CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Incidence of Recurrent Tuberculosis and Associated Factors In Category I and Category II Treatment Regimens

by

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A thesis submitted in partial fulfillment for the degree of Master of Science

in the

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Dedicated to Almighty ALLAH and the Holy Prophet Muhammad (P.B.U.H)and
My Loving Family

CERTIFICATE OF APPROVAL

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Abstract

Tuberculosis is (TB) world deadliest disease, about one third population is suffering from tuberculosis which is caused by *Mycobacterium tuberculosis* bacteria. It is transmitted through the air borne particles not by surface area, a person in close contact with the patient having tuberculosis have more chances to get infection. Gender, age, site of occurrence, categories of treatment and the smear results play key role in the recurrence of tuberculosis. Recurrence of tuberculosis is defined as the second episode of tuberculosis after the completion of first episode, this term is generic and it integrates all kind of relapse or re-infection i.e. recurrence due to the same strain of *Mycobacterium tuberculosis*or due to new strain known as relapse and re-infection respectively. Statistical Package of Social Science (SPSS) software used to analyze the data of tuberculosis patients, to measure the association among factors as well as identify the important factors which are responsible for its recurrence. It was found that the people with age group 20-40 have more chances to get infection due to the environment exposure and those with age group 40-60 are at higher risk for recurrent tuberculosis either due to the presence of any co-infection which suppressed the immune system or due to any other environmental factor i.e. smoking, air pollution, alcoholism etc. it is observed that males have more recurrent tuberculosis as compared to females and males show more smear positive results than females. The chances of recurrence is higher in pulmonary tuberculosis as compared to extra-pulmonary tuberculosis, it is easy to cure tuberculosis if it is detected as earlier as possible. Smear microscopic examination is best technique which is widely used to identify its presence. In this research it has been proved that pulmonary tuberculosis had easily detected with smear positive results while those who have smear negative results are either suffering from extra-pulmonary tuberculosis or from any co-infection which compromises the immune system by which it escape from the host immune system and cannot be easily detected.

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Abbreviations

BMRC	British Medical Research Council
CDC	Center of Disease Control
DNA	Deoxyribonucleic Acid
DOT	MDirect Observed Therapy
DR	Drug Resistance Diseases
HIV	Human Immunodeficiency Virus
MDR-TB	Multi Drug-Resistance Tuberculosis
RFLP	Restriction Fragment Length Polymorphisms
SPSS	Statistical Package of Social Science
TB	Tuberculosis
WGS	World Genome Sequencing
WHO	World Health Organization

Chapter 1

Introduction

1.1 Background

Tuberculosis (TB) is centuries old infectious disease caused by *Mycobacterium tuberculosis*. It majorly affects lungs but other parts of the body can also be affected. The tuberculosis is transmitted through the air droplets containing tuberculosis bacteria, when person having tuberculosis sneeze, cough, sing, laugh and talk in the environment. Therefore, the chances of tuberculosis in close contact of patients are higher. Sometimes it is also observed that the person infected with *Mycobacterium tuberculosis* never develops disease; if it is detected early orproperly treated it could be totally cured.

Tuberculosis is the world's most fatal disease and about one fourth of the world's population is suffering with tuberculosis. In 2017, ten million people around the world were infected with tuberculosis and there were 1.3 million tuberculosis related deaths worldwide [1]. It is a primary killer of people who are HIV infected. Pakistan ranked 5th in occurrence of tuberculosis in worldwide due to high burden of tuberculosis cases. The prevalence of tuberculosis among 100 thousand people is 348 with incidence of 276 and mortality rate of 34 individuals (Tuberculosis Controlled Program, GOP, 2019) [2].

People who are HIV infected are more vulnerable to get active tuberculosis due to their compromise immune system. It is very difficult to detect tuberculosis in patients who are already infected from HIV as the sputum sample mostly shows no infection even when one exists (known as being smear negative). There are more chances of HIV infected individuals to develop extra pulmonary tuberculosis. In addition to this some anti tuberculosis drugs cannot be used along with certain HIV medications [3].

Direct observed therapy OR DOT is tuberculosis management strategy which helps patients to adhere to therapy. It is the practice where skilled or well trained workers observe and document patient's daily intake of medications and motivate to do it regularly. DOT is preferred management strategy suggested by the Centers for Disease Control (CDC) for treatment of tuberculosis disease. DOT can reduce the development of drug resistance, treatment failure or relapse after the end of the treatment [4].

When *Mycobacterium tuberculosis* bacteria replicate, some of them naturally mutate and develop drug resistance against the anti-tuberculosis drugs. Treatment then kills non mutated bacteria leading to the survival of mutated drug-resistant bacteria. In Pakistan during 2017, it is estimated that about 480 thousand people developed drug resistant tuberculosis. People infected with drug-resistant tuberculosis are treated with the second- line drugs which are less safe, more expensive, less effective and take longer to treat the disease. Drug resistant tuberculosis often develops when treatment is interrupted or not completed due to any reason or when appropriate drugs required for treatment are unavailable. Drug resistant tuberculosis can also be transmitted from person to person.

Tuberculosis could be easily controlled by adapting effective infection control programs and early suitable treatment. The major problem which is faced by controlled programs is the development of multi drug resistant strains of *Mycobacterium tuberculosis*; and the recurrent disease especially due to the emergence of drug-resistant strains. The poor adherence to the treatment is becoming a global problem not only affecting developing countries but also affecting the developed

countries. World Health Organization (WHO) reports treatment failure, relapse after completion of treatment and development of multidrug resistance world-wide. Recurrence could be due to relapse disease caused by the same strain or reinfection disease caused by different strain from the initial infection. In most of the cases recurrence occurs due to relapse caused by reactivation or incomplete cure of initial infection. One of the major risk factor for relapse is non- adherence to the initial tuberculosis treatment.

1.2 Tuberculosis Recurrence

The World Health Organization (WHO) in 2017, used the word "relapse" to define all the kind of recurrences. The word relapse itself is collection of recurrences either due to the relapse or due to the reinfection. The two main types of recurrences are the relapse and reinfection both exist as independent forms from each other. The two forms of tuberculosis infection have different treatment outcomes and different treatment regimens [6].

Relapse is mostly due to the incomplete treatment or the treatment failure, there are a lot of reasons behind it e.g. due to the development of drug resistance or due to the poor treatment adherence. It is observed that in case of relapse, the drug resistance has been developed in the strain of *Mycobacterium tuberculosis* and the rate of relapse of tuberculosis is higher in HIV patients, who need extra care and treatment to eliminate *Mycobacterium tuberculosis* [7]. In low-class areas the rate of relapse is higher so it is very difficult to treat with the tuberculosis in these areas. In case of HIV co-infection, relapse is defined as the failure to develop immunity against the second episode after the completion of first episode and thus patient becomes more vulnerable to the reinfection with *Mycobacterium tuberculosis*.

1.3 Pathogenesis of Recurrence

The risk of developing second episode of tuberculosis after reinfection by *Mycobacterium tuberculosis* depends on two things; either on the reinfection of this reinfection breaking down into second tuberculosis episode or on the risk of reinfection after the first episode which is also known as background tuberculosis incidence. It has been shown in different studies that HIV infection is a major risk for reinfection (infection) breaking down into first tuberculosis episode or into a second tuberculosis episode [8].

True relapse can only happen when tuberculosis bacteria persist into the host after the treatment although apparently the disease is cured so that relapse and the failure can be seen when bacteriological cure of first episode is insufficient [9]. In the British Medical Research Council (BMRC); the combine rate of recurrence and the failure was the standard sign to measure the efficacy of the treatment regimens [10]. Treatment plays an important role in the relapse either it is inappropriate treatment regimens or treatment duration both are the main cause of the relapse.

The regimens containing Rifampicin related to small rate of recurrence as compared to the regimens without Rifampicin of long duration. Under routine condition, adequate treatment regimens may not guarantee permanent cure of disease due to the already presence of drug-resistance and thus resulting in high relapse rate [11].

The extent of disease or residual radiological lesions has been seen to be linked with recurrence. Silicosis, advanced age, and being male are also the risk factors of recurrence. True relapse mostly occurs after the treatment completion for the first episode. True relapse may also occur more than fifteen years after first tuberculosis episode. Recurrence due to reinfection may be expected to be a constant risk over time. According to British Medical Research Council (BMRC) trials, the rate of occurrence of recurrence is higher at first six months post-therapy. Some have proposed to define as “Latent Failure” which is any recurrence in first six months after the end of treatment [12].

The identification of the main cause of recurrence is necessary to find out the strains of *Mycobacterium tuberculosis* involve in first and the second episode of tuberculosis. To identify the strains of *Mycobacterium tuberculosis*, the recent genotyping method which rely on the high degree of polymorphism of some of the DNA insertion elements or short repetitive sequences are used. The patterns of the fingerprints show how the specific insertion element is spread throughout the whole genome. The main finger print technique which is widely used is the Restriction Fragment Length Polymorphism (RFLP) [13].

