbiologists and four laboratory technicians are provided by the RNTCP on a contractual basis to each NRL for the supervision and monitoring of the laboratory activities. The NRL microbiologists and laboratory supervisor/technicians visit each assigned state at least once a year for two to three days, as a part of onsite evaluation under the RNTCP-EQA protocol (Shafiq et al. 2006, ISTC 2009, John et al. 2010).

1.23 Herbal Treatment

India has rich floral biodiversity of herbal/medicinal plant wealth, constituting over 1,500 species distributed throughout India. Some of them have restricted distribution due to specific habitat requirements of species. Rapid depletion of forest, specific habitat needs, overexploitation, ever-increasing demand from the local pharmaceutical industry, and exports have brought medicinal plants to a very critical phase (Brijlal and Dubey 1992).

About 50 percent of tropical forest, the treasure house of plants and animal diversity, has already been destroyed, and the remaining half may not withstand the anthropogenic onslaught for another decade. This wanton

destruction has rendered almost 3,000-4,000 species of herbal/medicinal plants on the verge of extinction (Saxena 1986, 2012). Despite public awareness, this is likely to continue in the coming years, because the forest sector is the only source of supply of medicinal plants. Since the main source of the supply, i.e. forest, is unable to sustain the demand of medicinal/herbal plants alone, cultivation of these species in the farm sector remains the only answer to this catastrophic situation (Katiyar et al. 1997).

1.24 The Revised National Tuberculosis Control Programme (RNTCP): Response to the Challenge of Drug Resistant Tuberculosis

The main challenge before the revised national tuberculosis control programme (RNTCP) in making India tuberculosis-free is to halt the progress of ever-emerging drug-resistant strains by providing efficient diagnostics and therapy, through directly observed treatment short courses (DOTS). This will ensure the establishment of health services in line with international standards (Frieden and Munsiff 2005, Chandrashekharan et al. 2007, Frieden and Sbarbaro 2007, Jain et al. 2010). Drug abuse is one of the most serious threats, and a major contributor to the emergence of drug-resistant strains. Among other health needs, the RNTCP has also stressed the need for rational drug use under the supervision of qualified medical practitioners, to avoid the indiscriminate and injudicious use of anti-tuberculosis drugs. This program has also innovated the concept of universal and free access to anti-tuberculosis drugs under the umbrella of The Chennai Consensus Statement, to guide health service providers towards the effective management of drug resistance, outside the program's set-up. This program, in cooperation with the Medical Council of India (MCI) is educating, spreading awareness of, and sensitizing people and health workers at ground level to, the rational and judicious use of anti-tuberculosis drugs (Mehra et al. 2008). This program is also underwritten by a directive/legislation which ensures the selling of anti-tuberculosis drugs only against valid prescription. For strengthening diagnostics, which is crucial for conclusive therapy, a program has initiated a 'standard of care', with the management (diagnostics and treatment) of DR-TB as its integral component. The programmatic management of drug-resistant tuberculosis (PMDRT) for the effective and efficient management of DR-TB was launched in the states of Gujarat and Maharashtra in 2007 (Cox et al. 2008). PDMRT made satisfactory progress with respect to diagnosis and treatment in the initial year of its

launch. It was also decided to extend the services in states in a phased manner in the future, with the resolution to attain and achieve the objectives in time-bound manner. Further, drug susceptibility testing (DST) was extended to all smear-positive cases undergoing re-treatment, relapse cases, and to the new cases that were smear positive, early during the treatment regimen with first line anti-tuberculosis drugs. Laboratory set ups, with facilities for DST (culture based, solid and line probe assay, (LPA)) were also scaled up, in a phased manner, in the government-run public health sectors, (Sharma et al. 2009).

1.25 Achievements

Although tuberculosis is a curable infection, the continuous emergence of new drug-resistant strains, and co-infection with HIV/AIDS, have continued to baffle health service providers and rattle the human population, due to the lethal effect of the infection. The revised national tuberculosis control programme (RNTCP) was one major government initiative designed to put a brake on tuberculosis outbreak. The foremost activity enlisted under the RNTCP for control measures adopted and implemented through NGOs and governmental organizations, was the launch of programmatic management of drug-resistant tuberculosis (PMDRT) services in all the states (35 States and 704 districts), thus covering the entire population, along with 110 DR-TB wards which had airborne infection control measures. Additionally, 51 culture and drug susceptibility testing (C &DST) labs, utilizing 37 solid culture labs, 12 liquid culture labs, and 41 line probe assay (LPA) labs were also provisioned. About 181,021 suspected TB patients were tested for MDR-TB, and 20,763 patients were initiated into MDR-TB therapy. In addition to the above, each DR-TB center was provided with a counselor for patients and family, to ensure maximum compliance with the treatment, the identification of adverse drug reaction, drug and disease management, and overall social security.

With all these measures adopted and implemented, the country has witnessed a huge spurt in PDMT-based diagnostic services. Highly-focused PDMT review meetings were organized periodically with stakeholders, and progress was closely monitored. These initiatives have created a baseline for the spike in PDMT-driven approaches. In future, guidelines will be prepared for the use and regulation of new anti-TB drugs in India. As an initiative within this, a multicentric study was

finalized for selected cities in the country, to explore the possibility of incorporating Bedaquiline for TB treatment in India.

1.26 Health Management

1.26.1 Role of NGOs and Private Players

As a part of the health control measures, many non-governmental organizations (NGOs), public and private sectors, are complementing governmental organizations with a focused approach to controlling and eradicating tuberculosis. Tuberculosis is a great leveler, and the kind of effort that is being put into the control of it defines the value of these partnerships.

Various NGOs working at 'ground zero' have devised innovative strategies to reach affected people, for effective cure and management of the disease. In fact, they are the flag bearers and the real faces of government organizations for the implementation of government schemes. Various schemes that have been implemented successfully with the help of NGOs include directly observed short treatment courses (DOTS), and the revised national tuberculosis control programme (RNTCP). For the RNTCP, around 2,946 NGOs worked to make it a success in 2008. The National Anti-Tuberculosis Association, one of the oldest in the Indian sub-continent, has played an active role in tuberculosis control measures and continues to provide active services in India, Bangladesh, Nepal, and Sri Lanka. NGOs are playing a crucial role in spreading awareness of the pathophysiology, control, spread, and preventive strategies, related to tuberculosis, with active financial support from the national and international organizations, and also support at community level. Meaningful co-ordination between NGOs, private health service providers, and government organizations, is crucial for providing improvised services to patients, reduced incidence, and enhanced cure rates for tuberculosis (Sharma and Liu 2006, Sharma et al. 2009).

1.26.2 Implementation Arrangements (Linkages and Management Structure)

An inventory which will have various parameters of the patient, viz. clinical presentation, radiology, previous treatment history, demographic and socioeconomic data, and microbiology results, for the record, is being

made. This information will help in treating recurring infection or similar infection in others.

1.26.3 Management of Tuberculosis and Associated Co-infection: Special Issues Related to Tribal Populations

Madhya Pradesh has a sizeable population of tribal people, who, because of a lack of awareness, education, and their socio-economic conditions, fall prey to diseases like tuberculosis, a poor man's disease. At the outset, they avoid medical treatment, and resort to 'black magic/witchcraft, or sometimes, traditional medicine under the supervision of traditional healers, which is not enough to treat diseases like tuberculosis or its co-associated morbidities such as MDR/XDR-TB and AIDS (Maheswari 1964, Pama et al. 2014). Even if patients take treatment, they leave it midway, thus becoming reservoirs of infection and developing new resistant strains in the process. Further, tribes, because of their highly conserved gene pool and insular lifestyle, are not amenable to many treatments or clinical practices which a normal population would be able to accept. For instance, some tribes in Andaman and the Nicobar Islands cannot be vaccinated, as it impairs their overall physiology, leading to various morbidities. Against the backdrop of all this, it is very important to carry out an epidemiological survey to address the issues involved with respect to tuberculosis in particular, which has now switched to multidrug-resistant tuberculosis (MDR/XDR-TB) and HIV-AIDS co-associated with tuberculosis.

The proposed epidemiological study focuses on the selected regions of Madhya Pradesh, India, based on the previous records of tuberculosis prevalence, and will look into socio-economic and historical factors, as well as the clinical factors responsible for the emergence, prevalence, and its control. The strategy involves meta-analysis and statistical analysis of previously available data, as well as current data, laboratory analysis, and mathematical modeling, to study its projection and control.

1.27 Objectives of Proposed Study

The proposed study addresses the following objectives:

- Historical background and overview of tuberculosis in the identified regions of Madhya Pradesh, India.

- Efficacy of modem tuberculosis diagnostics, coupled with the herbal treatment protocol under traditional healers by the tribes of the study area.
- Epidemiology of tuberculosis including the rate of incidence, prevalence, and spread.
- Epidemiology of HIV and associated co-infection, particularly tuberculosis, including distribution and gender-based relationships.

1.27.1 Epidemiological Part

- Patterns, causes, and the effects of health and disease conditions in defined populations and informing policy decisions.
- Evidence-based practice by identifying risk factors for disease and targets for preventive healthcare.
- Identifying the risk factors for the disease and target for preventive healthcare.
- Study design, collection, and statistical analysis of the data, to draw appropriate conclusions.
- Meta-analysis of previous data to understand the pattern and demographic history of the disease to frame counter strategy for the future.

1.27.1.1 Detail Epidemiological Study of Tuberculosis

Data collection from hospitals, community health centers (CHC's), and the primary health centers (PHC's):

- Overall pattern of tuberculosis in identified regions of Madhya Pradesh.
- Socio-economic factors and demographic patterns.
- Prevalence among different working groups, viz. housewives, truckers, laborers, and sex workers.
- Modes of transmission.

1.27.1.2 Meta-Analysis and Statistical Analysis of Data

- Case-control, case series', and cohort study design to study the overall pattern of tuberculosis and its associated morbidities, i.e. HIV-AIDS.
- Analysis of temporal data related to tuberculosis, spanning over several decades, for meaningful interpretation.

- Statistical analysis of the data to draw conclusions about the current status.

1.27.2 Clinical/Laboratory Part

- Data collection on Recurrent tuberculosis, Re-infection, and Relapse.
- Data collection on the resistance patterns among tuberculosis patients over time.
- Assessment of co-associated morbidities of MDR/XDR-TB and HIV.

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CHAPTER II

TUBERCULOSIS UNDER HIV-AIDS CO-INFECTION

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Abstract

India, having less than one percent of the global land mass, houses more than 16 percent of the world's population, which is more than that of South America, Africa, and Australia, combined. The exceedingly large population, coupled with lack of education, poverty, and sub-human living conditions make India vulnerable to epidemics of HIV and its associated co-morbidity, especially tuberculosis. This threat is looming large in one of the states of India, i.e. Madhya Pradesh, which houses one of the largest tribal populations. This chapter investigates the HIV-TB epidemics in the four sub-divisions/blocks of the Anuppur district of Madhya Pradesh. The epidemiological investigation includes trend, prevalence, distribution, and management-related issues. Secondary data were collected from all the four subdivisions/blocks of the Anuppur district, through inventories and records in a district hospital. The collected data was analyzed through Chi-square summary statistics, such as Pearson Chi-square, likelihood ratio, and linear-by-linear association. Analysis investigates the relationship between the distribution of HIV and HIV+TB in different blocks and sub-divisions of the Anuppur district, and gender-based (male, female ANC, and female non-ANC) distribution of HIV and HIV+TB. The result was discussed in terms of viral spread through the socio-economic dynamics of hetero- and homosexual relationships, pregnant females, migrant workers, injectable drug users, and labourers. De-escalation of HIV-TB epidemics requires dispelling myths and the stigma associated with the disease, so that people with infection can come out into the open and seek palliative therapy.

"HIV is not something that 'guilty' people get. It is not a punishment for cheating, lying, using drugs or alcohol, having more than one partner, or not asking the right questions. It is a virus whose transmission is fuelled by poverty, ignorance, racism, sexism, homophobia, fear, violence, and many other factors – not by people with HIV."

Positive Women's Network of the United States of America

2.1 Background

Tuberculosis manifests itself in the resource-limited set-up of under-developed and developing nations. These regions are plagued by poor nutrition, or malnutrition, disease, and infections. Weakened immunity due to tuberculosis might lead to Acquired Immunodeficiency Syndrome (AIDS) infection, via Human Immunodeficiency Virus (HIV). Tuberculosis and HIV-AIDS happen to be cause and effect of each other, acting as a major co-infection. HIV associated with TB is more frequent in Pattern II and Pattern III countries, which include India (Khursheed 1992, Vasudeviah 2007) (Table 1). Recently, Kala Azar infection has also emerged as a major co-infection of HIV, making the treatment extremely complicated. Among the organs, the lungs are one of the organs most affected by tuberculosis, as well as HIV-AIDS. Breathing difficulties due to tuberculosis, pneumonia, phlegm, blood in sputum, and chest pains, are common features. Infection of the gut leading to gastrointestinal symptoms is common in particularly poor environmental conditions.

2.2 Mycobacterium: Geographical variation

Geographically, differences have been noted for several AIDS-associated illnesses. For instance, *Mycobacterium tuberculosis* is most frequently seen in patients in the industrialized western world, while in Africa and other countries of patterns II and III, bacterial infection is more common (Table 1). Similarly, tuberculosis associated with HIV is much more common in the countries of patterns II and III, including India, whereas among other members of the same group of bacteria, mycobacteria is a typical variety which is more frequent in the countries of pattern I (Khurshed 1992).

Table 2-1 Global Patterns of HIV infection

Pattern I: Expansion of HIV began in the late 1970s/ early 1980s. Homosexual males or Gays and Intravenous drug users (IVUD) were predominantly affected populations. Heterosexuals transmission is also increasing.

Western Europe, North America, some area in South America, Australia and New Zealand

Pattern II: Epidemics of HIV began in the mid-to-late 1970s/early 1980s. Heterosexual transmission continues to increase.

Africa, Caribbean, some area in South America

Pattern III: Epidemics of HIV not reported until mid-to-late 1980s. Extensive spread of HIV is now being reported in several countries in South East Asia but the prevalence of HIV in most countries classified within this pattern, remains relatively low. Countries in Asia are included in pattern III, where HIV was introduced later. Although the number of cases of AIDS in India is still low, it is likely to scale-up in the near future.

Asia, the Pacific region (minus Australia and New Zealand), the Middle East, eastern Europe, some rural areas of South America

2.3 History of HIV-AIDS as Co-infection of Tuberculosis

AIDS is a set of symptoms and opportunistic infections. This includes tumors, and other infections which manifest themselves on account of the damage to the human immune system caused by HIV. The process is gradual, ultimately leading to the absolute depletion and collapse of the body's defense mechanism. The weakened immune system is not competent enough to offer resistance to even milder infection, and the patient may die of an infection that is otherwise absolutely curable and controllable in normal conditions. These infections, which set in due to the weakened immune system, are called 'opportunistic infections'. The diversity of opportunistic infections leading to mortality for varied reasons make AIDS a syndrome, rather than a disease of unitary clinical entity (Khurshed 1992).

The emergence of AIDS can be traced back to the 1980s, in the USA, Europe, and Australia, when patients were reported showing two unusual symptoms, namely pneumonia caused by a protozoan parasite *Pneumocystis carini*, and an unusual skin cancer, called Kaposi's sarcoma. These symptoms further found correlation with aberrant sexual behavior of individuals, usually gay people. The association of infection with gay people has given it the name 'gay related infectious disease' (GRID). A little later, AIDS was also reported in intravenous drug users (IVDUs). Over time, another group of patients emerged who had received the infection due to the transfusion of blood or related products, or children born to infected mothers. Its varied association with the diversity of people of different origin, race, caste, and creed, has given it the name of '4H disease' (Haitians, Homosexuals, Hemophiliacs and Heroin users).

2.4 Epidemiology of HIV Transmission

AIDS is the result of a deficient immune system. Immunodeficiency has been known in humans since time immemorial. Before the emergence of AIDS as an immunodeficiency syndrome, three different types of immunodeficiencies were recognized in humans. These were: genetic or hereditary immunodeficiency, which transmits from the parent to the progeny; medically-induced immunodeficiency, as in the case of kidney transplants to avoid rejection; and finally, genetically-acquired immunodeficiency, prevalent in the African and Asian continents. Additionally, immunodeficiency could result from nutritional factors. However, retrovirus-induced immunodeficiency was entirely new, and no reported or documented case was known until the 1980s.

2.5 HIV as Causative Agent for AIDS

The causative agent for AIDS remained undiscovered till 1983, when Dr. Luc Montagnier's group at the Pasteur Institute in Paris first isolated a retrovirus from the lymph nodes of a lymphadenopathy patient (Barre-Sinoussi et al. 1983). This virus was subsequently named 'lymphadenopathy-associated virus (LAV)'. Montagnier and Françoise Barré-Sinoussi were awarded the Nobel Prize in Physiology and Medicine for their discovery. Another human retrovirus named HTLV-III (Human T-cell leukemia virus) was reported by Dr. Robert Gallo's group at the University of Maryland. This virus was isolated from peripheral blood mononuclear cells (PBMC) of a patient suffering from AIDS (Gallo et al. 1983). Later, both LAV and HTLV were found to be the same, which led the

International Committee on Taxonomy of Viruses to discard the previous names and come out with the new nomenclature of 'human immunodeficiency virus (HIV)' in 1986 (Coffin et al. 1986). Subsequently, two new subtypes of the virus, HIV-I and HIV-II were reported (Clavel et al. 1986, 1987).

There are a number of theories regarding the origin of HIV in primates, and its zoonotic transmission to humans around the late 19th or early 20th century. The first documented case of HIV dates back to 1959 in the Democratic Republic of Congo, from where it spread to the USA in 1966, and to India in 1986 (Zhu et al. 1998). Since then, it has expanded its area of influence, encompassing almost the whole globe, and assuming pandemic proportions. According to the 2011 UNAIDS report, 34 million people are currently living with HIV, with approximately two million new infections happening every year. The situation is alarming for under-developed and developing nations with resource-limited setup, which bear the maximum global burden of the infection (Raviglione et al. 1995, UNAIDS 2014).

2.6 HIV/AIDS Epidemic in India: Evolutionary Perspective

The first HIV case was reported in female sex workers, in Chennai, in 1986. This was followed by a rapid increase in other states (Table 2). Currently, the national prevalence is 0.26 percent, as compared to the global 0.2 percent. The prevalence rate in high risk groups such as female sex workers is 7 percent. HIV epidemics in India are characterized by low prevalence in the general population and high prevalence in high risk groups. HIV in India spreads through four main routes, i.e. through commercial sex workers, promiscuous heterosexual transmission, intravenous drug users, and finally among homosexuals or gay men (Paranjape and Challacombe 2016).

The beginnings of HIV-AIDS epidemics have seen high prevalence in the south Indian states like Tamil Nadu, Andhra Pradesh, Karnataka, Mumbai (Maharashtra) and the northeastern states, such as Manipur and Nagaland. The populations in the age group 15-49 years are among the worst affected. This includes 39 percent of the women, including both sex workers and housewives (Sharma et al. 2000, 2004). However, currently, the infection is most prevalent in injectable drug users (7.14 percent), followed by men having sex with men (MSM) (4.4 percent), and migrant workers and their wives (2.7 percent) (Paranjape and Challacombe 2016).

Table 2-2 Chronology of HIV-AIDS in India

Period	Event
April 1986:	First cluster (ten prostitutes) of HIV seropositive reported in Madras, Tamil Nadu.
May 1986:	First patient with terminal illness detected in Bombay (Mumbai), Maharashtra. Suspected to be the recipient of infected/unscreened blood transfusion during cardiac surgery in USA.
December 1986:	First seropositive male detected from STD clinic in Tamil Nadu.
July 1987:	First seropositive blood donor in Vellore, Tamil Nadu.
July 1987:	Spouse to spouse transmission (the same donor's wife).
October 1987:	Detection of seropositive infant (born to the above parents).
April 1988:	First indigenous case of full- blown HIV-AIDS.
January 1989:	Evidence of HIV antibodies in indigenously produced blood products.
Onward:	Evidence of exposure to HIV-AIDS among a high proportion of donors engaged by commercial manufacturers. Government ban on production.
July 1989:	Government gazette notification for mandatory screening of blood donors for HIV antibodies.
January-February 1990:	Recognition of a cluster of seropositive in IVUDs in North- East India; Incident of embalming a body of a patient of HIV disease.
July 1992:	Constitution of the National AIDS Control Organization (NACO); at state levels.
October 1992:	Establishment of the National AIDS Research Institute (NARI), Pune by Indian Council of Medical Research (ICMR).

Management and control strategies focus on education, information, and awareness, to prevent transmission. It has not yet included pre-exposure prophylaxis (PrEP) (Ramakrishnan et al. 2015). Fortunately, HIV-AIDS is now on a downward spiral, thanks to the availability of economical, generic, antiretroviral therapy (ART) drugs, manufactured by Indian pharmaceutical companies, and the free distribution of these generic drugs to patients from the government of India. This has led to a sharp decline in AIDS-related deaths in India. Currently, all known modes of the viral spread have been identified. In the early 1990s, an epidemic of HIV-AIDS in the north-eastern state of India was recognized among thousands of

intravenous drug users (Table 2). The virus might have started late, but it is racing rapidly to create large scale epidemics, especially in some metropolitan cities of India. Only a few countries started surveillance to detect HIV infection when the number of AIDS cases was very low. In other countries, because of complacency and lack of implementation, cost-effective strategies for sero-surveillance, followed by education and intervention programmes, are directed only at those in high risk situations.

2.7 Issues of HIV-AIDS in India

According to the national AIDS control organization (NACO), Mumbai (erstwhile Bombay), Chennai (erstwhile Madras) and Imphal are the three major epicenters of HIV infection in India. AIDS is spreading from these regions to other parts of the country, chiefly via transporters, migrant workers, and paid blood donors. Promiscuous multi-partner hetero- and homosexual lifestyles are major contributors to the infection. The majority of HIV-AIDS cases from Imphal in Manipur is due to IVUDs, and, to a lesser extent, by hetero- and homosexual relationships. This is because of easily available drugs (heroin) from the 'Golden Triangle', through the Myanmar (Burma) border. Mumbai and Chennai, being the major metropolitan cities of India, attract a large number of migrants. This leads to a higher probability of promiscuous sexual relationships (Khurshed 1992).

As shown in Table 2, the first case of HIV-AIDS was reported in 1986, which was due to the transfusion of infected blood to the patient during cardiac surgery in the USA. Another case also traced its origin to the USA, when a hemophiliac was given an infected blood product. These two cases, along with many others, have generated myths and irrational misconceptions among Indians that HIV-AIDS is a 'foreign disease', that it has mostly foreign origin, and that Indians, by and large, are immune to it. Subsequently, cases were reported from India as well (Table 2). The number of HIV-AIDS cases reported among Indians has outnumbered the cases reported in foreigners, thus dispelling the notion of HIV-AIDS being a foreigners' disease. India, with less than one percent of the global land mass, and supporting more than 16 percent of the world's population, is vulnerable to the threat of HIV-AIDS, due to a multitude of socio-economic factors (Jain 1994).

2.8 Tuberculosis and HIV-AIDS: Global Scenario

About one-third of the world's population is infected by the *Mycobacterium tuberculosis*. In 2006, approximately 9.2 million new cases of tuberculosis were identified, of which nearly 70,000 were infected with HIV (WHO Report 2005). In 2006, there were 1.7 million TB deaths, including 23,000 cases infected with HIV (WHO Report 2008, Tripathi et al. 2009). These deaths comprise 25 percent of all the avoidable deaths in developing countries. Ninety-five percent of TB cases, and 98 percent of TB deaths, occur in developing countries. Three-quarters of TB cases in developing countries occur among the economically productive age group, i.e. 15 to 40 years.

This situation is further complicated by the rapidly spreading HIV pandemic (Narain 1997). According to the recent joint update by the World Health Organization (WHO), and the United Nations programme on HIV-AIDS (UNAIDS), the revised global estimate of the people living with HIV-AIDS (PLHIV) has been calculated as 33.2 million (range 30.6 to 36.1 million). This was a reduction of 16 percent, compared with the estimate of 39.5 million in 2006 (Dandona et al. 2007, UNAIDS 2008). The major contribution to this new estimate has been the revised figure from India. According to the new estimate, 2.5 million people (range 2 to 3.1 million), or about 0.4 percent of the adult population in India are HIV-seropositive, and these estimates are less than half the earlier reported figure of 5.7 million (range 3.4 to 9.4 million) people (Joint United Nations Programme 2008). In light of these data, it is likely that the estimates related to HIV-TB co-infection will be revised in the near future.

The HIV epidemic has reached a generalized stage at national level in three countries of Asia, i.e. Cambodia, Myanmar, and Thailand (Narain et al. 2004, WHO 2005). In 2003, in Cambodia, the prevalence of HIV infection among those aged between 15 and 49 years was estimated to be 2.6 percent. In Myanmar, the prevalence was 0.7 percent, and in Thailand, the prevalence was 1.4 percent. The HIV epidemic is generalized in five states of India, namely Andhra Pradesh, Karnataka, Maharashtra, Manipur, and Nagaland (Narain 2004, Sharma et al. 2005).

In many parts of the world, TB is the most common opportunistic infection among HIV infected people (Sharma et al. 2004, WHO 2005). As per WHO/UNAIDS estimates (World Health Organization 2005), up to 50 percent of people with HIV or AIDS had developed TB. Of the 3.6 million adults with HIV infection in the south-east Asian region, nearly

half are likely to be infected with TB. In Myanmar, 80 percent of AIDS patients have pulmonary TB, while this estimate is 75 percent in Nepal, 60 percent in Thailand, and 56 percent in India (WHO 2005). In the Chiang Rai province in northern Thailand, a case-control study between 1990 and 1998 has shown that the proportion of TB cases attributable to HIV rose to 72 percent in male patients, and 66 percent in female patients (Ministry of Public Health Thailand 2003). This continuing increase in TB cases attributable to HIV has occurred even when there is a marked reduction in HIV prevalence in the region (Narain et al. 2004).

The HIV pandemic has entered both the epidemiology of TB, and the measuring of approaches to its control, in populations at high risk of TB and HIV infection, particularly in developing African and Asian nations. HIV-related TB continues to increase, even in the countries with well-organized national TB control programmes that are successfully implementing DOTS, the internationally recommended strategy for TB control (WHO 2005).

2.9 Diagnosis and Pathology

The clinical presentation of TB in HIV-infected patients varies, depending on the severity of immunosuppression. In patients with earlier stages of HIV infection, the clinical presentation of TB tends to be similar to that observed in persons without immunodeficiency. Pulmonary disease is most common, often with focal infiltrates and cavities. Diffuse pulmonary disease without cavitation, often involving the lover lobes and prominent meditational or paratracheal adenopathy, is seen in patients with advanced HIV infection (Pitchenik et al. 1988, Parriens et al. 1995, WHO 2006, Shah et al. 2007).

2.9.1 TB against HIV-AIDS Background

TB in patients with an HIV-AIDS background is difficult to diagnose, as routine TB diagnostics may not detect the TB bacterium. Sputum smear rarely reveals acid-fast bacilli (AFB) in HIV-seropositive patients with pulmonary TB (Pitchenik, et al.1984, Sunderam et al. 1986, Theuer et al.1990, Assoc et al. 1996, Havlir et al. 1999). In HIV-seropositive patients, the histopathological appearance of lymph nodes shows cessation lesions with few or no AFB. In such a situation, other clinical specimens like bronchoscopy and biopsy specimens from HIV-seropositive patients should be subjected to the mycobacterial smear and culture examinations

(Murray et al. 1984, CDC 1992, Spellman et al. 1998, Gandhi et al. 2006). Therefore, TB in HIV-AIDS background requires modern state-of-the-art DNA-based molecular diagnostics, such as nucleic acid amplification test (NAAT). However, this becomes a big limitation for the resource-limited setups of under-developed and developing countries (CDC 1992, Narain et al. 1998, 2007, Saket et al 2017).

2.9.2 Tuberculosis as HIV Co-infection in Children

As in the case of adults, clinical presentation of TB in HIV-infected children with early HIV disease is similar to that observed in immune-competent children without HIV infection. However, TB bacilli are more likely to disseminate to the other parts of the body in a child who has HIV infection. Tuberculosis meningitis, miliary TB, and generalized enlargement of lymph nodes are more likely to occur. HIV-seropositive children may have other opportunistic infections, apart from TB. In India, provisions are available under the revised national tuberculosis control programme (RNTCP) and the national AIDS control programme, to evaluate patients with TB for HIV infection, and vice versa, to maximize the case detection rates (National Framework for joint TB-HIV collaborative activities 2007).

2.9.3 Immune Reconstitution Inflammatory Syndrome (IRIS) in HIV-Tuberculosis co-infection

Anti-retroviral therapy (ART) has dramatically reduced the morbidity and mortality associated with HIV-AIDS. However, ART initiation is plagued with irresolvable complications, like HIV-associated immune reconstitution inflammatory syndrome (IRIS) particularly in the first six months of initiation of ART. This usually manifests in the form of considerable mortality and morbidity in patients who start ART with advanced immunosuppression. In such a situation, immune recovery following ART initiation is associated with pathological inflammatory response directed towards microbial antigens (Meintjes et al. 2008, Walker et al. 2015). The timing of ART initiation is crucial to reduce IRIS-associated morbidity. Improved understanding of the overall pathophysiology of IRIS may lead to the evolution of diagnostic protocols that will equip us with better and improvised therapeutic strategies (Sharma et al. 2005, McIlleron. et al. 2007, Walker et al. 2015).

2.10 Tuberculosis in HIV-AIDS Background: Management Issues

Effective diagnosis and targeted therapeutics are the first essential pre-requisite for the effective management of diseases like TB and HIV-AIDS. HIV-seropositive patients may develop TB while on antiretroviral therapy (ART) due to the weakened immune system. This may aggravate either of the infections, thus complicating its treatment. Untreated, or partially-treated cases may act as a reservoir of infection that can spread it to others. In India, RNTCP employs thrice-weekly directly observed treatment (DOTS) in the initial intensive phase, and in the continuation phase of chemotherapy. New cases are treated with category I treatment, while relapse cases are treated in category II (Narain et al. 2002, WHO 2003, 2006, 2008).

Different HIV-related infections, like the *Pneumocystis jiroveci* pneumonia and other bacterial infections, lead to considerable morbidity during the treatment of HIV-TB co-infection (Hira et al. 1998). Effective and targeted therapy against these inter-current infections might pave the way reducing morbidity and mortality in HIV-TB patients (Sharma et al. 2005).

2.10.1 Highly Active Antiretroviral Therapy (HAART)

HAART has revolutionized the treatment of HIV-AIDS since its introduction in 1996. HAART includes six classes of drugs that are used in combination to treat HIV infection. These drugs depend on the life cycle of a retrovirus, patient's viral load, CD4+ cell count, and the strain of the virus (Pallela et al. 1998). ART does not eliminate the virus, but it leads to near, or complete, suppression of HIV and reduction in transmission. This is achieved by the impairment of viral replication, with the simultaneous restoration of immune function (Pallela et al. 1998). Benefits of HAART are aplenty, but there are obvious limitations, like potential side effects of the drug, required to be taken in bulk, and also the huge cost of the drug involved, which may not be affordable in the resource-limited set-ups of developing and under-developed nations (Chaisson et al. 1996, Santora-Lopes et al. 2002, Saket et al. 2017).

2.10.2 Combinatorial Therapy

The majority of HIV-seropositive patients with pulmonary TB respond to the standard treatment regimen. However, chances of relapse or re-

infection of TB is greater in HIV-seropositive patients than in HIV-seronegative patients. In such a situation, prolonging the continuation phase of the treatment may reduce the chances of relapse. In the case of poor IVDU compliance, a fully supervised regimen is recommended. Additionally, sterilization of syringes or use of disposable syringes and their complete destruction to prevent reuse is highly recommended (Tahir et al. 2007).

2.11 Data Collection

Fig. 2-1 Different sub-divisions / blocks of district Anuppur

Secondary data were collected from all the four subdivisions/blocks of the district of Anuppur, through inventories and records from the Anuppur district hospital (Figure 1). The collected data was further organized into

categories, like HIV positive (HIV+) and HIV negative (HIV-) status, HIV with TB (HIV+TB) and HIV without TB (HIV-TB), HIV+ among male, antenatal female (ANC) and non-antenatal female (ANC) (Table 3-7).

2.11.1 Storage of Data

Collected data were initially entered into an electronic database machine using Sybase Central Software (Sybase, Inc. Dublin, CA). To ensure confidentiality, it was expunged to create a secondary data set which was used for the final analysis.

2.12 HIV-AIDS Diagnostics

Patients who experienced symptoms such as weakness, unexplained loss of body weight, loss of appetite and diarrhoea, etc., for a long time, were referred for HIV testing. HIV kits (ELISA) for testing anti-HIV antibodies or plasma HIV-RNA, and Western blot analysis, were employed as tests for HIV diagnosis. The Anuppur district hospital served as a nodal center for testing HIV from all the four blocks/sub-divisions, i.e. Anuppur, Jaitahari, Kotma, and Pushparajgarh.