1.4 Categories for Treatment of Tuberculosis

There are many factors involved in the development of drug resistant tuberculosis. One of the most important factors is the previous history of treatment with anti-tuberculosis drugs. Considering the importance of this factor, tuberculosis has been divided into two main groups as following: - Category-I is defined as the newly diagnoses cases of tuberculosis who have never received anti- tuberculosis treatment before. The chance of development of drug-resistant in this category are minimal and they can be started on the routine standard treatment of tuberculosis i.e., four-drug regimens (rifampicin(R), Isoniazid(H), pyrazinamide(Z), ethambutol(E) or streptomycin) and two-drug regimens (rifampicin(R), Isoniazid (H)) [14], [WHO, 2013].

Category-II is for patients who have previously received anti-tuberculosis treatment. This includes treatment failure cases, relapse cases or those who have not completed their treatments and now returned after treatment interruption. The chance of category-II patients to have drug-resistance are very high so they are treated with intensive anti-tuberculosis regimens which includes five drugs in first two months (S,R,H,Z,E), four drugs for next one month (R,H,Z,E) and then three drugs (R,H,Z) for subsequent five months. Raise in the incidence of drug resistant tuberculosis along with the endemic of HIV and increased population movement are big obstacle to control tuberculosis all over the world [15].

1.5 Risk Factors Associated with Recurrence

Recurrent tuberculosis is due to the endogenous reactivation of same strain of *Mycobacterium tuberculosis* (relapse) or the exogenous infection with new strain (re-infection) [16]. Whole Genome Sequencing (WGS) provides scientifically valuable data with advance technologies which have greater importance. As more genome sequence data become available the more association between genotype– phenotype emerges, their effects on disease and transmission will be better defined. The factors associated with the recurrence of tuberculosis helps tuberculosis controlled programs and clinical providers to identify those patients with previous history of tuberculosis and those who have greater risk for recurrence of tuberculosis so that they can explore ways of reducing the risk [17]. For clinical and social reasons, there are many factors which plays an important role in developing recurrences cause emergence of MDR tuberculosis i.e. individuals with mental health issues, those living with HIV and marginalized and susceptible population.

Currently, there are immune biomarkers which predicts the risk of recurrence of tuberculosis; however, in the future, the development of these biomarkers help us to design host directed therapies and clinical management. The optimum duration of treatment which is required to yield suitable risk of recurrence is a key question facing trails in both drug susceptible and multi drug resistance. To strengthen the investigation of the transmission of tuberculosis disease, the researchers, epidemiologists and public health teams should combine clinical, epidemiological and Whole Genome sequencing (WGS) data. Quick diagnosis and treatment of tuberculosis will help us to identify those patients who are at higher risk of recurrence [18, 19]. Recently, World Health Organization (WHO) End Tuberculosis strategy emphasizes the importance of integrated, patient–centered tuberculosis cases. Most importantly, information on progressive and geographical trends of tuberculosis cases and genotyping will be critical to fully understand tuberculosis recurrence and differentiate between reactivation and new infection [20].

1.6 Aims

To identify the rate of recurrence and relapse in the patients is important to understand the reasons or facts which are responsible for its recurrence. It is essential to identify and characterize the disease categories so that it will help to find out more suitable drug for its treatment.

1.7 Objectives

The study is designed with following major objectives:

1. To determine the frequency of pulmonary and extra pulmonary recurrent tuberculosis.
2. To determine the efficacy of category I and category II treatment regimens.
3. To determine the associated factors responsible for recurrence and relapse in Rawalpindi region.

Chapter 2

Literature Review

2.1 Tuberculosis

Individuals may either have active or latent tuberculosis when exposed to the infection. In active infection patient have symptoms and signs made by actively replicating tuberculosis. Pulmonary tuberculosis is contagious and the patients who are suffering from pulmonary tuberculosis have symptoms like cough, chest pain, shortness of breath, weight loss, fever, and night sweats. The individuals who are suffering from the latent infection do not show any signs and symptoms of tuberculosis as well as it is not transmittable; but they are at threat of developing tuberculosis during their lifetime. The time when individual get exposed to *Mycobacterium tuberculosi*s and the development of active infection is called incubation period and it varies from patient to patient [21].

The airborne particles which carry *Mycobacterium tuberculosis* called droplet nuclei are 1-5 microns in diameter. It is transmitted through air, not by surface contact [22]. Infectious droplet nuclei are produced when a person having pulmonary tuberculosis disease coughs, sneezes, shouts or sings. These particles can persist in the air for several hours depending on the environment. Transmission occurs when a person inhales the nuclei droplet containing *Mycobacterium tuberculosis* and it crosses the mouth, nasal passages, upper respiratory track, and

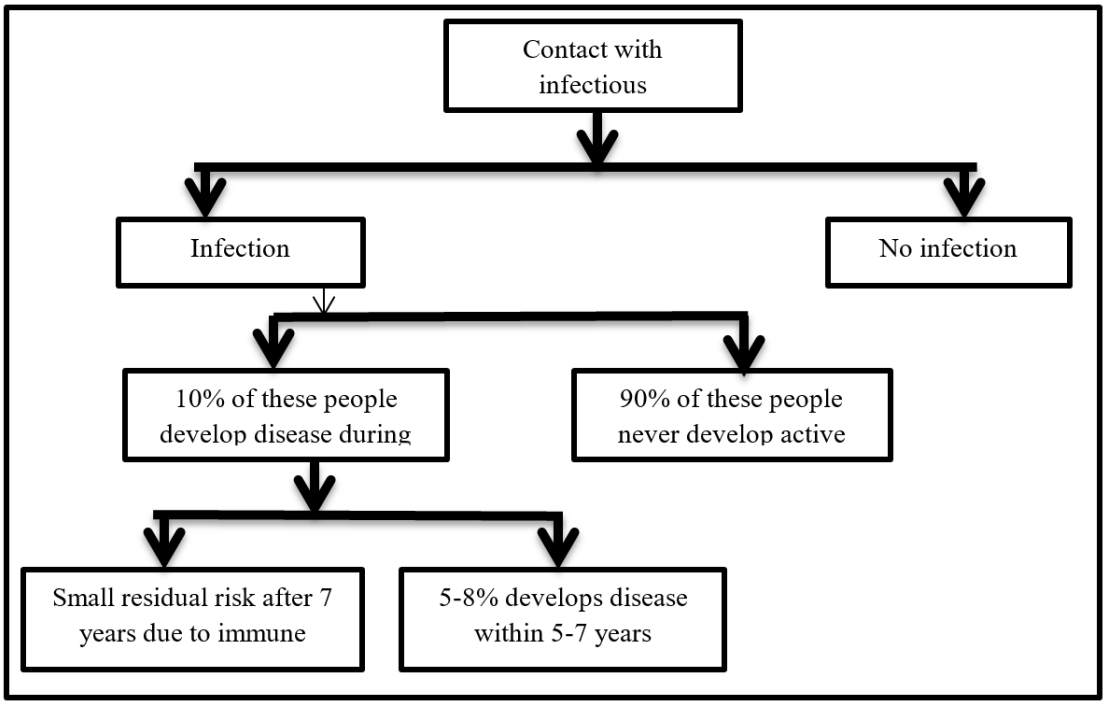

FIGURE 2.1: History of Tuberculosis in Newly Infected Host

bronchi to reach the alveoli of the lungs [23]. Young children are less expected to be infectious with pulmonary tuberculosis disease as compared to adults. This is because children do not produce sputum when they cough but transmission from children can happen. Therefore, children and the adolescents with tuberculosis disease should be measured for infectiousness using the same criteria as adults. These criteria contain presence of cough lasting for 3 weeks or longer, cavitations on chest radiograph, or respiratory track disease with association of lungs, airways or larynx.

2.1.1 Prevalence of Tuberculosis

Tuberculosis occurs in almost every part of the world. Tuberculosis is one of the top ten reasons of death worldwide. In 2017, 1.6 million people died from tuberculosis disease and about 10 million people were ill with tuberculosis (including 0.3 million among people having HIV). In 2017, the largest number of new tuberculosis cases occurred in South-East Asia and Western Pacific regions and about 87% of these cases occurred in the 30 high tuberculosis loaded countries; in which 8 countries

are contributing for the two third of the new tuberculosis cases: India, China, Indonesia, Philippine, Pakistan, Nigeria, Bangladesh and South Africa [24].

Pakistan is on the 5th place among 22 countries with the highest occurrence of the tuberculosis cases. The country is also estimated to have the fourth highest prevalence of multi drug-resistant tuberculosis (MDR-TB) globally. The rate of increase in occurrence of tuberculosis is gradually increasing in Pakistan from 2013-2017 [25].