2.13 Meta-Analysis of Data

2.13.1 Chi-Square (χ^2) Test

The Chi-square (χ^2) test was employed to compare the observed results with the expected results. This was done to check whether the difference between the observed and the expected data is due to some chance event, or to the relationship between the variables. The lower the asymptotic value (p-value), the less likely are the chances that the two variables are independent. Chi-square does not provide the strength of association between the variables.

2.13.2 The Likelihood Ratio

The likelihood ratio was utilized to compare the goodness of fit of the null and alternative models. This test is based on the likelihood ratio, which shows the greater likelihood of the data to fit under one model than the other. The likelihood ratio is the ratio of likelihood of an observed outcome under the null hypothesis, and maximum likelihood of an observed outcome, varying parameters over the_whole parameter space.

The likelihood ratio ranges between 0 and 1. A low value means that the observed results are less likely to occur under the null hypothesis, as compared to the alternative hypothesis, while a higher value means that the observed results are more likely to occur under the null hypothesis than the alternative hypothesis. This is used to calculate the p-value, or critical value, to accept or reject the null model.

2.13.3 Linear-by-Linear Association

This provides the Chi-square statistics with one degree of freedom (df=1). This tests the null hypothesis that there is a non-linear relationship between the variables, and provides a powerful tool against a more restricted null hypothesis. This test is applied for the ordinal (ordered) categories, and assumes equal and ordered intervals. The linear-by-linear association is usually applied for cases larger than 2x2 tables. The linear-by-linear test is best suited for ordinal (ordered) data, and assumes equal and ordered intervals.

The Chi-Square was calculated using cross-tabulation (crosstab). Crosstab forms a two-way table and provides the Chi-square test of association. This gives the frequencies of co-occurrences between the two variables. The first hypothesis tested here is whether there is a relationship between the sub-divisions/blocks of the district of Anuppur and the number of HIV+TB or HIV-TB cases. The null hypothesis is that there is no association between the two variables, the alternative is that there is an association of some sort (crosstab 1). The second hypothesis tested is, if there is a relationship between the sub-divisions/blocks of the district of Anuppur and the number of HIV+ cases within the total number of diagnosed cases. The null hypothesis is that there is no association between the two variables, the alternative is that there is an association of some sort (crosstab 2). The third hypothesis tested is whether there is a relationship between the number of HIV+TB and HIV-TB cases amongst the male, female (ANC) and female (non-ANC) in the district of Anuppur. The null hypothesis is that there is no association between the two variables; the alternative being that there is an association of some sort (crosstab 3). The hypothesis was tested using a value of alpha (α) = 0.05. The test was run in the following order: analyze – descriptive – crosstab.

2.14 The Spread of Tuberculosis in HIV-AIDS Background: Indian Scenario

The HIV data from April 2013 to March 2017 was collected from all the four blocks (sub-divisions) of the district of Anuppur, Madhya Pradesh (Figure 1) and categorized into two groups; HIV with TB (HIV+TB) and HIV without TB (HIV-TB). As given in Table 3a, a total of 280 cases (HIV without TB and HIV with TB) were reported from all the four sub-divisions of the Anuppur district. Anuppur shows the maximum number of cases in both the categories, while Jaithari shows the minimum (Figure 2). In all the sub-divisions, cases of HIV-TB (HIV not associated with TB) are more than HIV+TB (HIV associated with TB). However, Anuppur shows a maximum number of HIV+TB and HIV-TB cases, followed by Kotma and Pushprajgarh, while Jaithari shows the minimum. The same is depicted in the bar chart (Figure 3), showing HIV+TB in blue and HIV-TB in green. Table 3b provides the sum of valid cases of all the four sub-divisions (280, 100% diagnosed) and no missing cases. Sub-division-wise, HIV+TB and HIV-TB were counted separately, and their percentage calculated by dividing from the vertical sum of given data; 109 (HIV+TB), 171 (HIV-TB) and 280 (total) respectively (Table 3c). As evident from Table 3c, in all cases, the percentage abundance is similar for both the categories (HIV+TB and HIV-TB) in all the four blocks. Column 1 of Table 3d shows the summary statistics of the Chi-square which includes the Pearson Chi-square, likelihood ratio and linear-by-linear association. The likelihood ratio test can be interpreted in a similar way to the Chi-square test. Column 2 shows the value of different test statistics as mentioned above, and column 3 gives the degree of freedom (df). Column 4 gives the p value, here referred to as 'asymptotic significance'. Here, the p-value for Pearson Chi-square is 0.796 (χ^2 = 1.022; $p > 0.05$; df=3), which means the relationship between the two variables is statistically insignificant. The same is true for the likelihood ratio; the p value is 0.794 (1.028; $p > 0.05$; df=3), and the linear-by-linear association p-value is 0.680 (0.170; $p > 0.05$; df=1).

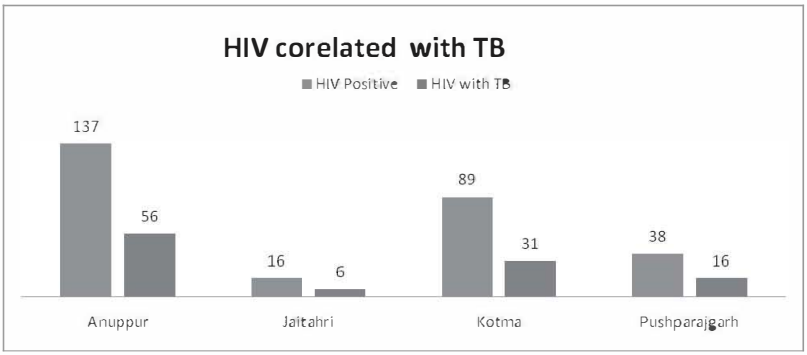

Fig. 2-2 Quantitative depiction of HIV+ and HIV+TB status in different subdivisions/blocks of district Anuppur

Table 2-3a Block wise distribution of HIV and TB in district Anuppur

S.No.	Sub-division/Block	HIV + TB	HIV - TB	Total HIV+
1.	Anuppur	56	81	137
2.	Jaitahri	6	10	16
3.	Kotma	31	58	89
4.	Pushparajgarh	16	22	38

Table 2-3b Case Processing Summary

Division * HIV	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
	280	100.0%	0	0%	280	100.0%

Table 2-3c Division * HIV Cross tabulation

			HIV		Total
			HIV +TB	HIV - TB	
Division	Annupur	Count	56	81	137
		% within HIV	51.4%	47.4%	48.9%
	Jatahari	Count	6	10	16
		% within HIV	5.5%	5.8%	5.7%
	Kotma	Count	31	58	89
		% within HIV	28.4%	33.9%	31.8%
	Pushprajgrah	Count	16	22	38
		% within HIV	14.7%	12.9%	13.6%
Total		Count	109	171	280
		% within HIV	100.0%	100.0%	100.0%

Table 2-3d Summary Statistics of Chi- Square Test

Summary Statistics	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	1.022[a]	3	0.796
Likelihood Ratio	1.028	3	0.794
Linear-by-Linear Association	0.170	1	0.680
N of Valid Cases	280		

a. 0 cells (.0%) have expected count less than 5. The minimun expected count is 6.23.

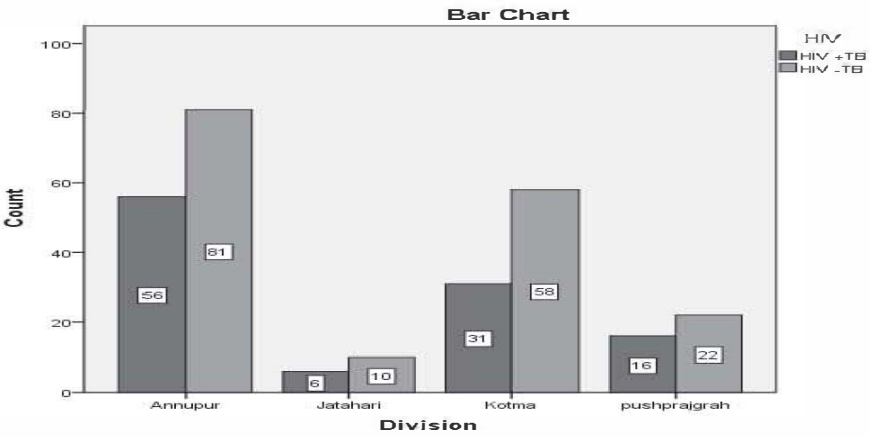

Fig. 2-3 Distribution of HIV+TB and HIV-TB status in different subdivisions/blocks of district Anuppur

2.14.1 Spread of Tuberculosis in HIV-AIDS Background in High Burden Districts of India

Table 4a gives the sub-division/block-wise number of HIV positive (HIV+) cases among the total tested, irrespective of their causal relationship with TB. Here also, the total number of suspected cases is at the maximum for Anuppur, followed by Kotma and Pushparajgarh, while Jaitabrai shows the minimum number of suspected cases (Figure 4). Table 4b provides the total number of valid cases from all the four blocks /sub-divisions and no missing cases (49,049, 100% diagnosed). Table 4c gives the HIV+ cases, along with their percentage from each of the different sub-divisions/blocks. As evident from Table 4c, Anuppur records the maximum number of HIV+ cases, while Jaitahri records the minimum. Table 4d gives the summary statistics of the Chi-square, that includes the Pearson Chi-square, likelihood ratio and linear-by-linear association. The asymptotic significance (p-value) for the Pearson Chi-square is 0.00 (χ^2 = 52.357; $p < 0.05$; df=3); for likelihood ratio p-value is 0.00 (47.391; $p < 0.05$; df=3) while the p-value for linear-by-linear association is 0.074 (3.201; $p > 0.05$; df=1) indicating a statistically significant relationship between the variables.

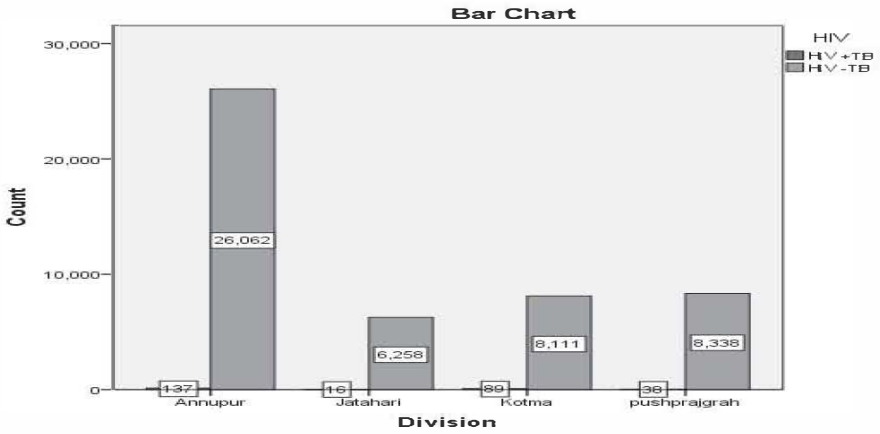

Fig. 2-4 Distribution of HIV+ and HIV- status in different subdivisions/blocks of district Anuppur

Table 2-4a Block wise distribution of HIV-seropositive and seronegative status in district Anuppur

S. No.	Sub-division/Block	HIV +ve	HIV -ve	Total Tested
1.	Anuppur	137	26062	26199
2.	Jaitahri	16	6258	6274
3.	Kotma	89	8111	8200
4.	Pushparajgarh	38	8338	8376

Table 4b. Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Division * HIV	49049	100.0%	0	.0%	49049	100.0%

Table 2-4c Division * HIV Cross tabulation

			HIV		Total
			HIV +	HIV-	
Division	Annupur	Count	137	26062	26199
		% within HIV	48.9%	53.4%	53.4%
	Jatahari	Count	16	6258	6274
		% within HIV	5.7%	12.8%	12.8%
	Kotma	Count	89	8111	8200
		% within HIV	31.8%	16.6%	16.7%
	Pushprajgrah	Count	38	8338	8376
		% within HIV	13.6%	17.1%	17.1%
Total		Count	280	48769	49049
		% within HIV	100.0%	100.0%	100.0%

Table 2-4d Summary Statistics of Chi- Square Test

Summary Statistics	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	52.357[a]	3	0.000
Likelihood Ratio	47.391	3	0.000
Linear-by-Linear Association	3.201	1	0.074
N of Valid Cases	49049		

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 35.82.

2.14.2 Gender-wise Affliction of Tuberculosis in HIV-AIDS Background

Tables 5, 6, and 7a provide the gender-based data of HIV with TB (HIV+TB) and HIV without TB (HIV-TB) for the study period (2013-17). For females, this data was further bifurcated into females with antenatal case (ANC) and those without antenatal case (ANC) having HIV+TB and HIV-TB. As evident from the table, females (ANC) shows the maximum number of HIV+ cases, of which HIV+TB constitutes half of the HIV-TB cases. A similar trend follows for the female (non-ANC) and males where HIV+TB measure half of the HIV-TB cases. However, female (ANC) records the maximum number of HIV cases, followed by males and females (non-ANC) (Figure 5, 6). Table 7b provides the total number of valid cases, which includes HIV+TB and HIV-TB cases amongst the male, female (ANC) and female (non-ANC) (396, 100% diagnosed cases; no missing case). Table 7c provides the counts and percentage of HIV+TB and HIV-TB cases from amongst the male, female (ANC) and female (non-ANC). As shown in Table 7c, female (ANC) records the maximum percentage of HIV+TB cases while female (non-ANC) shows the minimum. Table 7d provides the summary statistics of the Chi-square, which includes the Pearson Chi-square, the likelihood ratio, and linear-by-linear association, as earlier. The asymptotic significance (p-value) for the Pearson Chi-square is 0.587 ($\chi^2 = 1.066$; $p > 0.05$; df=2); for the likelihood ratio, the p-value is 0.580 (1.090; $p > 0.05$; df=2) while the p-value for linear-by-linear association is 0.330 (0.947; $p > 0.05$; df=1) indicating a statistically insignificant relationship between the variables.

Table 2-5 Block wise HIV/AIDS data from April 2013 to March 2017 in district Anuppur M.P.

S. No.	Year/ Session	Division/ Block	Male		Female Non ANC		Female With ANC		Total	
			Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive
1.	2013-2014	Anuppur	107	04	649	03	1405	00	2755	07
		Jaitahri	246	00	217	00	551	01	1014	01
		Kotma	197	00	241	00	958	00	1396	00
		Pushparajgarh	328	02	416	01	1358	24	2102	27
Total			1472	06	1523	04	4272	25	7267	35
2.	2014-2015	Anuppur	1080	15	1300	07	4788	47	7168	69
		Jaitahri	93	06	101	02	885	07	1079	15
		Kotma	75	02	257	00	1828	86	2160	88
		Pushparajgarh	129	01	244	00	1817	10	2190	11
Total			1377	24	1902	09	9318	150	12597	183

3.	**2015-2016**	Anuppur	963	21	1323	08	5188	06	7474	**35**
		Jaitahri	198	00	199	00	1592	00	1989	**00**
		Kotma	90	00	53	00	2111	00	2254	**00**
		Pushparajgarh	152	00	232	00	1026	00	1410	**00**
Total			**1403**	**21**	**1807**	**08**	**9917**	**06**	**13127**	**35**
4.	**2016-2017**	Anuppur	945	18	1836	03	6021	05	8802	**26**
		Jaitahri	327	00	267	00	1598	00	2192	**00**
		Kotma	112	00	56	01	2222	00	2390	**01**
		Pushparajgarh	412	00	575	00	1687	00	2674	**00**
Total			**1796**	**18**	**2734**	**04**	**11528**	**05**	**16058**	**27**

Table 2-6 Block wise HIV associated TB cases from April 2013 to March 2017, Anuppur M.P.