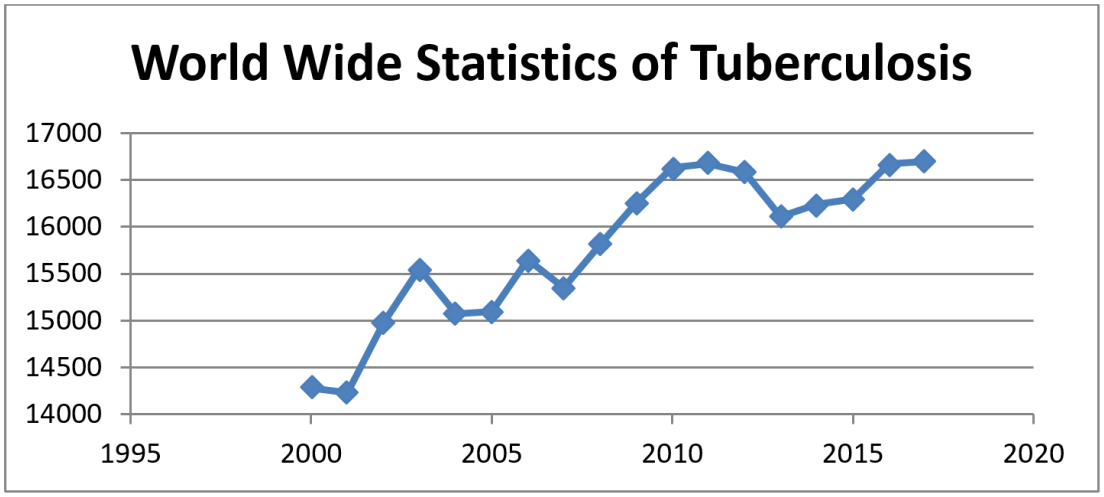

FIGURE 2.2: Statistics of Prevalence of Tuberculosis Worldwide (WHO, 2017)

2.1.2 Diagnosis of Tuberculosis

Tuberculosis is identified by finding the presence of *Mycobacterium tuberculosis* bacteria in the clinical specimen taken from the patient. There are several other diagnostic techniques which are used to investigate the *Mycobacterium tuberculosis* but they cannot confirm its presence. A complete evaluation for tuberculosis must contain a medical history, a physical examination, a chest X-ray and microbiological examination. It may also include tuberculin skin test, other scans, X-rays and surgical biopsy [26].

There are numerous tests available that can be used to diagnose tuberculosis depending on the type of tuberculosis and the resources available for testing. Some of the diagnostic tests are summarized in the figure below:

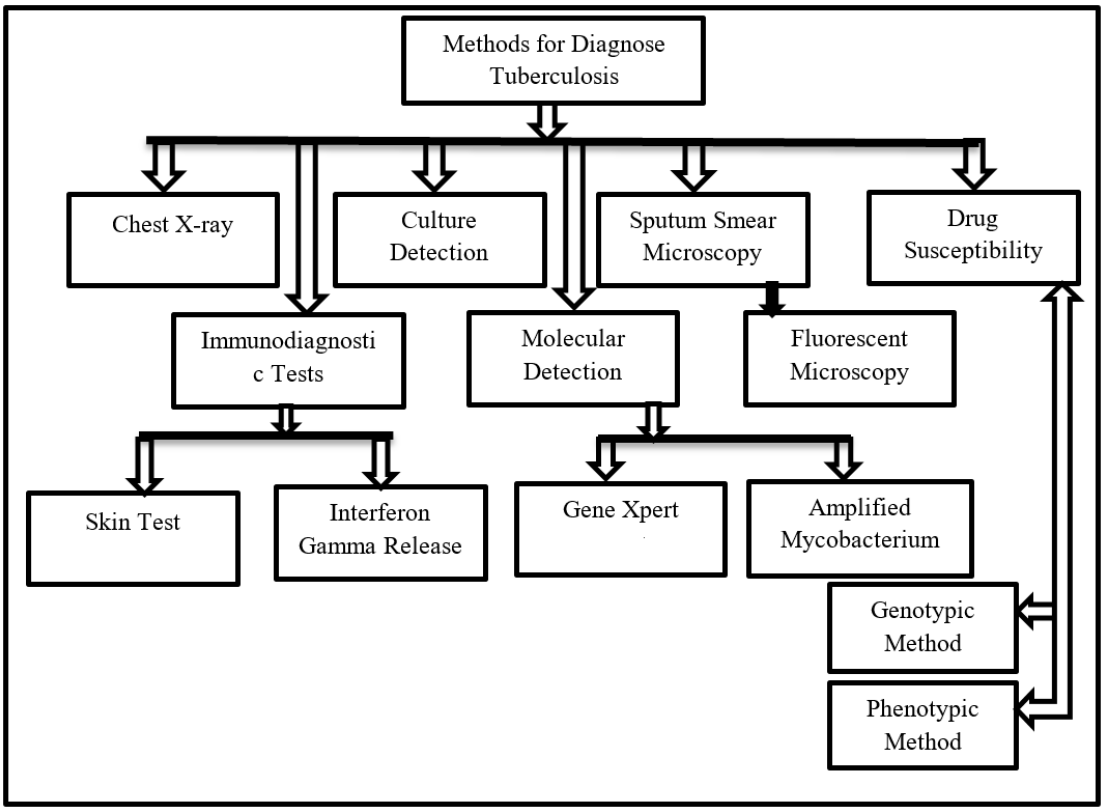

FIGURE 2.3: Methods for Diagnosis of Tuberculosis

2.1.3 Types of Tuberculosis

Tuberculosis is divided into two main categories depending on the site of occurrence i.e., pulmonary tuberculosis and extra pulmonary tuberculosis. Pulmonary tuberculosis mainly affects the lungs. Extra pulmonary tuberculosis is a type of tuberculosis which invades into other organs of the body than the lungs. Thus occurs in 15-20% of active cases causing other kinds of tuberculosis. On the other hand, tuberculosis is divided into two main categories on the basis of their onset i.e. active tuberculosis and the latent infection. Active tuberculosis is an illness in which the tuberculosis bacteria is multiplying and invading into the different organs of the body along with showing proper signs and symptoms of disease while in latent infection the tuberculosis bacteria is present in the body of the host, it is linked with the damage at cellular and tissue level and also don't show any signs and symptoms related to the disease. Although *Mycobacterium tuberculosis* is present in the body but any medical test cannot confirm its presence at this level. [27].

2.2 Recurrence of Tuberculosis

Recurrent tuberculosis is defined as the diagnosis of the successive episode of tuberculosis after the completion of treatment of first episode of tuberculosis. Recurrence mostly occurs when the first episode of disease is completely cured. The term cure is defined by the World Health Organization (WHO) as the negative samples of the sputum tests taken from the last month of treatment [28]. As recurrence of tuberculosis is second episode of tuberculosis happening after the first episode of tuberculosis has been completely cured. It has been challenging to find out that the subsequent episode of tuberculosis disease is due to endogenous reactivation of previous infection, or to a new reinfection. Molecular techniques have been developed which shows that the recurrence are due to the reinfection by a different strain, rather than relapse with the same strain that has causes first episode. The fact that the first episode of tuberculosis does not protect from the second episode caused by different strain has implication for vaccine development.

2.2.1 Relapse

Relapse is the second episode of tuberculosis due to the same infecting strain of *Mycobacterium tuberculosis* in the previous episode determined by the genotypic methods [29]. It is reported that *Mycobacterium tuberculosis* strains are homogenous in nature as well as in the rate of transmission [30].

2.2.2 Reinfection

Reinfection is the second episode of tuberculosis disease due to the new strain of *Mycobacterium tuberculosis* that is different from the infecting strain of previous episode [31]. It is very difficult to identify the main cause of reinfection in high endemic areas because patients may expose to the re-infected strain of *Mycobacterium tuberculosis* which is the cause of primary infection [32].

2.3 Epidemiology of Tuberculosis

At some extent the data about the treatment completion of recurrent tuberculosis is still missing. The average estimation of recurrent tuberculosis is 2290/ 100000 person–year cases [33]. The rate is high as 7850/ 100000 person-year cases in high occurrence settings. The recurrence rate of 3010 and 2290 per hundred thousand person-year following 6 & 12 months of treatment under measured trails reported by Punjabi et al. investigated in 32 studies [33]. They also reported that rates were high in countries with higher tuberculosis occurrence and at observational studies rates were high as compared to the controlled trails. For specific regions the recurrence rates are increasing; many of which are delineated as high burden countries by the World Health Organization (WHO). The recurrence rate of 24.4% cases in HIV-positive individuals, while 4.7% in HIV-negative individuals are reported by Glymm et al. [34]. Charalambous et al. reported the overall recurrence rate of 7.89%. The recurrence rate was 8.86% cases of HIV – positive individuals compared to 3.35% cases in HIV negative individuals [35]. Narayanan et al. reported the cohort of 306 patients from South India which was HIV/ TB co-infected; the recurrence rate was 14%. 88% of recurrent infections were due to re-infection. Sun et al. reported the rate of recurrence of 65% cases of patients with drug-resistant tuberculosis and 35% cases for patients with drug sensitive tuberculosis [36]. Luzze et al. reported in the cohort of Uganda, that the overall recurrence rate is 8.4%. They also reported that in HIV positive individuals recurrence rate was higher which were 9.4% compare to 6.7% in HIV-negative individuals [37]. The recurrence rate of 8.6% in the cohort of 244 patients in Vietnamese reported by Vree et al. [38]. Datiko et al. reported the recurrence rate of one case per hundred person-year in cohort of Ethiopia. Reported in Iran recurrence rate of 8.3% reported by MoosaZadeh et al. [39]. The frequency of recurrence in England and Wales as 4.1% in HIV-positive individuals reported by Crofts et al. [40]. (Table 2.1).