S. No.	Year/session	Anuppur		Jaitahri		Kotma		Pushparajgarh		Total	
	Category of patients	**HIV**	**HIV+TB**	**HIV**	**HIV+TB**	**HIV**	**HIV+TB**	**HIV**	**HIV+TB**	**HIV**	**HIV+TB**
1.	2013-2014										
	Male	04	01	00	00	00	00	02	01	06	02
	Female Non ANC	03	02	00	00	00	00	01	00	04	02
	Female with ANC	00	00	01	00	00	00	24	10	25	10
	Total	**07**	**03**	**01**	**00**	**00**	**00**	**27**	**11**	**35**	**14**
2.	2014-2015										
	Male	15	05	06	02	02	00	01	00	24	07
	Female Non ANC	07	03	02	01	00	00	00	00	09	04
	Female with ANC	47	22	07	03	86	31	10	05	150	68
	Total	**69**	**30**	**15**	**06**	**88**	**31**	**11**	**05**	**183**	**79**

3.	2015-2016										
	Male	21	07	00	00	00	00	00	00	21	07
	Female Non ANC	08	03	00	00	00	00	00	00	08	03
	Female with ANC	06	02	00	00	00	00	00	00	06	02
	Total	**35**	**12**	**00**	**00**	**00**	**00**	**00**	**00**	**35**	**12**
4.	2016-2017										
	Male	18	07	00	00	00	00	00	00	18	07
	Female Non ANC	03	02	00	00	01	00	00	00	04	02
	Female with ANC	05	02	00	00	00	00	00	00	05	02
	Total	**26**	**11**	**00**	**00**	**01**	**00**	**00**	**00**	**27**	**11**

Table 2-7a Block wise distribution of HIV-seropositive, HIV-seronegative status along with TB in males and females in district Anuppur

April 2013 to March 2017			
Sex	**HIV + TB**	**HIV - TB**	**Total**
Male	23	69	92
Female (Non-ANC)	11	25	36
Female (ANC)	82	186	268

Table 2-7b Case Processing Summary

	Cases					
	Valid		**Missing**		**Total**	
	N	**Percent**	**N**	**Percent**	**N**	**Percent**
Gender * HIV	396	100.0%	0	0%	396	100.0%

Table 2-7c Gender * HIV Cross tabulation

			HIV		**Total**
			HIV +TB	**HIV – TB**	
Gender	**Male**	Count	23	69	92
		% within HIV	19.8%	24.6%	23.2%
	Female (Non-ANC)	Count	11	25	36
		% within HIV	9.5%	8.9%	9.1%
	Female (ANC)	Count	82	186	268
		% within HIV	70.7%	66.4%	67.7%
Total		Count	116	280	396
		% within HIV	100.0%	100.0%	100.0%

Table 2-7d Summary Statistics of Chi-Square Test

Summary Statistics	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	1.066[a]	2	0.587
Likelihood Ratio	1.090	2	0.580
Linear-by-Linear Association	0.947	1	0.330
N of Valid Cases	396		

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 10.55.

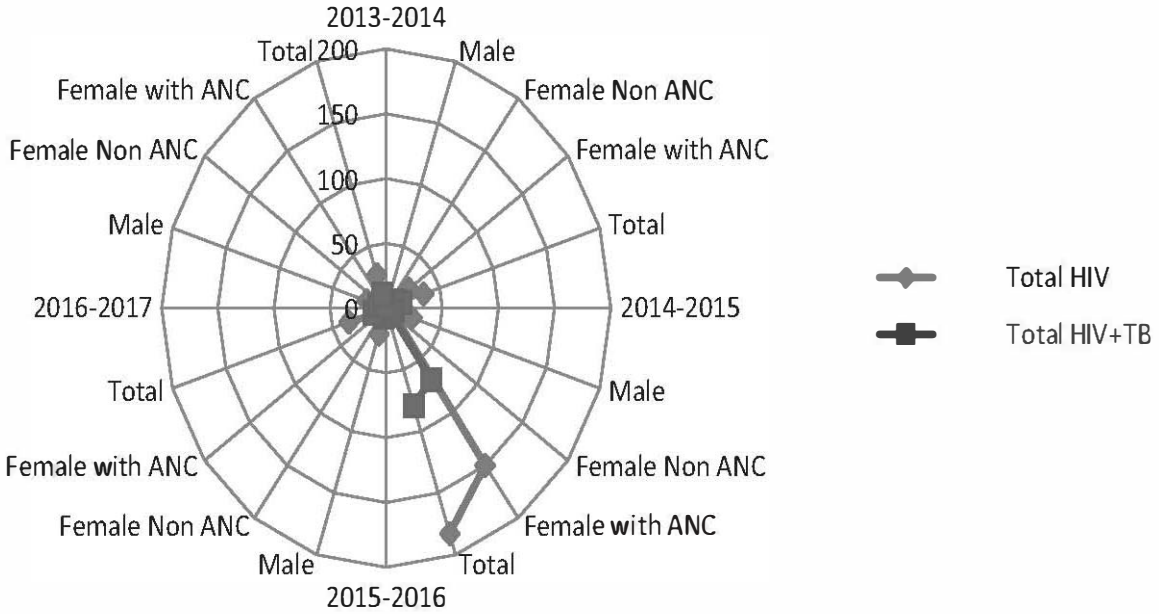

Fig. 2-5 Annual gender wise correlation of HIV and Tuberculosis during the study period (April 2013 to March 2017)

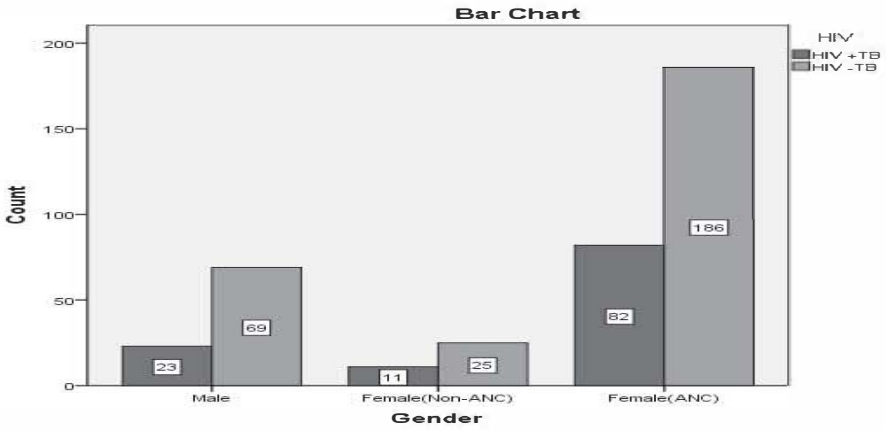

Fig. 2-6 Gender wise distribution of HIV+TB and HIV-TB status in different subdivisions/blocks of district Anuppur

2.14.3 Female Non-Antenatal Case (ANC)

Females constitute 40 percent of the HIV/AIDS burden in India, and the rate is increasing. The major contributors among females are paid sex workers engaged in unprotected sex with random multiple partners. An unequal power equation and relatively low status in society, when compared to males, weakens the ability of women to negotiate using protection during sex, both within and outside the marriage. This increases their vulnerability to AIDS. Data available through various studies also suggest that the females get the majority of HIV infection through their husbands or male partners, who work as truck drivers, migrant labourers, or through male sexual infidelity (UNAIDS 2014, NACO 2015). A study by Lucas and coworkers (2015) has shown that females taking injectable drugs are several times more vulnerable to contracting HIV infection than males. Additionally, nose or ear piercing practices among females, in both urban and rural areas, also push them towards the risk of getting AIDS.

2.14.4 Female Antenatal Case (ANC)

Data related to HIV-related opportunistic infection is scarce. To date, there is no proof that demonstrates differential $CD4^+$ T lymphocyte counts in pregnant and non-pregnant females. However, $CD4^+$ T lymphocyte counts may fall during pregnancy, due to the dilutional effect of increased plasma

volume. $CD4^+$ T lymphocyte serves as an index of immunosuppression during pregnancy (Miotti et al. 1992, Toumala et al. 1997). Further, physiological changes occurring during pregnancy might impact opportunistic infections. These changes include: increased cardiac output with simultaneous increase in glomerular filtration rate and renal clearance; increased plasma volume and relatively lower increase in red blood cell volume thus leading to dilutional anaemia; increased tidal volume and pulmonary blood flow, leading to the absorption of aerosolized medications; placental transfer of drugs; increased renal clearance; and increased gastrointestinal absorption and metabolism by fetus which might influence the maternal drug levels. All these factors may affect the pharmacokinetics of drugs taken to cure opportunistic infections. Hence, the increased number of cases of HIV+ and HIV+TB cases in females with ANC (Cruickshank et al. 1996).

2.14.5 Preponderance in Males

Males having HIV-seropositive status might be due to reasons which include unprotected sex involving multiple partners. Migrant workers, truck drivers, and labourers, act as bridges, linking rural and urban populations and also low risk and high-risk groups. The worst part is, that HIV diagnostics among these people is very low, which only aggravates the matter, as these people act as a potential reservoir, infecting others. According to UNAIDS (2014), 75 percent of women with a husband who is a migrant laborer tested positive for HIV, while one percent of people who have migrated from a rural to an urban area, tested positive for the disease (UNAIDS 2014). This indicates HIV-AIDS to be largely an urban phenomenon. Migrant workers, both male and female, are engaged in a high level of extra-marital promiscuous sex, without using protection, thus inflating the percentage of HIV amongst them (Saggurti et al. 2011). According to national AIDS control organization (NACO) estimates, around 2.5 percent of two million truckers are living with HIV (NACO 2015). Truckers are usually engaged in unprotected sex with sex workers, getting infected or transmitting infection, thus spreading the infection to the general population. NACO (2015) has also reported lower testing rates among truck drivers, as they are grossly unaware that HIV could be transmitted through a heterosexual act (Nasir et al. 2015).

Males with sexually transmitted diseases (STD) having sex with affected partners, or having STD themselves increase the propensity towards AIDS. Males having homosexual tendencies and male sex workers are particularly vulnerable to contracting AIDS. A study has shown that 70

percent of males suspected with HIV infection are engaged in male prostitution. Males engaged in prostitution show 43 percent of HIV infection, compared to the males engaged in homosexual acts (18 percent) (UNAIDS 2009, 2013, Narayanan 2013). Transgender people also show a very high prevalence of contracting HIV infection. This is largely due to high-risk behavior, alcohol and/or substance abuse, and low literacy rate (UNDP 2010, NACO 2015). Additionally, there are cases of drug users, particularly those using injectable drugs, which show the prevalence at around 10 percent. These people usually exchange needles, syringes, or opioid substitution therapy. The most usually used injectable drugs are buprenorphine, pentazocine, diazepam, and heroin. Injecting drugs forms the major transmission route of HIV in north-eastern states (Adhikary et al. 2013, NACO 2015). A study has shown that females taking injectable drugs are more likely to get HIV infection, as compared to the males taking injectable drugs (Lucas et al. 2015).

2.15 Conclusions

The HIV sentinel surveillance carried out among TB patients in many countries of the world shows that HIV prevalence rates are increasing quite rapidly. The HIV-seroprevalence among TB patients in different regions of India varies. Clinical and surveillance data show that TB is the major life-threatening opportunistic infection associated with HIV in Asia. There is a need to decriminalize and de-stigmatize the disease, as stigma associated with the disease leads to denial on the part of patients, who may hide the infection, or refuse or delay the treatment, thus making them reservoirs of infection, infecting others. An overall prevalence rate of HIV infection in India remains low. But even a slight increase in HIV infection rates in a country of more than one billion people may translate into large numbers of people becoming infected.

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CHAPTER III

SEASONALITY AND TREND PATTERNS IN TUBERCULOSIS: PREDICTABILITY ANALYSIS

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Abstract

Tuberculosis is a highly contagious disease that shows a seasonal pattern. Deciphering the season-specific risk, and the socio-economic factors, equips us with the forecasting ability and better counter strategy to control and manage the disease, which is one of the major causes of human mortality. Epidemiological studies were performed for the Indian central province, i.e. the state of Madhya Pradesh (with highest tribal population) to analyze the seasonality in tuberculosis. Data on smear-positive pulmonary tuberculosis was collected from the RNTCP for the period spanning 2005-2010, for all the districts of Madhya Pradesh. Data were analyzed via R-software (R-3.2.3 version) with Poisson Regression model and Time series analysis to count the annual rate of incidence, seasonality, and trend factors, for smear-positive pulmonary tuberculosis cases. Results have established a definite seasonal pattern which peaks in summer. Results were also compared for the tribal-dominated districts. Various seasonal risk factors, spatiotemporal, and socio-economic factors were discussed along with nutrition, associated co-infection, immune-competence, vitamin D deficiency, and physiology, with respect to their effect on tuberculosis infection, transmission, and progression. The results from this study will have the potential to predict future TB incidence, and epidemics, with the potential to optimize TB control via positive intervention, using the predictions as reference information.

3.1 Background

Tuberculosis (TB) is one of the leading causes of mortality in humans. Around one-third of the global human population is suspected to be infected with the *Mycobacterium tuberculosis* (Zaki 1971, Suarez et al. 2001, Glaziou et al. 2009, 2014). Of the 9.6 million global cases of TB infection, India alone carries the burden of 2.2 million cases. Around 40 percent of the population lives with the TB bacterium, while 22.7, 23.7 and 9.2 percent live with TB, MDR-TB and TB-HIV, respectively (WHO 2015, Zumla et al. 2015). Maternal mortality in India is more attributed to TB than to all other causes combined. Most TB patients are in the reproductive age group. More than 300,000 school-aged children have to leave school on account of TB, and around 100,000 women are abandoned by their families, owing to TB. Therefore, TB incurs a huge socio-economic cost in India (Central TB Division 2008).

3.2 Incidence Rate

TB can be pulmonary (PTB, with lungs as the primary site of infection) and extra-pulmonary (EPTB, where other organs, excluding lungs, are the primary site of infection). TB can be manifested in a variety of forms, including primary infection, latent TB, and relapse infection or reactivation cases (Suarez et al. 2001, Glaziou et al. 2009, 2014). The rate of incidence of tuberculosis shows high variability in different countries. Africa shows a maximum of 365 cases per 100,000 persons per year, followed by Spain (21 cases), UK (five cases), and the USA (four cases) (Fallah et al. 2012, Moosazadeh et al. 2012, Nasehi et al. 2012).

3.3 Seasonality Pattern

Tuberculosis shows a seasonal pattern, and therefore shows variations with the climate and weather patterns, and also with spatiotemporal, as well as socio-cultural factors (Thorpe et al. 2004, Nagayama and Ohmori 2006). Variations in seasonal and climatic factors affect the transmission of TB in a significant manner by forcing people to stay indoors, making it conducive for bacteria to spread, hence the transmission (Fares 2011). Season-determined diet patterns, and the resulting patient metabolisms also affect the rate of incidence. A particular time period may see an upsurge in incidence, as well as reporting, of the cases. For example, post-festival time, the reporting of cases is enhanced. Malnutrition is the major cause of TB epidemics, particularly in resource-limited, poor countries, as

it weakens the immune system, making malnourished individuals vulnerable to TB. Malnutrition, coupled with co-infection of HIV-AIDS, poor ventilation, poverty, sub-human living conditions, lack of awareness, and poor education, further increases the vulnerability towards TB. The disease is a major economic burden on government resources, which could be utilized for other welfare and development measures.

3.3.1 Seasonal Patterns Across the Globe

The variability in the seasonal pattern of TB is reported on a global scale (Douglas et al. 1996, Schaaf et al. 1996, Rios et al. 2000, Yamamoto et al. 2003, Thorpe et al. 2004, Leung et al. 2005, Nagayama and Ohmori 2006, Akhtar and Mohammad 2008, Luquero et al. 2008). South Africa shows the highest incidence of TB in late winter and early spring (Schaaf et al. 1996, Khaliq et al. 2015). Whereas the UK (Douglas et al. 1996), Hong Kong (Leung et al. 2005), and Pakistan (Onuzaka and Hagihara 2015) show the highest incidence in summer; Spain and Japan (Rios et al. 2000, Yamamoto et al. 2003, Luquero et al. 2008) are highest in summer and autumn, while Taiwan has reported its peak in late spring and early summer (Liao et al. 2012). The analysis clearly reveals the non-uniform pattern of TB incidence globally, with respect to the geoclimatic attributes and time intervals (Rios et al. 2000, Fares 2011). This attests to the underlying mechanism that is driving the seasonal variations in TB, with the involvement of many factors, which, if understood properly, will help in controlling the same. Many studies have shown the increasing trend for TB cases during the closing of winter and the start of summer (Rios et al. 2000, Naranbat et al. 2009, Li et al. 2013).

3.3.2 Vitamin D Deficiency

Vitamin D deficiency, coupled with impaired host immune defense, may also affect the rate of incidence. Vitamin D shows seasonal variations at the physiological level, displaying reduced levels in spring and winter (Maes et al. 1994). Solar radiation shows a multitude of benefits, like synthesis of vitamin D, killing bacteria, and synthesis of an immune regulator of cellular immunity, ensuring protection against TB infection (Maes et al. 1994, Nagayama and Ohmori 2006, Coussens et al. 2009). However, these factors apply to primary TB infection and not the relapse or the reactivation cases.

3.4 India: Case Scenario

India, with 17.5 percent of the global population, bears the burden of 26 percent of the global cases of TB (RNTCP 2005). TB is a giant killer in India, killing almost 500,000 people annually. It is a major public health issue in India with 2.2 million cases in 2015 alone as against the global incidence of 9.6 million cases (Chakraborty 1993, WHO 2015, Zumla et al. 2015). Around 40 percent of the Indian population is living with infectious TB bacteria (RNTPC 2005). India shares 22.7 percent, 9.2 percent, and 23.7 percent, respectively of TB, TB/HIV and MDR-TB in the top 20 countries, in terms of absolute numbers and the burden of severity in 2015 (Ricks et al. 2011, WHO 2015). TB is a notifiable disease in the state of Madhya Pradesh in India (Rao et al. 2010), which is the fifth largest state in India by population size, having the highest percentage of tribal population (New Delhi TB 1999). In 2015 alone, 103,108 patients were registered for treatment (RNTCP 2005). The incidence and mortality rate of TB in Madhya Pradesh has been consistently increasing for the last 15 years (New Delhi TB 1999, Murhekar et al. 2004).

3.5 Madhya Pradesh: Tribal Dominant State

Madhya Pradesh is home to the largest tribal population, with 46 recognized tribes, including Gond, Baiga, Bhil, Bhariya, Korku, Kaul and Sahariya (Chakma et al. 1996, Murhekar et al. 2004, GOI 2001, 2010, Rao et al. 2010, Yadav et al. 2010). This constitutes 21 percent of the total population. The state has a total of 51 districts, of which Dhar, Jhabua, and Mandla consist of more than 50% of the tribal population, whereas the Anuppur district has about 21% of the tribal population (Bhat et al. 2009).

3.5.1 Revised National Tuberculosis Control Program (RNTCP)

In the Indian context, the 'revised national tuberculosis control program (RNTCP)' predominantly spearheads TB treatment and control, followed by the government as well as private players. In 2015 alone, the RNTCP has covered 1.28 billion people affected with TB (RNTCP 2016). However, TB notification is still less than half, as one- third of cases are not diagnosed, diagnosed but not treated, or diagnosed and treated but not reported to the RNTCP (Grassaly et al. 2006, Fisman 2007, WHO 2013). Epidemiological and statistical reports prepared by the RNTCP for Madhya Pradesh advised implementing the internationally recommended, 'directly observed treatment short-course (DOTS) strategy for the best

cure results in tuberculosis treatment' (RNTCP 2000, RNTCP 2005, CTB 2011).

3.5.2 Tuberculosis in India: Seasonality Pattern

Thorpe and coworkers (2004) have pioneered the seasonality analysis of TB in India for a period of almost a decade (2008-2014). Narula and coworkers (2005) have reported seasonality in the northern region of India, with a peak reported in the second quarter from April to June, while a fall was reported in the fourth quarter from October to December (Narula et al. 2015). However, no seasonal pattern was observed for the southern part of India (Thorpe et al. 2004). Kumar and coworkers have also reported a falling seasonal trend in TB cases in Delhi for the period 2007-2012 (Kumar et al. 2014). The determination of temporal and seasonal patterns of TB has forecasting potential, and could help in the management and control of TB. It is possible to predict the outbreak of the disease well in advance, based on the previous history of the disease and taking into account the parameters associated with it (Douglas et al. 1996, Rios et al. 2000, Naranbat et al. 2009). The incidence pattern could serve as a guiding force, and will channel the efforts of healthcare providers for the efficient management of the disease, at the planning as well as the execution level (Akhtar and Mohammad 2008).

The findings of seasonality and trend patterns are important in adopting preventive measures for TB control, and to determine risk factors.

3.6 Study Area

The state of Madhya Pradesh lies in the central part (central province) of India, between the latitude 21.2° N-26.87° N and the longitude 74.02'-82.49'E (Figure 1). Madhya Pradesh experiences a subtropical climate, with a hot dry summer (April–June) followed by monsoon rains (July–September), and cool and relatively dry winters. The average rainfall is about 1,371mm (54in). The southeastern districts have the heaviest rainfall, with some places receiving as much as 2,150mm (84.6in) while the western and the northwestern districts receive 1,000 mm (39.4in) or less.

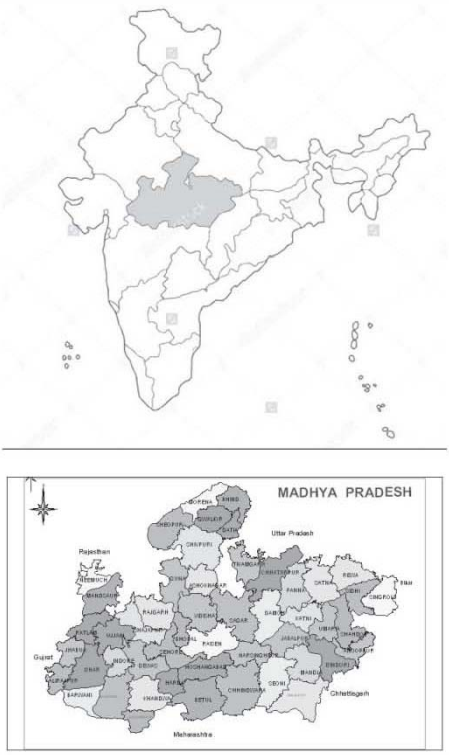

Fig. 3-1 Map of India showing Madhya Pradesh (Central Province) on Left and different districts of Madhya Pradesh on Right.

3.7 Data Collection

Laboratory-confirmed TB cases from all the 45 districts of Madhya Pradesh, India, were reported to the RNTCP from 2005-2010. The six-year study period constituted 24 epidemiological quarters for each district. For example, data was made available in the form of a quarterly report of districts, i.e. monthly data grouped into four quarters in a year, (q): q1 (January-March); q2 (April-June); q3 (July-September); and q4 (October-December). Data collected over a period of six years and 24 epidemiological quarters were analyzed for the seasonality and trend pattern (Figure 1).

Laboratory confirmation was based on the positive acid-fast bacilli (AFB) smear examination, which was further confirmed by the chest X-ray radiographs. Directly observed short course treatments (DOTS) notified the cases after three tests of sputum smear analysis, where two positive cases were considered as true cases of TB, and registered with DOTS as smear-positive cases (CTB 2010). The test was performed through the Ziehl-Neelson stain method, as per the guidelines of the World Health Organization (Tadesse 2015).

3.7.1 Meta-Analysis

Seasonality in TB can be analyzed by several methods, which include Fourier analysis (Parrinello et al. 2012), Cosinor analysis (Douglas et al. 1998), the sinusoidal harmonic model (Leung et al. 2005, Akhtar and Mohammad 2008), spectral analysis (Atun et al. 2005), seasonal autoregressive integrated moving average model (SARIMA) (Parrinello et al. 2012, Korthals et al. 2012), the time-series decomposition method (Douglas et al. 1996, Behera and Sharma 2011, Willis et al. 2012), and others. The present study has adopted a time series decomposition analysis to examine the seasonal variation in active TB cases in all the districts of the state of Madhya Pradesh from 2005 to 2010 in India. Seasonal amplitude (the difference between the monthly highest and lowest mean case counts) was calculated for the evaluation of seasonal variation in TB.

3.7.2 Statistical Analysis

Microsoft Excel (2010 version) was utilized to analyze the data. We assessed the seasonality of TB in Madhya Pradesh from 2005 to 2010, using R-software (R-3.2.3 version), and finally, we used the Poisson Regression model to assess the potential impact of seasonality (Christensen et al. 2011). Data were entered in R-software and accessed through the time series package. R-software is an integrated suite of software facilities for data manipulation, calculation, and graphical display. Data pertains to the incidence of TB cases in districts of the state of Madhya Pradesh, India, where each district represents a spatial point. The count of incidence is recorded over 24 quarters, from the first quarter of 2005, to the last quarter of 2010. In effect, data is available for 24 time points. The incidence is measured in terms of counts, rather than the rate that is a common measure of the incidence in the epidemiological studies (Kumar et al. 2014). For each spatial point, the data on the counts show

seasonal variations which also give the trend visible across some spatial points. The counts for the quarters are independent stochastic events.

For the counts that are observed in a quarter, the Poisson model is proposed, with

$$E(Ct) = St + Tt$$

Here, Ct represents the count in quarter t, St represents the effect of season, and Tt represents the effect of the trend. Therefore, the effects pertaining to the seasonality and the trend may, for a given spatial point, be estimated through the Poisson Regression model, as follows:

$$\log(Ct) = \alpha 0 + \alpha 11 sin\left(\frac{2\pi t}{4}\right) + \alpha 12 cos\left(\frac{2\pi t}{4}\right) + \alpha 2\left(\frac{t}{4}\right) \quad (1)$$

The terms $\alpha 01 sin\left(\frac{2\pi t}{4}\right) + \alpha 01 cos\left(\frac{2\pi t}{4}\right)$ model the component of seasonality present in the counts pertaining to different quarters. The amplitude, δ indicates the extent of the seasonal variation of log of counts over the year given by the following equation:

$$\delta = 2max(|\alpha 11|, |\alpha 12|) \quad (2)$$

Hence, $exp(\delta)$ gives the amplitude in terms of counts.

Here, the peak can be defined through different numbers estimated for all the four quarters/seasons. A total log of the count will provide the final result of the maximum mode for each district for the given quarter, where the peek is high or low.

3.8 Seasonality Indices

The study period from 2005-2010 constitutes 24 epidemiological quarters. According to the amplitude of seasonal indices, a maximum number of TB cases was observed in the second quarter (q2) from April to June (summer) and a minimum number was observed in the first quarter (q1)

from January to March during the study period (2005 to 2010). The peak of the second quarter (q2) of every year for each district shows positive amplitude (Table 1). This means the environmental factors of the summer season contribute towards the enhanced number of TB cases.

Table 3-1 List of seasonal indices for seasonality during 2005 to 2010 in the districts of Madhya Pradesh

S. No.	District	Seasonal Indices (from 2005-1 to 2010-4)			
		q1	q2	q3	q4
1	Balaghat	0.03	0.1	-0.07	-0.05
2	Barwani	-0.03	0.23	-0.07	-0.13
3	Betul	-0.06	0.09	0.01	-0.05
4	Bhind	-0.1	0.16	0.07	-0.12
5	Bhopal	-0.09	0.11	0.04	-0.07
6	Chhatarpur	-0.12	0.18	0.06	-0.11
7	Chhindwara	-0.05	0.23	0.02	-0.2
8	Damoh	-0.04	0.12	0.04	-0.12
9	Datia	-0.03	0.17	0.04	-0.17
10	Dewas	0	0.13	-0.03	-0.1
11	Dhar	0	0.13	-0.04	-0.09
12	Dindori	-0.1	0.24	-0.06	-0.08
13	Guna+ashoknagar	-0.11	0.15	0.12	-0.16
14	Gwalior	-0.07	0.18	0.07	-0.18
15	Harda	-0.11	0.11	0.13	-0.12
16	Hoshangabad	0	0.09	-0.01	-0.07
17	Indore	0.02	0.11	-0.03	-0.1
18	Jabalpur	-0.06	0.15	0.03	-0.11
19	Jhabua+alirajpur	-0.12	0.17	0.04	-0.09
20	Katni	-0.08	0.18	0.05	-0.15
21	Khandwa+burhanpur	-0.04	0.07	0.01	-0.04
22	Khargone	0.01	0.17	-0.02	-0.15
23	Mandla	0.02	0.14	0.02	-0.18
24	Mandsaur	-0.05	0.14	0.08	-0.17
25	Morena	-0.1	0.22	0.02	-0.14

26	Narsinghpur	-0.08	0.21	0.01	-0.15
27	Neemuch	0.02	0.09	0.02	-0.13
28	Panna	-0.04	0.13	0.02	-0.11
29	Raisen	0	0.15	-0.02	-0.13
30	Rajgarh	0	0.12	0.05	-0.17
31	Ratlam	0	0.14	0.01	-0.14
32	Rewa	-0.05	0.12	0.02	-0.08
33	Sagar	-0.09	0.16	0.02	-0.1
34	Satna	-0.03	0.15	0	-0.12
35	Sehore	0.05	0.2	-0.02	-0.22
36	Seoni	-0.02	0.28	-0.02	-0.24
37	Shahdol+APR	-0.08	0.14	-0.02	-0.04
38	Shajapur	-0.04	0.08	0.05	-0.1
39	Sheopur	-0.08	0.21	0.01	-0.15
40	Shivpuri	-0.1	0.16	0.1	-0.15
41	Sidhi+singrauli	-0.16	0.06	0.06	0.04
42	Tikamgarh	-0.03	0.14	0.09	-0.19
43	Ujjain	-0.08	0.18	0.05	-0.15
44	Umaria	0.05	0.12	-0.05	-0.12
45	Vidisha	-0.02	0.12	0.03	-0.13

3.9 Annual Tuberculosis Detection

The annual TB incidence rate per 100,000 of population shows highly increasing trends for the districts of Chhatarpur, and Indore for the study period (Figure 2). There has been a steady, increasing, trend observed for the aforementioned districts. For other districts, Bhopal records the maximum number of reported cases, 5,054 per 100,000 of population, while Balaghat showed the minimum number of reported cases, at 1,433 per 100,000 of population (Table 2). As expected, the time-series displays a number of peaks, all appearing in the second quarter (q2) of the study period, which represents the summer season. A definite seasonal pattern is observed, with the peak of registered cases across an equal interval of quarters. Shahdol and Anuppur combined (predominantly tribal districts) show a slightly increasing trend for the study period (Fig. 2 and Fig. 3). Some districts, like Chhindwara, Datia, Guna, Ashoknagar, Morena, Raisen, Rajgarh, Seoni, and Tikamgarh, show a continuously decreasing

trend in registered cases, relative to the other districts during the study period (Figure 4).

Table 3-2 Annual total case detection rate per 100,000 populations in different districts of Madhya Pradesh (MP)

		Year						
S. No.	District	2005	2006	2007	2008	2009	2010	Total
1	Balaghat	312	233	227	222	198	241	1433
2	Barwani	388	393	565	537	449	533	2865
3	Betul	252	425	377	476	458	433	2421
4	Bhind	410	392	516	444	419	365	2546
5	Bhopal	662	681	869	883	1057	902	5054
6	Chhatarpur	412	404	579	557	592	732	3276
7	Chhindwara	335	379	350	320	315	543	2242
8	Damoh	547	407	444	505	524	558	2985
9	Datia	532	486	557	544	468	466	3053
10	Dewas	334	353	431	389	368	594	2469
11	Dhar	432	402	459	440	434	472	2639
12	Dindori Guna+	265	280	319	381	426	504	2175
13	Ashoknagar	395	439	348	344	384	447	2357
14	Gwalior	548	547	705	775	794	573	3942
15	Harda	294	281	384	351	354	550	2214
16	Hoshangabad	541	627	676	599	604	559	3606
17	Indore	431	588	595	673	824	718	3829
18	Jabalpur Jhabua+	374	377	401	390	378	642	2562
19	Alirajpur	450	409	444	401	426	520	2650
20	Katni Khandwa+	410	375	370	362	345	477	2339
21	Burhanpur	365	412	424	379	385	509	2474
22	Khargone	485	366	404	415	400	561	2631
23	Mandla	384	423	438	484	497	555	2781
24	Mandsaur	423	421	509	472	437	439	2701
25	Morena	546	337	464	439	361	449	2596
26	Narsinghpur	326	422	321	374	430	497	2370
27	Neemuch	582	652	827	708	686	611	4066

28	Panna	258	265	275	274	240	426	1738
29	Raisen	346	307	282	223	227	305	1690
30	Rajgarh	435	339	370	353	282	405	2184
31	Ratlam	413	375	439	384	340	436	2387
32	Rewa	276	429	431	441	494	384	2455
33	Sagar	429	406	425	418	396	408	2482
34	Satna	365	298	302	331	314	360	1970
35	Sehore	335	284	318	308	323	368	1936
36	Seoni	272	251	233	194	187	408	1545
37	Shahdol+ APR	287	313	315	332	375	424	2046
38	Shajapur	475	357	419	359	335	394	2339
39	Sheopur	723	499	490	516	452	440	3120
40	Shivpuri	415	349	386	387	419	444	2400
41	Sidhi+ Singrouli	276	377	356	368	426	452	2255
42	Tikamgarh	312	222	236	236	218	328	1552
43	Ujjain	458	425	435	443	383	397	2541
44	Umaria	280	250	267	268	231	395	1691
45	Vidisha	625	480	413	374	375	383	2650