TABLE 2.1: Worldwide Studies on Recurrences

Country	No. of Patients Analyzed	Recurrences (Total)	Reinfection (%)	References
Kenya	196	11	1(33)	Hawken et al. (1993)
Hong Kong	NA	Not reported	5(12)	Das et al. (1993)
Kenya	NA	Not reported	1(20)	Godfrey-Faussett et al. (1994)
India	NA	30	3(23)	Das et al. (1995)
India	52	44	9(31)	Sahadevan et al. (1995)
USA	NA	2	1(100)	EL-Sade et al. (1998)
USA	71	8	0(0)	Vernon et al. (1999)
Rawalpindi, Pakistan	NA	50	0(0)	Khurram et all. (2009)
South Africa	698	48	12(75)	Van Rie at al. (1999)
Uganda	291	17	0(0)	Johnson et al. (2000)
Brazil	32	12	3(25)	Lourenco et al. (2000)
Gran Canaria Island	92	23	8(44)	Caminero et al. (2001)

Table 2.1 continued from previous page

Country	No. of Patients Analyzed	Recurrences (Total)	Reinfection (%)	References
Italy	2127	32	5(16)	Bandera et al. (2001)
South Africa	65	65	14(36)	Sonnenberg et al. (2001)
Spain	2567	172	14(33)	Gracia de Viedma et al. (2002)
Uganda	1100	40	9(23)	Fitzpatrick et al.(2002)
Vietnam	2901	168	0(0)	Lan et al. (2002)
USA	Not reported	100	8(21)	El Sahly et al. (2003)
Canda,USA	1244	79	3(4)	Jasmer et al. (2004)
South Africa	447	61	24(77)	Verver et al. (2005)
South Africa	87	9	1(25)	Scaaf et al. (2005)
China	202	54	32(62)	Shen et al. (2006)
Spain	645	20	1(13)	Cacho et al. (2007)
South Africa	609	57	11(69)	Charalambous et al. (2008)
India	Not reported	74	44(92)	Narayanan et al. (2009)

Table 2.1 continued from previous page

Country	No. of Patients Analyzed	Recurrences (Total)	Reinfection (%)	References
Denmark	4154	73	19(26)	Bang et al. (2009)
South Africa	309	203	66(51)	Marx et al. (2010)
Malawi	584	53	13(33)	Crampin et al. (2010)
Saudi Arabia	Not reported	223	39(17)	Vargese et al. (2012)
Malaysia, South Africa, Thailand	Not reported	50	3(9)	Bryant et al. (2013)
Malawi	1471	139	20(26)	Guerra-Assuno et al. (2014)
USA	3039	136	20(15)	Interrante et al. (2015)
Italy	4682	83	19(23)	Schiroli et al. (2015)—
Finland	8299	48	3(14)	Korhonen et al. (2016)
China	13417	710	59(42)	Shen et al. (2017)
Southern Africa	Not reported	51	3(9)	Whitney et al. (2017)

2.4 Risk Factors of Recurrent Tuberculosis

2.4.1 Treatment Response: Relapse

The most general cause of relapse is the irregular intake of medicines which usually cause incomplete cure. There are few risk factors which play an important role for recurrent tuberculosis infections i.e. poor treatment regimens and adherence, drug resistance, regimens with inappropriate period, inadequate drug choice, low bactericidal strength all attribute to recurrence. The drugs susceptibility testing plays an important role as well. The use of standardize treatment regimens without drugs susceptibility testing contribute to inappropriate regimes choice. Furthermore, it contribute to the development of drug resistance and relapse [41, 42].

Different studies had been reported the use of thiacetazone containing regimens and the relationship between the risk and the treatment regimens for recurrent tuberculosis. Thiacetazone is also an anti-tubercular drug that was used with isoniazed for the treatment of tuberculosis. Further studies substitute this drug with the Rifampicin that is widely used in standardized regimes now days. Poor adherence to anti-tuberculosis treatment is also the cause of development of drug resistance has also been reported for the relapse [43]. The problems related with the regimen choice and adherence include insufficient drug supply as well as drugs stock out and these are associated with the poorly resources high tuberculosis endemic countries. The risk of developing drug resistance is also linked with the recurrent tuberculosis; it has been reported that the increase in development of Refamycin-resistant tuberculosis in patients is due to receiving Refamycin based direct observed therapy. The important risk factors involve in the development of Refamycin resistance-acquisition is HIV co-infections with advanced immunosuppression [44].

Chiang et al. reported 33.3% prevalence of overall drug resistance and 12.9% prevalence of MDR-tuberculosis in a cohort of 93 relapse patients. Similarly, the recurrent rate of 65/1000 and 35/1000 percent-year, in a cohort of 100 multi drug

resistant and 150 non-multi drug-resistant tuberculosis patients respectively were reported by sun et al. [45]. The prevalence of drug resistance in relapse patients is increasing due to inefficacy of standardized treatment regimes and the treatment duration [46].

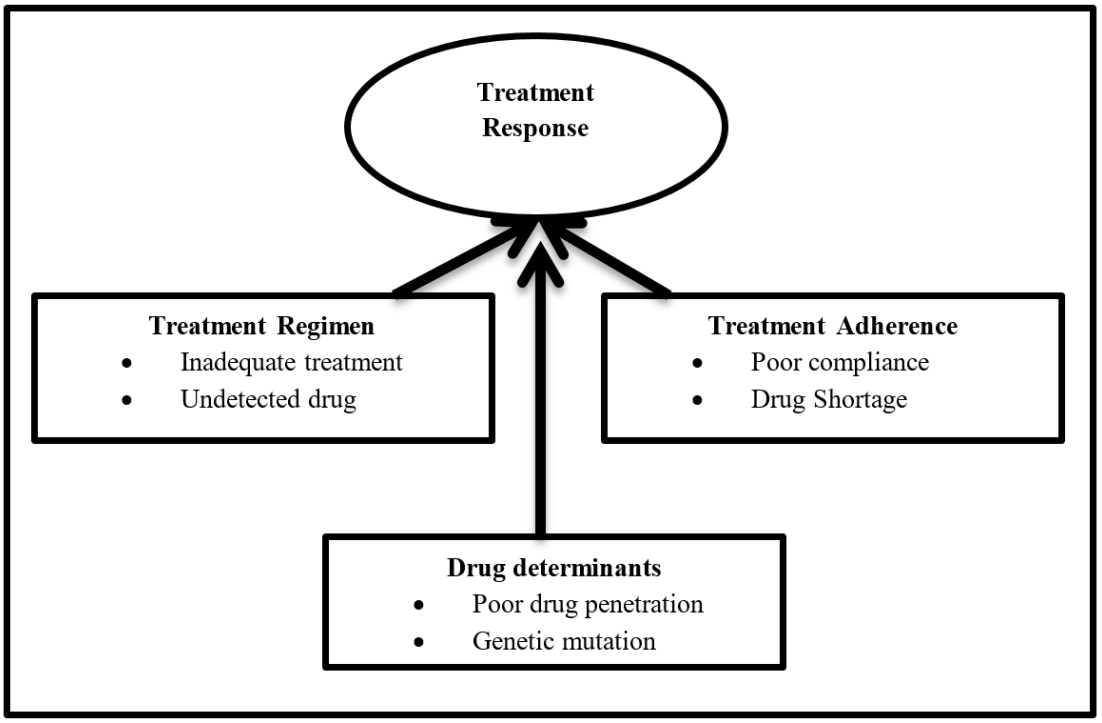

FIGURE 2.4: Risk Factors Associated with Treatment Response

2.4.2 Individual Vulnerability

2.4.2.1 HIV Infection

It is observed that HIV co-infection has been related to increase in the rate of recurrent tuberculosis disease especially in those countries where the rate of occurrence of tuberculosis is very high [47]. Tuberculosis relapse were similar with HIV-positive or negative individuals, it is exposed by the cohort study in Malavi [47]. In contrast, numerous studies have been reported that there is higher incidence of recurrent tuberculosis in HIV positive patients as compared to HIV negative patients [48].

In some studies, it is reported that the recurrent rate is higher in the HIV-infected compared to HIV-uninfected individuals. The study in analysis that reported important association in a multi variate analysis was presented by Polido et al. A complementary explanation is that the patient who is already suffering from compromise immune system may die from any other disease before getting the infection of recurrent tuberculosis [49]. It was reported in different studies that the most powerful predictor of tuberculosis recurrence is anti-tuberculosis treatment for less than 37 weeks. In a review of two studies, Chaisson et al. also reported the rate of recurrent tuberculosis between HIV infected patients and HIV uninfected patients [50, 51].