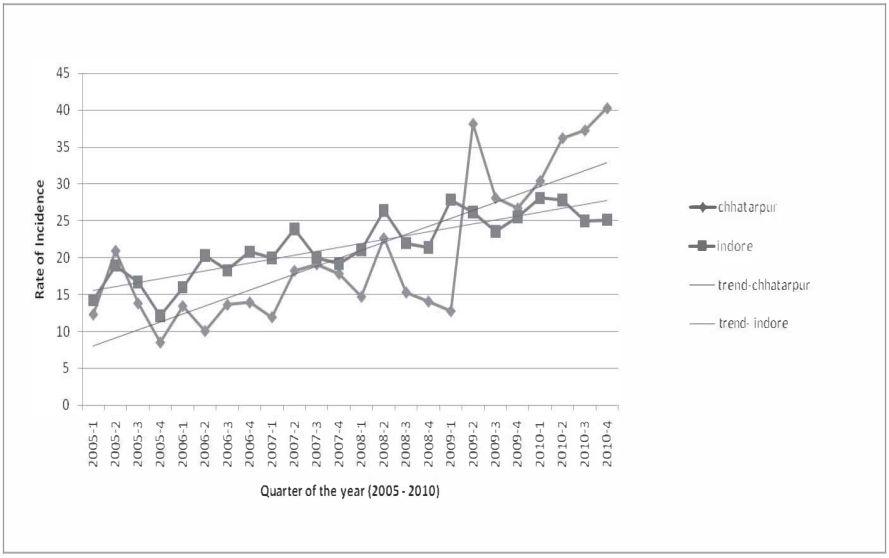

Fig. 3-2 Annual highly increasing trend of two districts in Madhya Pradesh

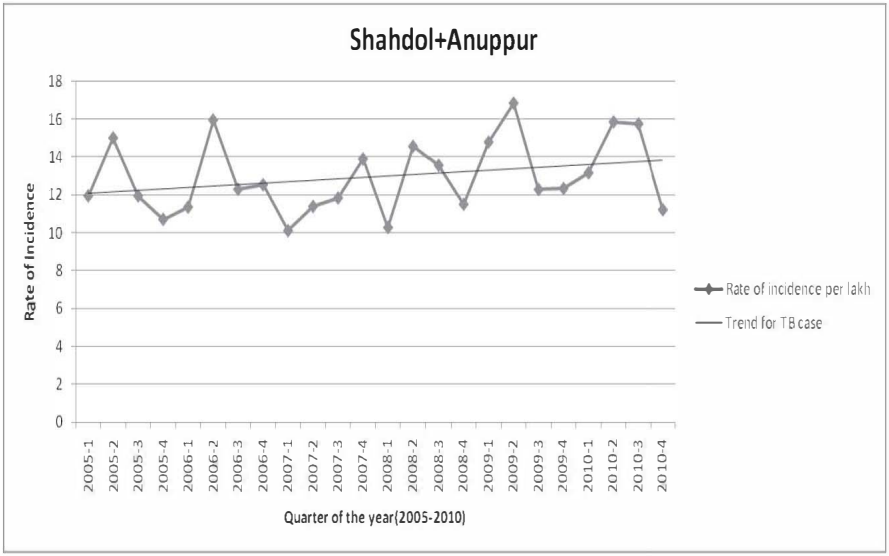

Fig. 3-3 The quarterly incidence rate and trend of TB of Shahdol + Anuppur districts especially for Baiga tribes

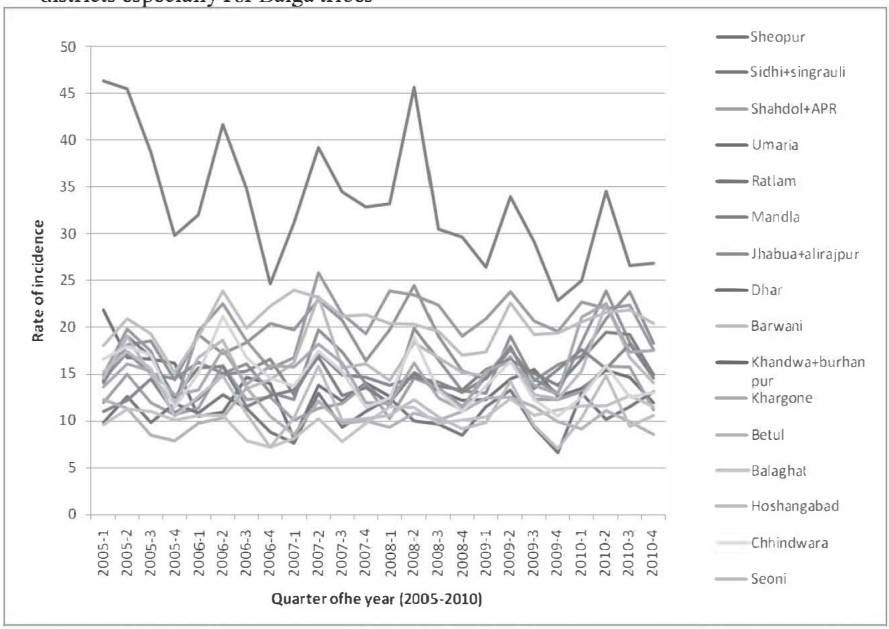

Fig. 3-4 TB incidence rate of 19 tribal districts in Madhya Pradesh during study period (2005-2010)

3.10 Annual Case Detection in Tribal Populations

We have also estimated the annual case detection rate of the districts which have more than 50 percent tribal population, as shown in Table 3. An average number of cases notified per quarter for the tribal-dominated districts (Dhar, Jhabua, Mandla, and Anuppur + Shahdol) was 422 (Table 3). As given in Table 3, Mandla showed the maximum number of notified cases, while Anuppur + Shahdol combined, showed the minimum number of notified TB cases in the study period, for the tribal-dominated districts. The annual number of notified cases also showed variability, with Mandla showing the maximum number of cases (555 in 2010), while Anuppur + Shahdol combined, showed the minimum (287 in 2006) cases (Table 3). Surprisingly, the year 2010 showed the maximum annual reported cases for the tribal-dominated districts (Table 3).

Table 3-3 Annual total case detection rate per 1,00,000 in districts of MP where tribal population is more than 50 percent

	Year						
District (tribal population more than 50%)	2005	2006	2007	2008	2009	2010	Total
Dhar	432	402	459	440	434	472	2639
Jhabua	450	409	444	401	426	520	2650
Mandla	384	423	438	484	497	555	2781
APR+SDL*	287	313	315	332	375	424	2046

*Anuppur and Shahdol (APR+SDL) - maximum accuracy of Baiga tribes

3.11 Seasonality and Regression Analysis

We implemented the time series analytical package in R-software using R-stat command, which shows the periodic fluctuation of the season, and the data trends with their peaks (Douglas et al. 1996). Seasonality indices were calculated for the value of 1 in the incidence rate of TB cases reported quarterly for the four different seasons across the study period

(2005 to 2010). Table 1 shows the result for seasonality with the amplitude of indices for every quarter observed by the Poisson Regression model. The highest peak, and the maximum indices, point between +0.20 to +0.30 for many districts in the second quarter (q2) from 2005 to 2010. All the counts of the Poisson model for indices have a positive number for each district (Thorpe et al. 2004, Corbett et al. 2010).

As given in Table 1, the highest count of seasonality was +0.28 for the Seoni district, followed by +0.24 for Dindori, while Chhindwara and Barwani got the values of +0.23 each. Here, the value 0.23 meant that the Poisson model explained 23 percent of the observed variation in the series of 24 quarters for the period 2005-2010. As an output of seasonality for TB, a report of the RNTCP has confirmed the highest peak in the second quarter through the Poisson Regression model and the time series analysis. Overall, the current study validates the findings from the earlier studies, that TB cases are higher in the summer (April - June), and the TB incidence rate is relatively higher in the 19 tribal districts of Madhya Pradesh. Results establish a definite pattern of seasonality, with a peak in the summer and trough during the winter. This indicates that the summer peak might be due to the reactivation of latent TB, increased concentration of the particulate matter, or the enhanced winter transmission, due to indoor crowding in winter, and reduced vitamin D synthesis, due to reduced sunlight (as happens during winter).

3.12 Seasonal Variation in Tuberculosis: Mechanistic Details

Tuberculosis shows a definite seasonal variation in the study region of Madhya Pradesh. Earlier studies have also confirmed similar findings (Douglas et al. 1996, Rios et al. 2000, Yamamoto et al. 2003, Thorpe et al. 2004, Leung et al. 2005, Narula et al. 2005, Luquero et al. 2008, Onuzaka and Hagihara 2015).

3.12.1 Extrinsic and Intrinsic Infection

The principal findings that emerged out of this study are the higher number of TB cases registered during the summer (April to June), and secondly, that cases are more frequent in the 19 tribal districts, relative to the other districts of Madhya Pradesh. The reason could be the increased reactivation of latent TB. This reactivation might result in 'extrinsic infection', due to increased overcrowding and enhanced transmission, and

'intrinsic infection' due to vitamin D deficiency and high epidemics of other respiratory diseases. Slow-growing tubercular bacteria take 7-8 weeks to develop into full-fledged clinically identifiable tuberculosis. So, there might be a possibility that winter overcrowding has led to the spread of the infection, which has subsequently developed into TB. Enhanced winter transmission due to indoor crowding in winter is seen, as the *Mycobacterium* spreads quickly in closed surroundings with too many people around (Rios et al. 2000, Naranbat et al. 2009, Li et al. 2013).

3.12.2 Prolonged Confinement in Dark and Crowded Places

Confinement in dark and dingy environments for long periods deprives people of sunlight and increases the risk of transmission, as solar radiation kills the bacteria. The winter season is characterized by short days with reduced photoperiod (sunlight), and hence, vitamin D deficiency. Foggy weather during winters leads to increased particulate matter in the environment which causes respiratory distress, leading to intrinsic infection. Overcrowding of public transport during festivals and marriages also adds to the burden, as people downplay the symptoms, and delay reporting, or consulting a physician. This leads to delay in taking proper medical care, thus allowing bacteria to complete their incubation period, leading to tuberculosis.

3.12.3 Effect of Seasonal Variation on Physiology

The majority of respiratory infections are driven by the season, hence they show variations with climate and weather patterns (Thorpe et al. 2004). Tuberculosis shows fluctuations in rate of incidence which varies with spatiotemporal, as well as socio-cultural, factors. These include temperature, sunlight, humidity, crowding, person-to-person contact, etc. (Nagayama and Ohmori 2006). Physiologically, vitamin D deficiency (< 12ng/ml against 20-50ng/ml of normal range), which shows seasonal fluctuation, and impaired host immune defense, may also account for its occurrence. The most accurate measure of vitamin D deficiency is 25-hydroxy vitamin D (25-OH D), which decreases in the spring and winter (Maes et al. 1994).

3.12.4 Effect of Seasonal Variation on Immune system

Immunologically, exposure to solar radiation leads to the formation of an immune regulator of cellular immunity, thus providing protection against

TB infection (Coussens et al. 2009). Variable immune competency through the year, with significant periodicity in cell function, and proliferation and abundance of peripheral blood leucocytes, can also be held responsible for the observed pattern. Peripheral B lymphocytes show a winter fall, while CD4+T lymphocytes show a summer fall, coinciding with the CD8+T peak (Maes et al. 1994, Nagayama and Ohmori 2006). However, these factors apply to primary TB infection, and not the relapse or reactivation cases.

3.12.5 Malnutrition

Seasonally-determined diet patterns, and the resulting patient metabolism, also affect the rate of incidence. A particular time period may see an upsurge in incidence, as well as in the reporting of the cases. Malnutrition, particularly among children below five years of age, is the main determinant for TB epidemics, as undernourishment weakens the immunity, hence increasing vulnerability towards TB. The high prevalence of HIV-AIDS, poor ventilation, poverty, sub-human living conditions, lack of awareness, and poor education, are other major drivers of TB epidemics.

3.13 Tuberculosis: Economic Challenges

TB is a huge economic drain on government resources. For the period 2006 to 2014, TB cost the Indian economy a massive USD 340 billion, which could have been utilized for welfare and development measures. The RNTCP alone has covered 1.28 billion people affected with TB in 2015, which is in addition to the cases reported by other health service providers, such as government hospitals, PHCs, CHCs, and private clinics. However, TB notification is still less than half, as one-third of cases are not diagnosed, diagnosed but not treated, or diagnosed and treated, but not reported to the RNTCP (Grassaly et al. 2006, Fisman 2007, WHO 2013).

3.14 Tuberculosis in the Tribal Population

This study has emphasized the tuberculosis incidence in the tribal-dominated regions, and the results obtained were as per expectations. The 19 tribal districts show more incidences of TB when compared to the non-tribal districts. Tuberculosis is largely a 'poor man's' disease, as it manifests itself in poverty-stricken, low socio-economic setups. Socio-economic constraints are further worsened by the reluctance of tribal

communities to accept the modern treatment which is necessary to cure the disease. As an alternative, they resort to 'occult practices', 'witchcraft', or traditional herb-based medicine, which is not good enough to cure the disease. This makes them a potential reservoir of infection, transmitting the disease to others, and forming a chain. Reluctance may also arise out of the lack of health centers and other health service providers. Tribal or rural people avoid going into cities to receive treatment, due to long travel distances and the lack of sufficient resources and money. This makes them fall prey to the local 'quacks' or traditional healers, which only aggravates the disease. Therefore, the government needs to create fully-equipped health facilities, such as primary health centers, community health centers, hospitals, and pathology laboratories in rural areas. This will avoid the loss of crucial time wasted in the transportation of patients, or in the search for good hospitals. This could further be complemented with good road and transport facilities for smooth transportation of patients to highly equipped, city-based hospitals. However, the best way to tackle TB is through diet supplements rich in proteins, vitamins, minerals, and fats, and through improved living conditions, with adequate light and ventilation.

3.15 Conclusions

The current study investigates the seasonal fluctuations of tuberculosis to identify the relevant information about basic biology, immunology, epidemiology, and seasonal effects, related to tuberculosis. The identification of causative factors may provide possible preventive measures, leading to the development of targeted and effective policies for the rational use of resources more efficiently and effectively. The current analysis may have an advisory role for the stakeholders, i.e. the patients and the service providers. Health service providers, both at government and non-government level, need to focus on the vulnerable areas, like the tribal-dominated districts and the rural areas. This may include organizing awareness programs, health camps, and regular counseling sessions, to sensitize people about preventive measures, nutrition, hygiene, etc., to ward off the scourge of the disease. Additionally, there is a need to increase and improve the health infrastructure in tribal/rural areas to provide immediate relief.

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CHAPTER IV

NOVEL TREATMENT PROTOCOL FOR TUBERCULOSIS: COMBINATORIAL THERAPY

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Abstract

The present study has employed the novel concept of combining the best of modern TB diagnostics with the traditional herbal medicine system for TB treatment. It has enrolled over a hundred cases of uncured TB, and also relapse cases across different age groups, gender, and status, over a period of two years. The outcome has validated the efficiency of the proposed combinatorial model of treatment, as patients were completely cured of TB without any significant relapse cases. Additionally, it has a multitude of benefits, including economy of the costs involved, and lack of any potential side effects. Traditional systems of medicine have proven themselves to be robust systems for the treatment of TB. Findings from similar studies may pave the way for adopting the same model of treatment. This would ease the economic burden on the government exchequer by reducing the costs of treatment, and would also increase the cultivation and conservation measurement of medicinally important plants.

4.1 Background

Ayurveda is the traditional Hindu system of medicine, popularly known as the 'ancient Indian system of medicine'. This system of medicine has ample mention in the Atharva Veda, the last of the four Vedas. This art of medicine is largely based on homeostasis within the various systems of the body, and relies on herbal medicine and yogic breathing. The underlying

philosophy is based on the concept of life, and the view of life, 'as a whole', (Abraham et al. 1968). The utility of medicinal plants has its roots in the 'Uphanishads' and an Indian school of philosophy widely known as 'Saddarsanas'. The six channels of Saddarsanas are systematized and grouped together as six traditional schools, known as Samakhya, Yoga, Nyaya, Vaisesika, Purvamimansa, and Uttaramimansa. Among these six traditional schools, Ayurveda doctrine can trace its origin from Samakhya, Vaisesika, and Nyaya, for its pragmatic and scientific approach (Balasubramanian et al. 2000). Ayurveda follows the Samakhya philosophy for the cosmic origin and the sufferings of human beings during their mortal life on Earth (Ramchandran et al. 2010).

4.2 Ayurveda: Herbs and Yoga

Use of herbs and other plants for the prevention and cure of disease is an ancient practice in India which has more than 5000 years of history (Saxena 1970). The Harappan civilization, considered to be one of the oldest and most evolved civilizations, used herbs for medicinal purposes, including the treatment and cure of diseases. There is ample evidence available, in the form of seals and other artifacts, which attests to the use of herbs by the people of the Harappan civilization for the treatment and cure of diseases (Brijlal and Dubey 1992). However, with the advent of Ayurveda, and its evolution over the period, the use of plants for medicinal purposes spiked. A rich heritage of meticulously catalogued and coded healthcare systems, and use of plants, finds mention in the Ayurveda and in Yoga. The Indian system of medicine, or Ayurveda, has been practised in India since time immemorial, and includes Yoga as its integral part. The introduction of Patanjali Yoga, in about 1000 BC, has seen a great rise in the co-development of Ayurveda and Yoga for curing ailments and other bodily and physiological disorders. Ayurveda attained its peak during the time of Charak and Sushruta, when it was taken to the Greeks and further modified into another system of medicine, popularly known as 'Unani' (Bhargava et al. 1985, Bharat et al. 2011). It is believed that the Unani system of medicine evolved from Ayurveda several thousand years ago, when Hippocrates visited Taxila to study Ayurveda, and took it to Greece, where the Unani system was developed (Mukhopadhyay et al. 2001).