Narayanan et al. reported in an Indian cohort that there is higher rate of recurrent tuberculosis in HIV-infected individuals as compared to HIV-unifected individuals; 14% versus 9% [52]. Furthermore, 88% rate of reinfection was reported in HIV-infected while only 9% recurrence of tuberculosis was found in HIV un-infected individuals. The patients with co-infection with HIV were related with development of drug resistance as well as more clustering of strains [53]

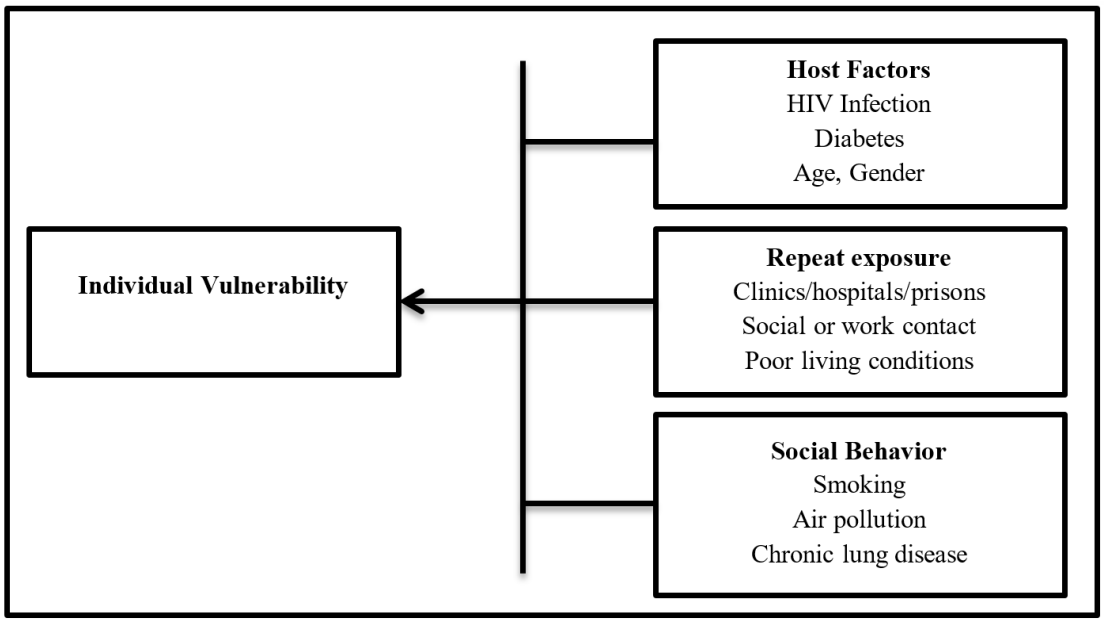

FIGURE 2.5: Risk Factors Associated with Individual Vulnerability

2.4.2.2 Lung Damage

The presence of cavities showing severity of lung disease is an important factor for recurrent tuberculosis. It has been reported that the lung cavities is a dominant associate of recurrent disease. It was reported by Sonnanen Berg et al. that residual cavities was a risk factor for tuberculosis relapse in a study of south African mine workers. The link among residual cavities and the recurrent diseases is the poor diffusion of anti-tuberculosis drug into the cavity. It is reported that the strains of *Mycobacterium tuberculosis* may have increased tendency for the infection of previously damaged tissues [54]. The residual lung cavity and the recurrent tuberculosis link play key role in investigation; to know about the number of cavities before and at the end of treatment of tuberculosis is a predictor of recurrent tuberculosis. However, there is no proper method to know the exact amount of lung damage; still it is needed to establish those measuring techniques which help to identify the tissue of lungs which are involved in the damage [55].

Mallory et al. has divided lung tissues into three parts. He reports dose-response relationship between the numbers of lung zone with fibrosis [56]. Tam et al. reported in their study, the score of lung involvement is determined by the total area of lung tissue with lesions [57].

2.4.2.3 Diabetes Mellitus

Diabetes mellitus plays an important role in tuberculosis reinfection and relapse infection after the treatment completion. It has been reported that the diabetic patients are at higher risk for developing pulmonary tuberculosis as compared to non diabetics [58]. There are several studies which state that diabetes mellitus co-morbidity badly affects tuberculosis treatments outcome. In addition to the interaction between diabetes mellitus and tuberculosis, the occurrence of diabetes mellitus is increasing day by day in multidrug-resistant tuberculosis which is approximately 10-23% of MDR-tuberculosis patients having diabetes mellitus

[59]. A suggested hypothesis for the link between diabetes mellitus and multidrug-resistant tuberculosis is that altered immunity with diabetes mellitus affects the transmission of multidrug-resistant tuberculosis (MDR-TB) as with other immunodeficiency diseases. A latest systematic review implied that the tendency of diabetes mellitus and tuberculosis patients according to the genotype of the infected *Mycobacterium tuberculosis* strain was quantitavely accessed. While the meta analysis of 4076 patients analyzed, 13% had diabetes mellitus and 27% of these patients isolates displaying clustering failed to define the transmission pattern of tuberculosis in diabetes mellitus [60]. These highlighted the requirement of monitoring diabetes mellitus patients who have completed their treatment for incident tuberculosis. In an addition, management for diabetes mellitus tuberculosis patients is very important to reduce the relapse in these patients [61].

2.4.2.4 Extreme of Age

It has been reported that individuals with age between 15 and 44 years are at higher risk of recurrent tuberculosis while the individuals under 15 and over 65 years of age have low risk of recurrent tuberculosis [62]. Age does not have any direct association with the treatment but by default it is linked with the treatment regimens and treatment outcomes; however, no exact age is defined with the recurrence of tuberculosis.

It is hypothesized that there is higher rate of treatment completion in children due to the lower bacterial load as well as due to extra care and attention. There are very few data available on the recurrence of tuberculosis in children. Scaaf et al. reported in a cohort of 87 children that out of 87 children, 9 have second episode of tuberculosis and 2 of which had a third episode of confirmed tuberculosis while 11 recurrence of tuberculosis has been reported in the study. Due to non-availability of clinical isolates of the first episode of tuberculosis in few cases, study could not be completed in some areas [63].

2.4.3 Social Risk Factors

2.4.3.1 Tobacco Smoking

The tobacco smoke is linked with increased risk of tuberculosis infection as compare to non-smokers reviewed by Line et al. The risk of developing active tuberculosis and the rate of mortality increased with smoking [64]. It has been reported that smoking alters the lung immune response to *Mycobacterium tuberculosis*, contributing the higher vulnerability to individual tuberculosis infection as well as it affects bacteriological response and the treatment outcomes as well as the relapse in tuberculosis [65].

The chronic exposure to the pollutants and the tobacco harms the normal clearance of secretions from the surface of bronchial mucosal surfaces and thus allow *Mycobacterium tuberculosis* to escape from the host immune defense. By reducing phagocytic ability of the cells smoke hinders the activity of alveolar macrophages. Lung et al. reported in their study that the impact of smoking on the treatment outcomes of tuberculosis patients by monitoring 16435 patients getting anti-tuberculosis treatments at the Chest Clinic in the Hong Kong. The negative treatment outcomes of tuberculosis due to smoking were 16.7% [66]. 426 cases of relapse were detected, among 13349 patients who were effectively treated for tuberculosis. They reported clear gradient ratios of relapse risk from non-smokers to previous smokers and current smokers; (12.2%, previous smokers 7.2%) with an overall population attribute the risk of 19.4%.

2.4.3.2 Air Pollution

In addition to the tobacco, the different environmental pollutant also plays a very important role in developing the risk of tuberculosis. The quality of air which is impacted by atmospheric pollution and the presence of different amount of gases i.e. carbon monoxide have been reported to persuade the bacillary reactivation and to increase the occurrence of tuberculosis. In Brazil, air pollution is directly

linked with occurrence of tuberculosis reported by De Castro Fernandes et al. The association between the concentration of smoke, suspended particulate matter and tuberculosis in relation to nitric oxide and carbon dioxide levels has been reported by different studies conducted in US and Russia [67].

In Taiwan, the air pollution is produced by traffic directly linked to sulphur dioxide, ozone and carbon monoxide was linked to culture-confirmed tuberculosis. Similarly, in South Korea, the study was conducted and it exposed that the exposure of sulphur dioxide increased the risk of tuberculosis by 7%. In homes, air pollution is due the use of solid fuels for cooking which also plays a role as a risk factor for tuberculosis disease. The role of these factors in recurrent tuberculosis needs further study and investigation [68].

2.4.4 Repeat Exposure: Reinfection

Recurrent tuberculosis contribute to the significant proportion of the tuberculosis cases in tuberculosis controlled programs and it also contributed to the transmission of tuberculosis infection in close contact environment i.e. home community, offices etc. due to the high rate of occurrence of tuberculosis, the recurrence is mostly contributed as the reinfection with up to 75% of cases [69].

High rate of reinfection contributed to the urgent need for its identification and the treatment of tuberculosis as well as it has important consequences for tuberculosis control programs to reduce the transmission of tuberculosis in the environment. Co-morbidities i.e. HIV and diabetes reduce the immunity of the patient to become vulnerable to get infection [70]. Recent studies show that the transmission occurs only at community not within the household. A recent report, in South Africa on extensively drug-resistant tuberculosis (XDR-TB) cohort describe that 19% patients with secondary case of tuberculosis were discharged [71].

In the KwaZulu-Natal Province of South Africa, Shah et al. reported that the XDR tuberculosis was related to the disease transmission as opposed to an inappropriate drug treatment for MDR-tuberculosis [72, 73]. These studies highlighted that

epidemic control requires an increased focus on interrupting the rate of transmission of the disease as well as established community-bases containment strategies [74].

Chapter 3

Materials and Methods

This chapter includes the methodological steps and strategies used to meet the objective of the study.

3.1 Study Population

The present study was conducted in Rawalpindi region of Punjab, Pakistan. Government of Pakistan has considered tuberculosis as national emergency since 2001. All the hospitals including tehsil level hospitals have tuberculosis treatment facilities. We have targeted the tuberculosis patients registered in Tuberculosis Centers and getting the anti-tuberculosis treatment. During this study, various centers were contacted and data about patients were acquired. The Tehsil Headquarter Hospital Kahuta was willing to share their data of tuberculosis patients with us only for the purpose of research.

According to the present retrospective study; total 1500 TB patients were enrolled from December 2017 to March 2018, in which complete information of 1452 patients were registered in TB control centre. Different information i.e. initial diagnostic information, sociodemographic characteristics as well as information of occurrence of disease, that the patient were newly diagnosed or have any previous history were collected from THQ hospital Kahuta. All the information took from

patients by the staff of TB control centre, THQ hospital Kahuta at the time of registration. TB control centre had proper registers where they entered patients' information regarding their disease and all the other related information.

Study population includes patients of tuberculosis on anti-tuberculosis medication and registered in treatment facilities. We have included patients with different age groups with both pulmonary or extra pulmonary cases were considered, along with their sputum smear status. Data was acquired with approval from the center in charge and with consensus that all the information will remain confidential and will be used only for research purpose. Personal information of the patients will not be disclosed what so ever, only clinical information associated with tuberculosis onset and reoccurrence as discussed in Literature Review will be used.

There were the different standard parameters of Tuberculosis Control Centers according to which the patients have registered and enrolled for getting anti tuberculosis treatment as following:

- Gender
- Age
- Treatment Category (Category I or Category II)
- Site of Occurrence (Pulmonary or Extra-pulmonary tuberculosis)
- Sputum smear microscopy results (Positive or Negative)
- Type of Occurrence (new or Relapse)

3.2 Ethical Approval

This research was ethically approved from bioethical review committee Department of Bioinformatics and Biosciences, Capital University of Science and Technology. Informed patient consent was also prescribed compulsory before data collection.

3.3 Inclusion and Exclusion Criteria

Inclusion criteria were the patients enrolled in tuberculosis control program during 2017-2018 at tehsil headquarter Kahuta. Patients with incomplete or insufficient data were excluded from the study. The data was collected based on literature survey in standard forms designed by TB control program.

3.4 Sampling and Testing Equipments

There are the different methods used for the diagnosis of tuberculosis but the two main tests which are practicing in TB centers of Rawalpindi region are sputum smear microscopy and the chest X-ray.

3.4.1 Sputum Smear Microscopy

Sputum smear microscopy is the first line test for the diagnosis of tuberculosis. It is cost effective and less time consuming test mainly used for the diagnosis of pulmonary tuberculosis. All the Basic Management Units (BMU's) and Tehsil Headquarter units (THQ's) have the facility of sputum smear microcopy test. It is used to diagnosed the presence of *Mycobacterium tuberculosis* in the smear which ensures that the patient have tuberculosis. Acid-fast staining is mostly used to diagnose the presence of TB bacteria in smear as *Mycobacterium tuberculosis* is acid fast bacteria.

3.4.2 Chest X-ray

Chest X-ray is also used for the diagnosis of pulmonary tuberculosis but it is not the best first line diagnosis test. It is mostly done for the patients who have smear negative and need to rule out the smear negative pulmonary tuberculosis. The patients who are diagnosed with sputum smear don't need chest X-ray. Chest

X-ray indicated the presence of tuberculosis but it is not specific. The method which is used by TB Control Program for targeting the smear negative pulmonary tuberculosis is as follows:

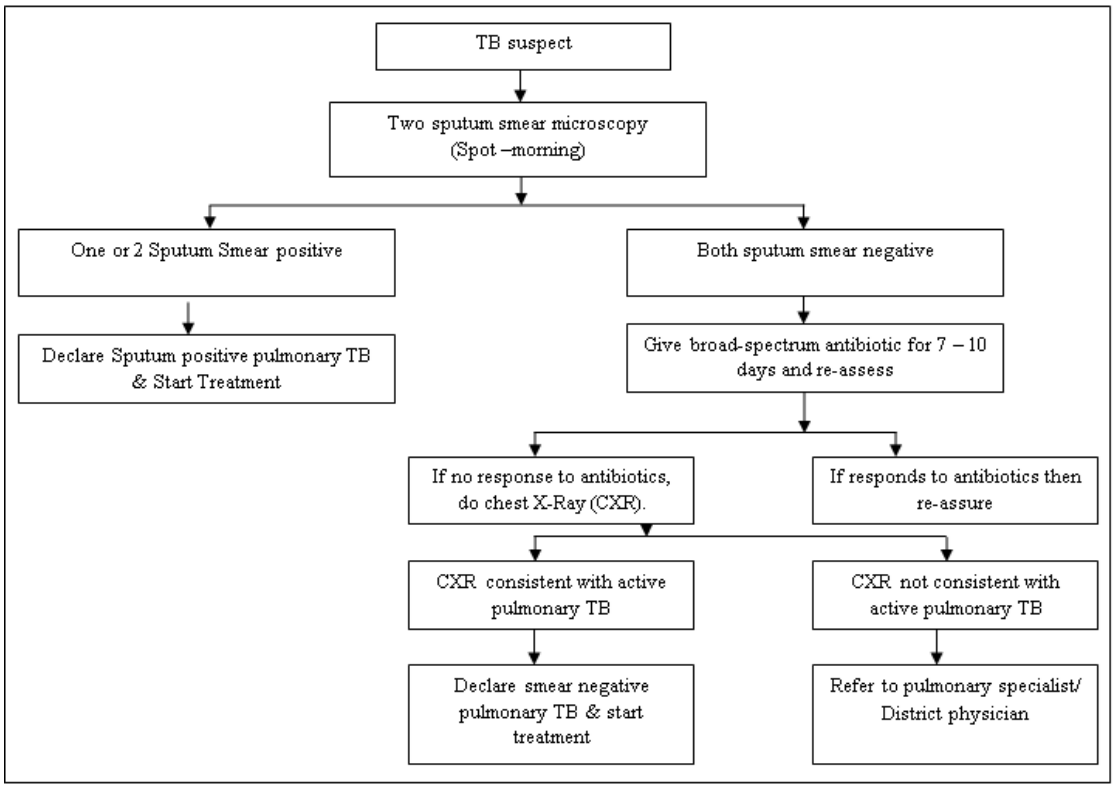

FIGURE 3.1: Management of TB Suspect

3.5 Data Acquisition

Different types of data were collected on the basis of which statistical measurements has been done. List of the data is given below:

3.5.1 Treatment Category

The patients were categorize into two different categories on the basis of their treatment either they are the Category I patients who don't have drug resistance in them and get the first line of anti tuberculosis drugs or either they are Category II patients have developed drug resistance in them and treated with the second

line of anti-tuberculosis drugs. Category I is usually assign to the new and relapse patients diagnosed with pulmonary tuberculosis with smear positive results while category II is assign to relapse cases with smear positive or negative results for pulmonary or extra-pulmonary tuberculosis. According to National TB control Program centre; TB patients are put into two main categories on the basis of smear results as shown in the table 3.1.

TABLE 3.1: Categories of patients on the basis of smear results

Smear Results	**Disease Classification**	**Patient Type**	**Category**
Positive	Pulmonary tuberculosis	New	Category I
		Re-treatment, Relapse Rx.after failure Rx.after default Other (S+ only)	Category II
Negative	Pulmonary or Extra-pulmonary tuberculosis	New & Others (S- only)	Category I

3.5.2 Site of Infection

The TB Control Centers recorded the information of the site of infection at the time of registration of patient for anti tuberculosis treatment that either the patient has pulmonary tuberculosis or extra pulmonary tuberculosis. Tb control centers usually use the history of TB drug intake in past to decide the patient type; pulmonary tuberculosis or extra-pulmonary tuberculosis.

3.5.3 Type of Patient

The Tuberculosis Control Centers also recorded the information of the type of the patient either he or she is new patient first time diagnosed with tuberculosis or relapse patient diagnosed the second episode of tuberculosis after the completion of first episode of tuberculosis.

3.5.4 Sputum Smear Test

Sputum smear microscopy test is the widely used test in Tuberculosis Control Centers in Rawalpindi. Basically when patient get enrolled for taking anti tuberculosis treatment the management staff took the sputum of the patient for the microscopic examination to diagnose the presence of *Mycobacterium tuberculosis* in the sputum which confirms that patient have tuberculosis; sometimes it does not identify the presence of tuberculosis bacteria in the sputum smear and results smear negative in case of newly diagnosed cases or in relapsed cases.