The Rigveda has rich mention of the use and significance of plants for medicinal purposes (Saxena 1970). Since the time of Rigveda, the use of different plants and their parts for preparing medicines has seen tremendous development. This has given a boost to the use of prepared

herbal products for the prevention and treatment of diseases. India has a rich diversity of plants with perceived use for medicine, with more than 3,500 species, and many more as yet unexplored for medicinal purposes (Ray et al. 1994). Almost all plant parts, including the root, tuber, stem, leaves, flowers, and fruits, are important for preparing concoctions or medicines.

Currently, the Indian system of medicine is endowed and enriched with Ayurveda, Unani, Siddha, Yoga, and Naturopathy. These arts of medicine have taken care of the health needs of people in historical times, before the western systems of medicine or allopathy set their feet on Indian soil, and across the globe. Almost half the population relies on these systems for their healthcare needs.

4.3 Medicinal Plants

Medicinal plants have played an integral part in the lives of humans and livestock as far as healthcare needs are concerned. India, with its varied floral diversity, is home to rare herbs and plants which have a very important medicinal role to play. More than 90 percent of the herbs find their use in medicines prepared for Ayurveda, Siddha, Unani, and Homoeopathy (Pama et al. 2014). These plants synthesize chemical compounds which have an important physiological role to play in humans, and other animals. To date, 12,000 such compounds have been identified, which is only 10 percent of the total available compounds yet to be explored. These chemicals work on the human body in a similar fashion to pharmaceutical drugs, with the distinct advantage of having no-side-effects (Lai and Roy 2004, Tapsell et al. 2006). Most of these drugs can be directly extracted from plants in ready-to-use mode, and do not require sophisticated industrial set-ups or corporate management (Chandrabose et al.1987, Cragg et al. 1995).

4.3.1 Advantages Associated with Herbal Medicine

According to an estimate of the World Health Organization (WHO), around 80 percent of the population of developing or underdeveloped countries relies on traditional medicines based on herbs or plants for their primary healthcare needs. However, the lack of any perceived side effects of these medicines has spiked the demand for medicinal plants throughout the world. Now, developed western countries and European nations are increasingly using herbal drugs for their primary healthcare needs, as

reported by the WHO (Cragg et al. 1995). However, herbal medicine is far from being a one-stop-solution, as there are a number of patients showing risks and negative health consequences. The major cause may be the substandard quality of the primary source, i.e. raw material used for the extraction and preparation of herbal medicine (Chunekar 1982).

4.3.2 Over-Exploitation of Herbal Plants for Medicinal Purposes

The extensive use of medicinal plants for medicine has seen their injudicious harvest and use, which has led to the decline in their numbers. In most cases, many rare plants are already extinct, or on the verge of extinction. The process of extinction is both man-made, and driven by evolution. However, the anthropogenic impacts are more severe than the natural ones. In the changing paradigm, there has been a growing concern for their significance in human life, and also as media to restore ecological and environmental balance. This has led to the enhancement of cultivation and conservation measures to protect these plants. Simultaneously, stakeholders are also working on, and exploring the alternatives for, the same (Reddy et al. 1986). This entails protecting or conserving not only endangered plants, but also their associated habitats, niches, and native regions, and the culture integral to protecting these plants. Medicinally important plants usually grow in biologically less conducive habitats, grow in small numbers, or take longer to grow. Therefore, the need of the hour is intervention at molecular and genetic levels to lessen their growth period, increase their number or population, and grow them in controlled artificial environments, such as botanical gardens, conservatories, orchidaria, greenhouses, etc., so as to ensure their sufficient availability and management (Ghosal et al. 1986).

4.4 National Forest Policy: Conservation Measures

The National Forest Policy (1988) and the Rio Declaration (1993), along with many other National and International foreign policy documents, have stressed the conservation and protection of biodiversity. India has a rich share of families of flowering plants, with 328 out of the global total of 425 families, comprising 16,000 species. Alarmingly, 3,000 to 4,000 of these are under the threat of extinction. Almost, all the plants available on Earth have some medicinal potential, but only 1,500 are recognized and characterized under the Ayurvedic system (Bisen et al. 2002). GPS, satellite-based surveys and vegetation maps made by the Tropical Forest

Research Institute in Jabalpur (TFRI), research wings of the State Forest Department (SFD), the Botanical Survey of India (BSI), and the Central Institute of Medicinal and Aromatic Plants, Lucknow (CIMAP), etc., have revealed an ominous picture of the loss of genetic plant resources (Bisen et al. 2002).

4.4.1 Conservation of Plants with Medicinal Values

Plant species or varieties with medicinal value, are the worst affected. The restricted distribution of medicinal plants, due to specific habitat requirements, overexploitation of forest resources from pharma industries and others, and the illegal export of such plants, have led them to the brink of extinction, or at least into the endangered zone (Bisen et al. 2002). Around half of the tropical forests that house the majority of floral and fauna diversity are destroyed to accommodate ever-increasing populations, industry, and farming requirements, thus destroying rare herbs and plants with high medicinal values (3,000-4,000 of Indian species) (Bisen et al. 2002). Increasing awareness about protection and enhanced cultivation, both in native habitats and protected artificial habitats, will be the key to replenishment measures (Bisen et al. 2002).

4.4.2 Protected and Notified Plants

The national government has woken up to the crisis of the loss of floral diversity, and declared many plant species and varieties as notified and protected. Many plant species and varieties have been prohibited from export. The TFRI, in Jabalpur has taken the initiative in this regard, and has established a germ-plasm of medicinally important plants found in Indian tropical deciduous forests (Brijlal and Dubey 1964, Sexena 1986). These include medicinal plants of both pharmaceutical and traditional importance (Bisen et al. 2002).

These plants are categorized with respect to their principal plant products, like steroids, flavonoids, glycosides, cathartics, plant gums, and oils and fats, insecticidal and insect repellant plants, and also those falling into the threatened or endangered category. The TFRI in Jabalpur is also encouraging research into traditional medicinal plants, in addition to educating and creating awareness among people regarding the conservation and protection of medicinal plants. It is also imparting training to cultivate plants and herbs of medicinal importance (Sarangthem and Haokip 2010).

4.5 Tuberculosis

Tuberculosis has emerged as a major global health issue. According to estimates of the World Health Organization (WHO), around 33 percent of the global population is afflicted with the *Mycobacterium tuberculosis*, making it a most significant burden on human health. Despite being a totally curable disease (WHO 2015), tuberculosis is a major cause of mortality in the human population across different age groups, gender, class, and strata. Tuberculosis is more common in resource-limited set-ups, hence it is rightly called a 'poor man's disease'. As a result, underdeveloped and developing nations are facing the maximum burden of tuberculosis.

4.5.1 Emergence of Drug Resistant Forms

The burden of tuberculosis is further aggravated by the emergence of drug-resistant strains of *M. tuberculosis*, i.e. multidrug-resistant (MDR) forms, showing resistance to the two best first-line drugs used to treat tuberculosis, i.e. rifampin (RIF) and isoniazid (INH). Extensively drug-resistant (XDR) tuberculosis shows additional resistance to fluoroquinolone (ciprofloxacin, moxifloxicin, etc.), and an injectable drug (kanamycin, capreomycin, or amakacin), the two best classes of second-line drugs (Maitre et al. 2016). The WHO estimates that five percent of new TB cases are MDR, with approximately 10 percent of those actually being XDR. Unfortunately, drugs effective for the treatment of MDR/XDR tuberculosis are still nowhere in sight (Sachdeva et al. 2012, Mishra et al. 2013). The limited number of antibiotics available to treat tuberculosis necessitates rapid diagnosis, not only to reduce the spread of drug-resistant strains, but also to monitor and limit the emergence of new resistant strains.

4.5.2 Mycobacterium tuberculosis

Mycobacterium tuberculosis is a slow-growing bacterial strain, so initial diagnosis takes up to six weeks, with an additional 12 weeks for drug susceptibility profiling (WHO 2010). This largely depends on the techniques available, which is a big limitation for resource-limited developing and underdeveloped countries. Lack of right and timely treatment further aggravates tuberculosis, leading to MDR/XDR TB.

Despite various control efforts, tuberculosis remains a major public health challenge in much of the developing and the transitioning world, with an estimated 9.4 million new cases, and nearly two million deaths in 2009

(WHO 2010). Expenditure on tuberculosis control efforts reached USD five billion in 2011. The emergence of MDR-TB threatens to overwhelm the recent gains in disease control, and faces substantially increased costs, given that it requires lengthy and expensive treatment regimens (CTB 2008).

4.6 Tribal Populations

The Achanakmar-Amarkantak Biosphere Reserve lies between 21° 15' -22° 58' North latitude and 81° 25' - 82 ° 5' longitudes (Tiple et al. 2010). The core region of the biosphere reserve (BR) is situated in the Chhattisgarh state, whereas the buffer and transition zones lie partly in both Madhya Pradesh and Chhattisgarh. Villages like Achanakmar, Chhaparwa, Tilaidabra, and Lanmi, with a population of 7,617 persons lie in the core area (Shukla and Singh 2007, Tiwari et al. 2012). Human population in the core area of the BR varies year on year, probably due to the temporary shifting of persons from one area of core forest to another, or to nearby buffer or transition zones, and occasionally outside the BR, in search of employment/better opportunities. Residents in the BR constitute 48.1 percent of scheduled tribes, and 8.46 percent of scheduled caste populations. The literacy rate is 26.48 percent. There are 27 communities living in different zones of the BR. These are Baiga, Gond, Dhanwar, Kol, Kanwar, Oraon, Sais (Sartgi), Basore, Lonia, Muslim, Sindhi, Brahmin, Rajput, Goswami, Baraith, Kalar, Kumhar, Kewat, Nai, Ahir (Raut), Panika, Sondhiya, Lohar, Sonar, and Baniya. Among these, the Oraon came from the Surguja district and settled in the BR in the early eighties. The major tribes residing in the BR are Baiga, Gond, Kol, Kanwar, Pradhan, and Panka (Prasad and Pandey 1993).

4.6.1 Tuberculosis is Invariably a 'Poor Man's Disease'

Tuberculosis manifests in low nutrition, and poor hygiene and sanitation conditions (Prasad and Pandey 1993). Madhya Pradesh, being home to the largest tribal population, is particularly vulnerable to the combined threat of TB, MDR/XDR-TB, and HIV, which are cause and effect to each other. This poses a huge threat and economic burden to the government exchequer, and most importantly, the loss of precious human resources. Due to its alarming rise, the government of Madhya Pradesh has declared it as a notified disease (Keshavjee and Farmer 2012). Madhya Pradesh state has nearly 90,000 patients, with the addition of 17 MDR-TB cases per month. The state has a poor showing in almost all the reported parameters

related to tuberculosis, and is well above the national average. For example, the Saharia tribe alone in Madhya Pradesh shows a very high prevalence of 1,270 reported cases per lakh population, against the national average of 216 per lakh. Indore alone has reported 200 MDR cases, while the state capital has 37 active cases.

4.6.2 Tribal Population of Madhya Pradesh, India

Madhya Pradesh has a sizeable population of tribal people, who, because of a lack of awareness, education, and socio-economic conditions, fall prey to diseases like tuberculosis, a poor man's disease. At the outset, they avoid medical treatment and resort to black magic, witchcraft, or sometimes traditional medicine, which is not good enough to treat diseases like tuberculosis or its co-associated morbidities, like MDR/XDR-TB and AIDS (Keshavjee and Farmer 2012). Even if patients take the treatments, they leave them midway, becoming a reservoir, developing new resistant strains, and infecting others in the process. Further, tribes, because of their highly conserved gene pool and insular life-style, are not amenable to many treatments and clinical practices that a normal population would be. For instance, some tribes in Andaman and Nicobar islands cannot be vaccinated as it impairs their overall physiology, leading to various morbidities. Tribes of the Amarkantak region have a long history of using 'wonder herbs' of the region to cure them of various morbidities, like tuberculosis (Jain et al. 1985, Saxena 1970, Jain and De Filipps 1991, Keshavjee and Farmer 2012).

4.7 Use of Ethno Medicinal Plants by Traditional Healers of the Amarkantak Region

Table 1 provides an exhaustive list of the medicinal plants used by traditional healers. The table provides complete information on the plants, including taxonomic details, the parts which are utilized for medicinal purposes, and the end product (Figures 1 to 8).

Table 4-1 List of Medicinal plants traditionally use by tribal for the treatment of Tuberculosis

S. No	Name of plants	Scientific name	Family	Availability	Collection area	Useful part	End Product
1.	Adusa	*Adhatoda vasica*	Acanthaceae	All India	Amarkantak	Root bark,stem bark,leaves and flower	Bark powder,whole leaf and paste (vr. Kwath) of flower.
2.	Ankol	*Alangium salvifolium*	Alangiaceae	Central India	Amarkantak hills	Stem bark, extract of flower and fruit.	Bark power, extract of flower and fruit.
3.	Arjun / Kahua	*Terminalia arjuna*	Combretaceae	All India	Amarkantak hills	Stem bark and leaves	Kwath/powder of stem and leaves
4.	Ganger	*Grewa Texan*	Tiliaceae	Indian hills	Amarkantak valley	Mucilage of bark	Mucilage of bark
5.	Amahaldi	*Curcuma amada*	Zingiberceae	Indian forest	Amarkantak valley	Rhizome	Powder/kwath
6.	Kalihaldi	*Curcuma caesia*	Zingiberaceae	Throughout India	Amarkantak hills	Rhizome	Powder/Kwath
7.	AK, Madar	*Calotropis procera*	Asclepiadacea e	Throughout India	Amarkantak valley	Bark of root and stem ands flower.	Powder/Kwath
8.	Koraiya,Kutaj	*Holarrhena antidysentrica*	Apocynaceae	Central India	Amarkantak	Stem bark and fruit	Powder

Fig. 4-1 *Adhatoda vasica Nees* showing stem, leaves and flowers

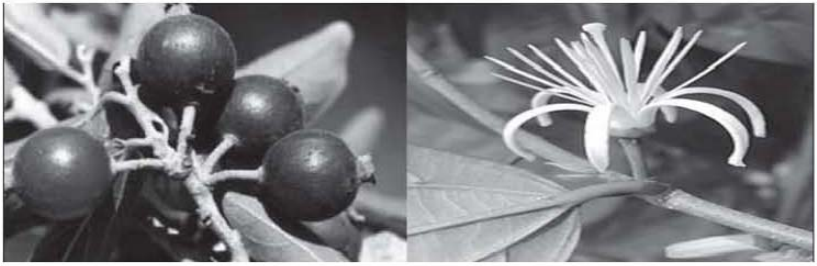

Fig. 4-2 *Alangium salvifolium* L.f. showing stem, leaves, flowers and fruits

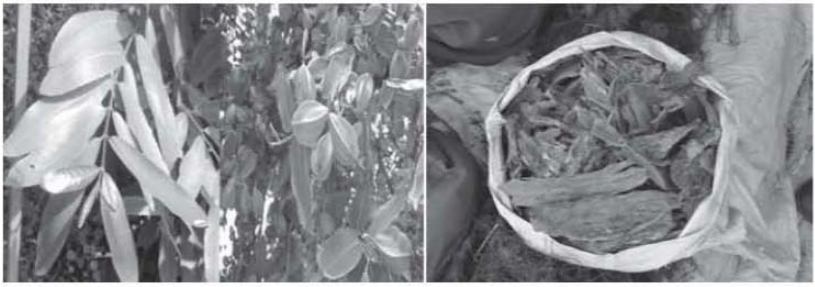

Fig. 4-3 *Terminalia Arjuna* showing bark and leaves

Fig. 4-4 *Grewia tenax* Forsk showing stem, leaves and fruits

Fig. 4-5 *Curcuma amada* Roxb. showing rhizome, leaves and flower

Fig. 4-6 *Curcuma caesia* Roxb. showing pieces of rhizomes and leaves

Fig. 4-7 *Calotropis procera* Ait. showing stem, leaves and flowers

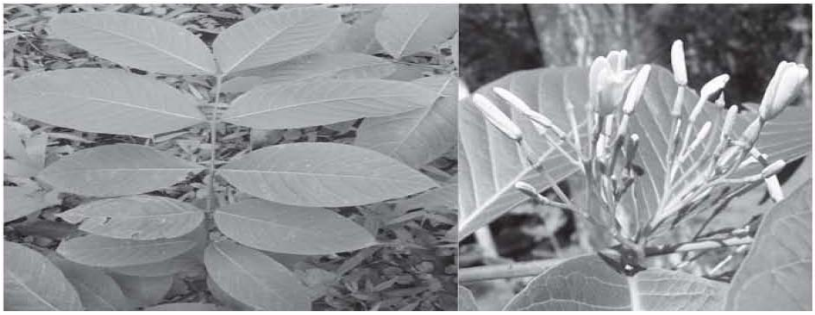

Fig. 4-8 *Holarrhena antidysenterica* Roxb.Ex-Fleming showing stem, leaves and flowers

4.8 Preparation of Herbal Extracts

Bark from roots was utilized for the preparation of extracts from plants like Adusa, Arjuna, Koraiya (Kutaj), Ankol, and Madar, while stem bark was utilized in the case of Bhanvarmal, Arjuna, Koraiya (Kutaj) Ankol, and Madar (Aiyer and Kolammal 1960, 62, 66, Aiyer et al. 1957, Kurup et al. 1979). The rhizomes of Amahaldi, Kalihaldi (Shyanuahaldi), and Jangali adarak (wild zinger), were utilized in the study. The flower of Adusa (Adhatoda), and the seeds of Datura (Datura metel), were also used for treatment regimens (Saxena 1986, Dubey et al. 2004).

The following process was applied for the preparation of herbal extracts/medicines by the tribes/vaidyas, for the treatment of tuberculosis:

(a) The root bark of Adusa, Arjun, Koraiya, Ankol, and Madar was removed by axe or sickle, sheared into small pieces, and dried. The dried root bark lobe was ground into a fine powder (Agarwal et al. 2011).
(b) The stem bark of Adusa, Arjun, Koraiya, Ankol, Madar, and Dhatura was removed by sickle or axe, cut into small pieces, sun-dried, and processed into fine powder. This powder was further mixed with jaggery or honey (Agarwal et al. 2011).
(c) A kwath, prepared with the root bark or stem bark of the Arjuna plant was also used with honey for the treatment of tuberculosis (Maheshwari 1964, Maheshwari et al. 1990).
(d) Chewing of the leaves of Adusa regularly, for up to six months, was recommended as a part of the treatment (Chopra et al. 1992).

4.9 Doses

In all cases, the end product, i.e. fine powder, was mixed with honey or jaggery to prepare the final dose. For children (< 10 years), tablets weighing 1-2gm were given twice a day, while for adults (> 10 years), tablets weighing 3-5gm were given thrice a day, with milk or hot water in both cases.

4.10 Case Study

Around 160 patients, including 115 males and 45 females were observed from July 2014 to May 2016 (Figure 9, Table 2-5). The age of patients ranged from the minimum age of 19 years, to the maximum age of 90 years. Out of 160 patients enrolled/under observation, 144 patients recovered completely within 4-18 months (Figure 9-11), but 16 patients have become relapse cases (Figure 9-11). This could be due to the improper/irregular intake of medicines, and also internal or external factors, or immunity.

Table 4-2 Details of patients enrolled from July 2014 to June 2015 for the treatment via herbal medicine under the supervision of traditional healers

S.No	Age of patients	No. of patient	Male	Female	Initial Symptoms	Duration	Diagnostics	Mode of treatment
1.	19-30	2	2	-	Cough, chest pain losing weight. Respiratory problems.	From one month	Microscopic analysis of sputum and X-ray	General medicines of allopathy
2.	31-40	6	4	2	Cough with blood and chest pain	From two weak	Do	Allopathy
3.	41-50	36	27	9	Cough with blood, loss of weight due to loss of appetite	From one month	Do	Allopathy and homeopathy
4.	51-70	11	8	3	Cough, chest pain, sputum with blood respiratory problems	From one month	Do	Allopathy and herbal.

Table 4-3 Details of patients enrolled from July 2014 to June 2015 along with pathological and dosage details

S.N	Age of patients	No. of patients	Male	Female	Full recovery	Time/duration	Doses /amount	Relapse cases
1.	19-30	2	2	-	2	4-6 Months	**TDS	None
2.	31-40	6	4	2	3	6-10 Months	TDS	3
3.	41-50	36	27	9	15	6-10 Months	TDS	12
4.	51-70	11	8	3	4	6-10 Months	TDS	7

** Thrice in a day

Table 4-4 Details of patients enrolled from July 2015 to May 2016 for the treatment via herbal medicine under the supervision of traditional healers

S.No.	Age of patient	No. of patients	Male	Female	Initial symptoms	Duration	Diagnostics	Mode of treatment
01.	00-20	04	04	None	Respiratory problems and, cough.	Two weeks	Microscopic analysis of sputum and X-ray	Allopthy medicines
02.	21-40	26	17	09	Chest pain, cough with blood	2-3 weeks	Do	Ayurvadic and allopathy medicine
03.	41-60	42	30	12	Respiratory problems, cough with sputum.	3 weeks	Do	Herbal and allopathy
04.	61-90	33	23	10	Respiratory problems, chest pain and sputum with blood.	2-3 weeks	Do	Allopathy and herbal medicines

Table 4-5 Details of patients enrolled from July 2015 to May 2016 along with pathological and dosage details

S.N	Age of patients	No. of patients	Male	Female	Full recovery	Time/duration	Doses /amount	Relapse cases
1.	00-20	4	4	None	4	4-6 Months	**TDS	None
2.	21-40	26	17	9	15	6-8 Months	TDS	11
3.	41-60	42	30	12	27	8-9 Months	TDS	15
4.	61-90	33	23	10	21	8-12 Months	TDS	12

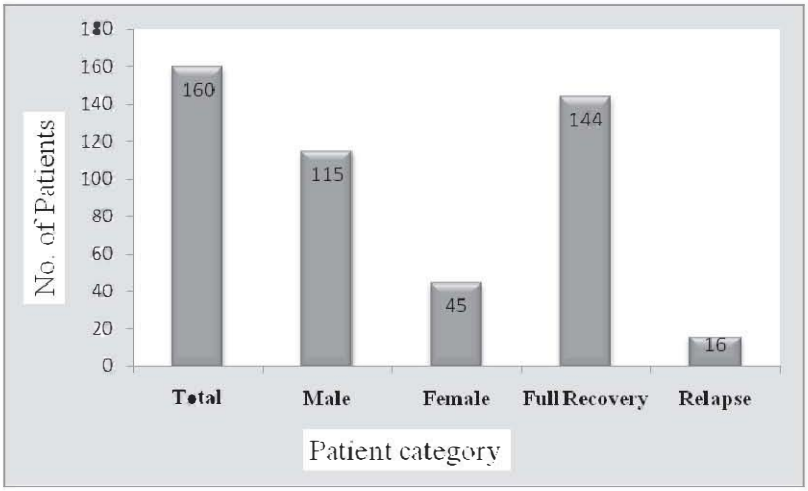

Fig.4- 9 Analysis of Tuberculosis Patients in Amarkantak

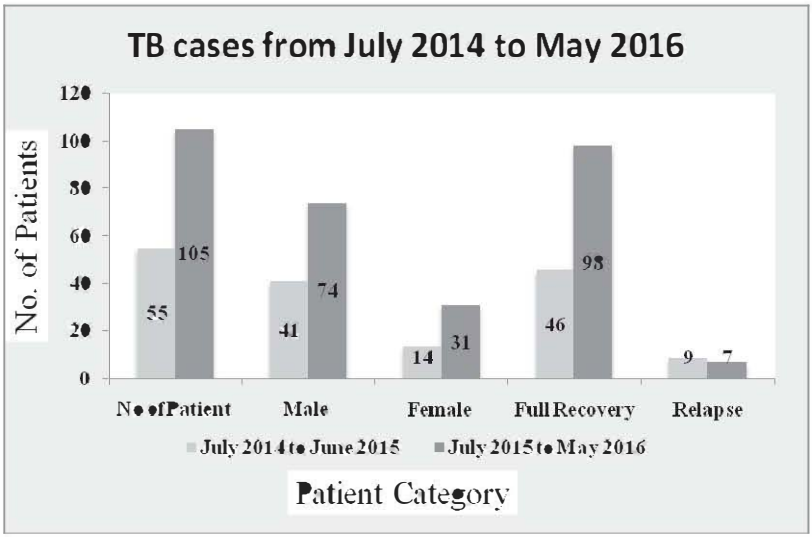

Fig. 4-10 Graphical analysis of cases which occurred during July 2014 to May 2016 included all 160 patients

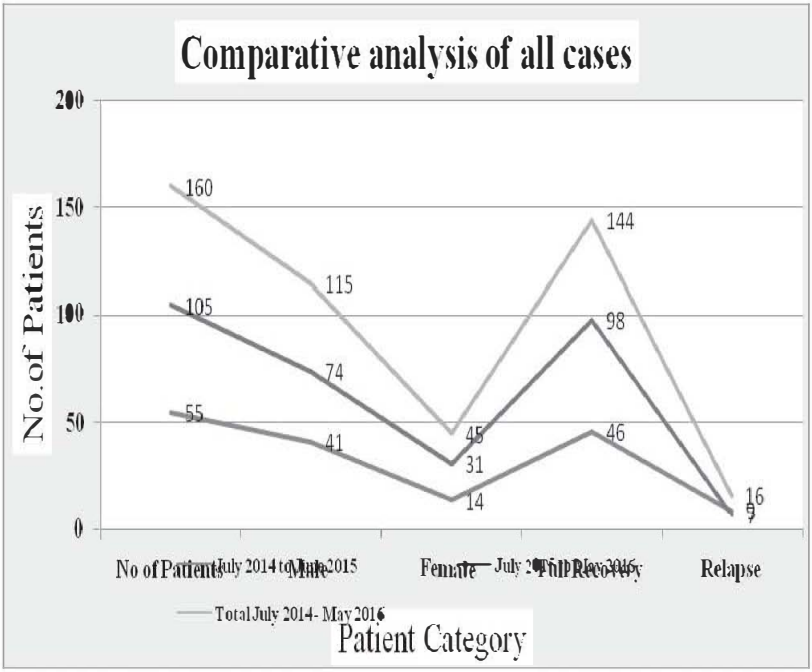

Fig. 4-11 Comparative analysis of all cases included decreasing relapse and increasing full recovery after treatment (showing in green line of total 2014 to 2016) with proper taking of medicine

4.11 Precaution

- Medicine to be taken properly on a regular basis.
- Spicy food to be avoided.
- Three months follow-up for a checkup.

A heightened sense of inferiority, due to illiteracy, poverty, and lack of awareness, means that most of the time tribal peoples refuse to acknowledge that they are suffering from some serious ailment. This prevents them from taking treatment, thus, not only aggravating the disease, but also acting as a potential reservoir of infection.

4.12 Treatment Outcome

Overall 160 people (115 male and 45 female), have enrolled for treatment, and were observed over a period of two years (July 2014 to May 2016). Of these, 144 (90 percent) were fully recovered after completing the treatment, while only 16 (10 percent) relapse cases were reported. These relapse cases were largely due to factors other than the treatment itself (Table 3, 5). Tables 2 and 4 give the number and history of patients, such as their initial symptoms and their persistence, modes of diagnosis and treatment. As is evident from the tables, in all cases, patients have initially gone for the allopathic treatment, along with herbal medicines in a few cases. However, not getting sufficient relief after continued treatment, they opted for the treatment offered by traditional healers based on herbal medicines. Surprisingly, the maximum frequency of TB was seen in the middle-age group (41-50 years), while the minimum was found in adolescents to youth (19-30 years). This could be attributed to the efficiency of the immune system, which weakens as age advances.

Tables 3-5 give the details of the treatment in terms of dosage, duration of treatment and relapse cases if any. As mentioned above, the maximum observed cases of relapse are in the middle age group (41-50 years) and the minimum in the case of adolescents to youth (19-30 years), which is again due to the efficacy of the immune system. A strong immune system follows the treatment regimen more effectively, and without any side effects, compared to a weak immune system. Duration of the treatment further attests to that, as for the youth a treatment duration of 4-6 months was sufficient to cure them of the disease without a single case of relapse. However, the duration of treatment was the maximum in the case of middle age group patients. Older patients have shown better results in terms of efficacy of treatment, as well as relapse cases, although, the immune system is highly compromised in old age, but the results observed here might be due to a decrease in physical activity.

4.12.1 Summary of Treatment Outcome

- Total no. of patients from July 2014 to May 2016 = 160
- Male - 115 (73.71 %)
- Female - 045 (28.84 %)
- Full recovery - 144 (90.00 %)
- Relapse cases - 016 (10.00 %)

4.13 Traditional-Healers

Amarkantak is presumed to be the home of a treasure-trove of medicinally important herbs endemic to the region (Brandis 1874, Kolamal 1979, Dey 1980, Nayar and Sastry 1988, Pal et al. 1999, Biswas and Ghosh 2011). This region is predominantly tribal, occupied by one of the most primitive tribes. The tribal people, due to their ignorance, beliefs, and abject poverty, refuse to follow the modem treatment regimen, and hence resort to hokum/occult practices, sometimes going to traditional healers, who largely use herbal preparations for treatment (Dubey and Bahadur 1996). These traditional healers inherit the systems and treatment protocols from their forefathers, and keep on transmitting them to the next generation, as heirlooms. These systems of treatment are mostly based on experience and beliefs, rather than on the foundation of pure scientific logic.

4.14 Combinatorial Therapy

The reported case study has tried to combine the best of traditional and modem medicine, as the traditional system has no means of accurate diagnosis. Diagnosis in such a system is based on a few observable symptoms which may lead to false positive or false negative results, and to wrong treatment. Therefore, we focussed on modern diagnostic tools, like 'microscopic analysis of the sputum', and chest X-rays to doubly confirm the suspected cases of tuberculosis which were primarily based on the initial symptoms. Once the diagnosis confirmed TB, the patient was taken under the 'traditional treatment regimen', conducted by traditional healers. The treatment protocol followed the age-dependent dosage of the medicines (Sikand and Pamra 1956, Aiyer et al. 1962, Prakash 1981, Raghunathan and Mitra 1982, Suresh et al. 1985, Roy et al. 1992, Rai and Nath 2005, Shafiq et al. 2006).

4.15 Age-Dependent Treatment Outcome

A study by Negina and colleagues (2015) has validated that TB-related deaths are more common in people over the age of 50 years. Elderly people are more prone to developing extra-pulmonary and atypical TB, which is difficult to diagnose. This is further complicated by the side effects of the prescribed allopathic drugs, as the immunity and strength of the body are weakened with advancing age. Older people also develop complications due to the presence of drug-resistant strains. Estimates of the global burden of disease have clearly shown that more than 57 percent

of deaths occurred among older people. For example, in Australasia, this estimate is 92 percent, and it is 93 percent in western Europe, while lesser developed economies show lower percentages, such as East Asia (79 percent) and tropical Latin America (65 percent) (Negina et al. 2015). A number of factors work together to inflate the TB estimates among older people, and diagnosis is one such factor. A meta-analysis by Perez-Guzman and co-workers (1999) has enlisted the difficulties in TB diagnostics. Prominent symptoms like haemoptysis, dysponea, and fever, are less observable in advanced age. Further, the tendency to cough out high quality sputum is also reduced in older people. The Mantoux skin test gives false negative results (Perez-Guzman et al. 1999, Rajagopalan 2001, Kobashi et al. 2008). Older people have compromised immune systems owing to associated chronic co-morbidities, like diabetes, organ transplant, cancer, and immune senescence combined with dementia. People co-infected with tuberculosis and diabetes are several times more likely to fail treatment due to disturbed glycaemic control (Mi et al. 2013, Riza et al. 2014). This is further aggravated by social marginalization, reduced mobility, and financial dependency, which demotivate them to seek curative treatment. Overall, tuberculosis treatment in older people often fails, or yields poor results, due to delayed diagnosis, drug-related complications owing to associated co-morbidities, and financial dependency.

4.16 Conclusions

Accurate and timely diagnosis of TB is the key to its successful treatment. However, slow growth of the TB bacilli delays diagnostic results, and hence treatment. This not only affects patients by aggravating it further to drug-resistant forms, but also makes them potential reservoirs of infection, infecting other people. In addition, prolonged treatment of TB with anti-tubercular drugs has some perceived potential side effects. These issues can be resolved by combining the best of modern diagnostics with traditional healing systems using herbal medicine. The added advantage of such treatment could be the lack of any side effects from herbal medicines, and the lower cost of treatment, relative to allopathic medicines. There is an urgent need for escalation of such studies showing and validating the efficacy of herbal medicine. Nature is replete with the bounty of wonder herbs waiting to be explored and characterized for such types of treatment. Modern advanced research and techniques need to focus on finding ways to empirically validate the protocols of such treatment, so that it can make a quantum leap from the confines of traditional healers to the broader

domain of the modem medicine system, and become an integral part of it, to create a TB-free, global society.

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