3.5.5 Gene Xpert

The Xpert MTB/ RIF is catridge-based nucleic acid amplification test. It is molecular tests for diagnosis of tuberculosis by detecting the presence of *Mycobacterium tuberculosis* as well as it identifies the resistance to one of the most widely used anti-tuberculosis drug i.e. rifampicin simultaneously. It was introduced in Pakistan 2-3 years ago at certain scale but now it is being used at all district levels, to detect the presence of TB bacteria along with resistance to anti-tuberculosis drugs.

3.5.6 Statistical Analysis

The chi-square and co-relation model were used to identify factors that independently affect recurrence. For the statistical analysis, Statistical Package for the

Social Sciences (SPSS) has been used which is world's leading statistical software which is used to solve many research and business related problems i.e. ad hoc analysis, hypothesis testing, geospatial analysis and predictive analysis. The data on which statistical measurement has been done is present in form of numbers and percentages to find out the relationship among them. With the help of chi-square and co-relation model we have calculated the P value among different factors to know the association between them. The P value or probability value for a given statistical model, the probability that when the null hypothesis is true. The P value helped us to determine the significance association among different factors.

Chapter 4

Results and Discussion

4.1 The Frequency of Pulmonary and Extra Pulmonary Recurrent Tuberculosis

Table 4.1: Frequency of pulmonary and extra pulmonary tuberculosis

	Number	**Percentage**	**Frequency**
Total Samples	1452	100%	1
No. of pulmonary cases	1314	90%	0.90
No. of extra- pulmonary cases	138	10%	0.10

Out of total 1452 calculated samples, 1314 were cases of pulmonary tuberculosis while 138 were the cases of extra pulmonary tuberculosis registered in Tehsil Headquarter Hospital Kahuta.

4.1.1 Association of Recurrence with Pulmonary and Extra Pulmonary Tuberculosis

On the basis of site of occurrence, tuberculosis categorize into two main groups pulmonary tuberculosis (tuberculosis of lungs) and extra-pulmonary tuberculosis

(tuberculosis of different organs i.e. kidneys, brain, liver other than lungs). There is very weak association of recurrence of tuberculosis with the site of infection (pulmonary or extra pulmonary tuberculosis). From the total 1452 patients there is only 138 cases of extra-pulmonary tuberculosis while rest of them are pulmonary tuberculosis cases as shown in table 4.2

TABLE 4.2: Association between rate of recurrence and site of infection

			Recurrence of Tuberculosis		**Total**
			New	Relapse	
Disease Site of Tuberculosis	**Extra pulmonary Tuberculosis**	Count	131	7	138
	Pulmonary Tuberculosis	Count	1211	103	1314
Total		Count	1342	110	1452

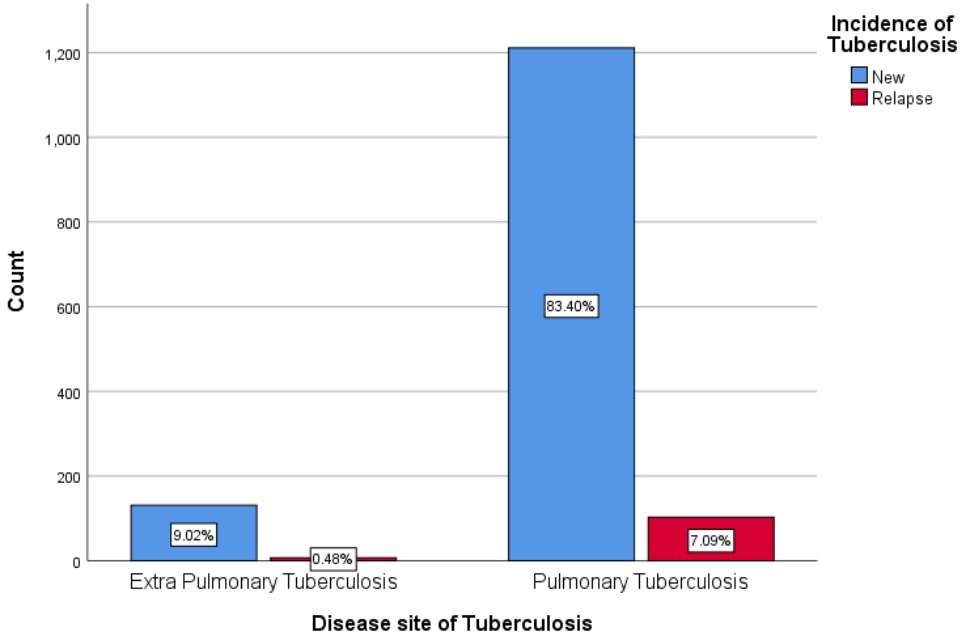

FIGURE 4.1: Association between rate of recurrence and the site of occurrence. Statistical analysis was done by chi square and p value was found to be 0.24. In the figure x-axis indicate site of infection and y-axis indicates the rate of occurrence.

The graph (figure 4.1) shows that the rate of occurrence of new cases of pulmonary tuberculosis is higher as compared to rate of occurrence of new cases of extra-pulmonary tuberculosis while the recurrence rate of pulmonary tuberculosis is higher as compared to extra-pulmonary tuberculosis because mostly the people with suppressed immune system or due to any other co-morbidity i.e. HIV, Diabetes mellitus etc are more vulnerable to get tuberculosis infection. They p value of about 0.24 indicates that there is weak association between the site of tuberculosis and the recurrence rate of tuberculosis.

4.2 The Efficacy of Category I and Category II Treatment Regimens

4.2.1 Association of Disease Categories with Disease Recurrence

TABLE 4.3: Recurrence of tuberculosis with respect to the categories

			Recurrence of Tuberculosis		**Total**
			New	**Relapse**	
Categories of Tuberculosis	**Category I Tuberculosis**	**Count**	1253	22	1275
	Category II Tuberculosis	**Count**	88	88	176
Total		**Count**	1342	110	1452

The occurrence of categories of tuberculosis is independent from each other. There are the very few cases in which the occurrence rate of category I and category II tuberculosis depend on each other by any mean. Out of 1452 patients there is 1275 patients are suffering from category I tuberculosis while only 176 is diagnosed

with category II tuberculosis. Only 110 recurrence cases are diagnosed while rest of them is newly diagnosed cases of tuberculosis as shown in table 4.3.

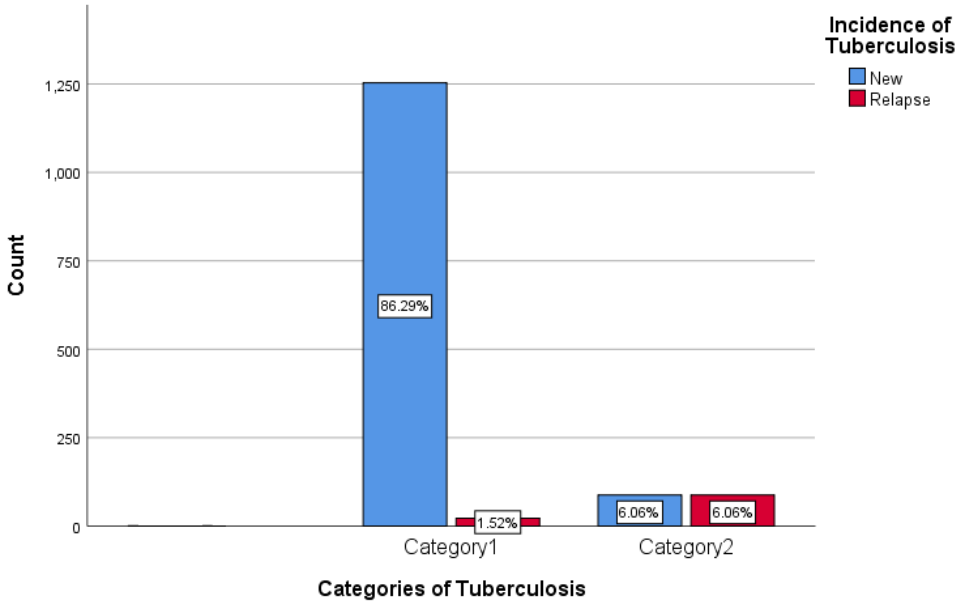

FIGURE 4.2: Association between the categories of tuberculosis and recurrence. Statistical analysis was done by chi square and p value was found to be 0.001. In the figure x-axis indicates categories of tuberculosis and y-axis indicates the rate of recurrence of tuberculosis.

The graph (figure 4.2) shows that the rate of recurrence of category II tuberculosis is higher as compared to the category I tuberculosis, the main reason which is responsible for recurrence of category II tuberculosis is that during the replication of tuberculosis bacteria some of them get naturally mutated so developed resistance against anti-tuberculosis treatment so that it persist in the host and relapse within few months post-therapy. On the other hand the rate of occurrence of new cases of category1 tuberculosis is higher as compared to the category II tuberculosis. To find out the association between the recurrence and the categories of tuberculosis, performed Chi-Square test and it is identified that the above mention factors have strong association with each other with P value of 0.001.

4.3 The Associated Factors Responsible for Recurrence in Rawalpindi Region

4.3.1 Association of Gender with Categories of Tuberculosis

Tuberculosis is categorized into two main groups on the basis of the drug resistance i.e. category I and category II; category I is defined as the newly diagnosed or relapse cases of tuberculosis have never received any anti-tuberculosis drug before or have not developed drug resistance so can easily treat with first line of anti-tuberculosis drugs where in category II patients have previously received anti-tuberculosis treatment. From the total observed data of about 1452 it is estimated that out of 1452, 1275 cases are of category I and about 176 cases are of category II tuberculosis (table 4.4).

Table 4.4: Association of Gender with Categories of Tuberculosis

		Categories of Tuberculosis	
Sex	Total Number	Category I	Category II
Female	797	706	90
Male	655	569	86
Total	1452	1275	176
		1452	

It is observed (figure 4.3) that the rate of occurrence of category I and category II tuberculosis is higher in females as compared to males. Category II is defined as the new or relapse cases of tuberculosis have received anti-tuberculosis before or have drug resistant tuberculosis so it cannot be treated with the first line of anti-tuberculosis drugs. The chances of recurrence of category II tuberculosis are higher in females because of many reasons either co-infected with any other disease or any other co-morbidity or either involve in any unconditional activity which

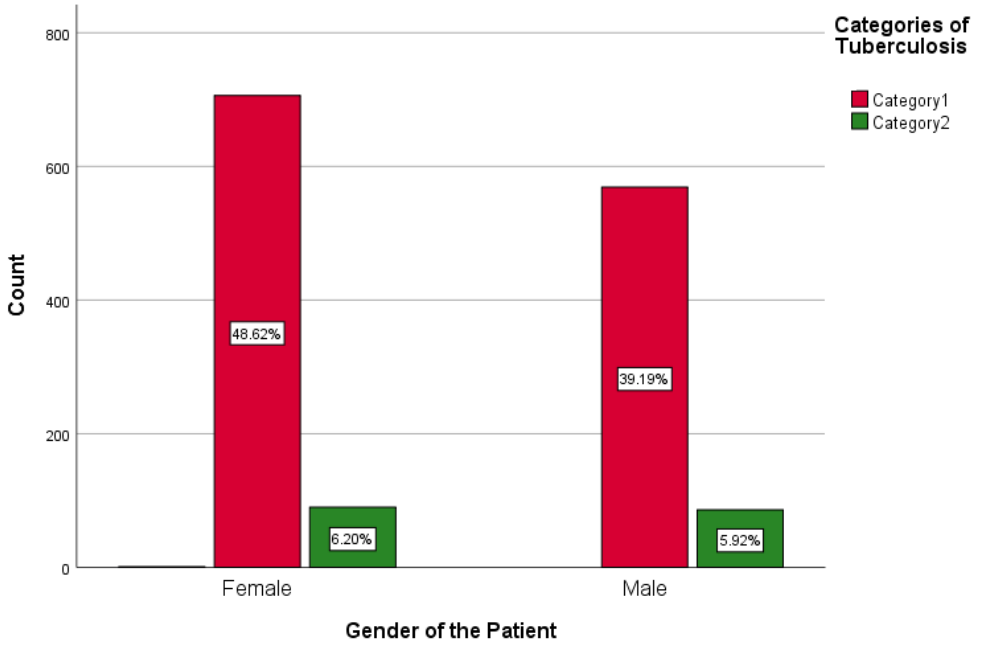

FIGURE 4.3: Occurrence rate of categories of tuberculosis with respect to gender. Statistical analysis was done by chi square and p value was found to be 0.3. In the figure x-axis indicates gender and y-axis indicates the categories of tuberculosis.

suppress the immune system. The estimated P value for the above mention factor is about 0.3 which indicates the weak association of gender with the occurrence of categories of tuberculosis

.

4.3.2 Association of Gender with Site of Tuberculosis

With respect to the site of occurrence tuberculosis is categorize into two categories i.e. pulmonary tuberculosis and extra-pulmonary tuberculosis. From the total observed data it is observed that the rate of occurrence of pulmonary tuberculosis is higher in females as compared to males where as in males the rate of occurrence of extra pulmonary tuberculosis is high. Out of 797 females it is observed that 729 are suffering from the pulmonary tuberculosis and the rest of them have extra-pulmonary tuberculosis and out of 655 males it is observed that 70 are suffering from extra-pulmonary tuberculosis and the rest of them have pulmonary tuberculosis. The overall ratio of the occurrence of extra-pulmonary tuberculosis

seems higher in males as compared to females while the ration of occurrence of pulmonary tuberculosis seems higher in females as compared to males as shown in table 4.5.

TABLE 4.5: Association of gender with disease site of tuberculosis

		Disease site of Tuberculosis	
Sex	Total Number	Extra pulmonary Tuberculosis	Pulmonary Tuberculosis
Female	797	68	729
Male	655	70	585
Total	1452	138	1314
		1452	

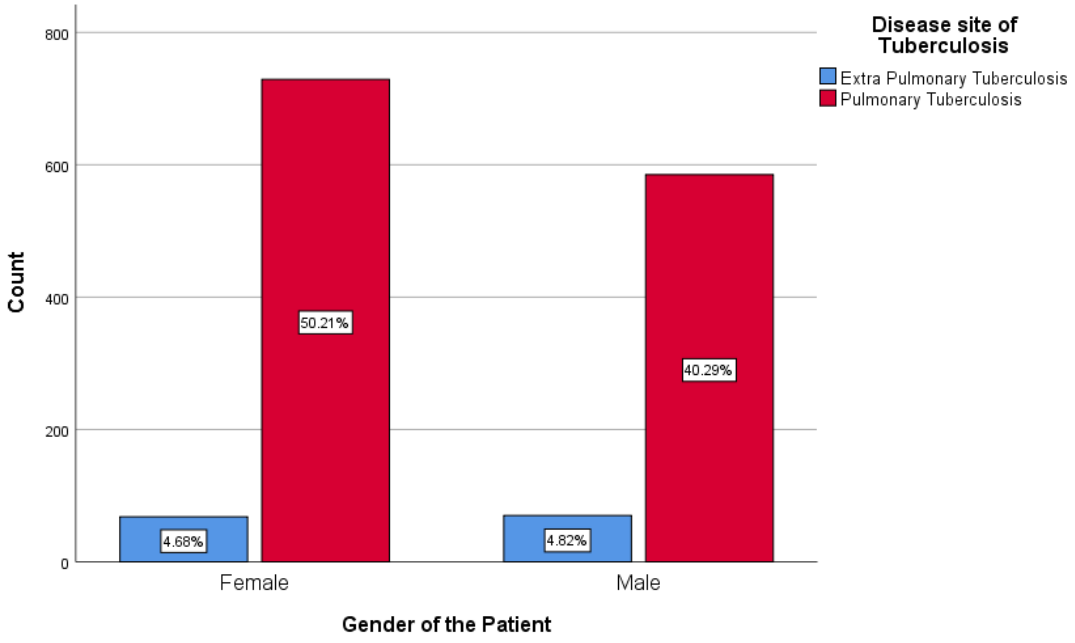

FIGURE 4.4: Graph shows the occurrence rate of pulmonary tuberculosis and extra pulmonary tuberculosis with respect to the gender. Statistical analysis was done by chi square and p value was found to be 0.16. In the figure x-axis indicates gender and y-axis indicates the disease site of tuberculosis.

The graph (figure 4.4) shows the rate of occurrence of pulmonary tuberculosis is higher in females whereas extra-pulmonary tuberculosis occurrence is high in males. There are the different factors which are responsible for their occurrence i.e. in males there are more chances to develop extra-pulmonary tuberculosis due

to the presence of different co-morbidities i.e. diabetes, cancer HIV, extreme age and due to the compromised immune system; whereas females are less co-infected with other diseases which are described earlier and the other reason is may be the earlier diagnosis of tuberculosis. The P value for above mentioned factor is estimated about 0.16 which means that the occurrence site of tuberculosis don't have any direct association with the gender of the patient and the gender don't play any role in site of occurrence of tuberculosis.

4.3.3 Association of Gender with Disease Relapse

Gender plays an important role in the recurrence of tuberculosis as it is defined that the total number of population with respect to the rate of occurrence of tuberculosis. Out of 1452 studied subjects, 797 were male and 655 were female population and it is estimated that the recurrence rate is high in males as compared to females while there is high occurrence of new tuberculosis cases in females as compared to male population as shown in Table 4.6.

Table 4.6: Association of Gender with Recurrence of Tuberculosis

		Recurrence of Tuberculosis	
Sex	Total Number	New	Relapse
Female	797	747	50
Male	655	595	60
Total	1452	1342	110
		1452	

The relapse rate is higher in males as compared to females, reason could be different factors i.e. males are more exposed to the environment containing tuberculosis bacteria e.g. in work place, community, home, gathering etc. as well as males are more involved in those activities which alters the immune response against the *Mycobacterium tuberculosis* i.e. tobacco smoking, use of alcohol and such other stuffs. On the other hand the rate of occurrence of new cases of tuberculosis is

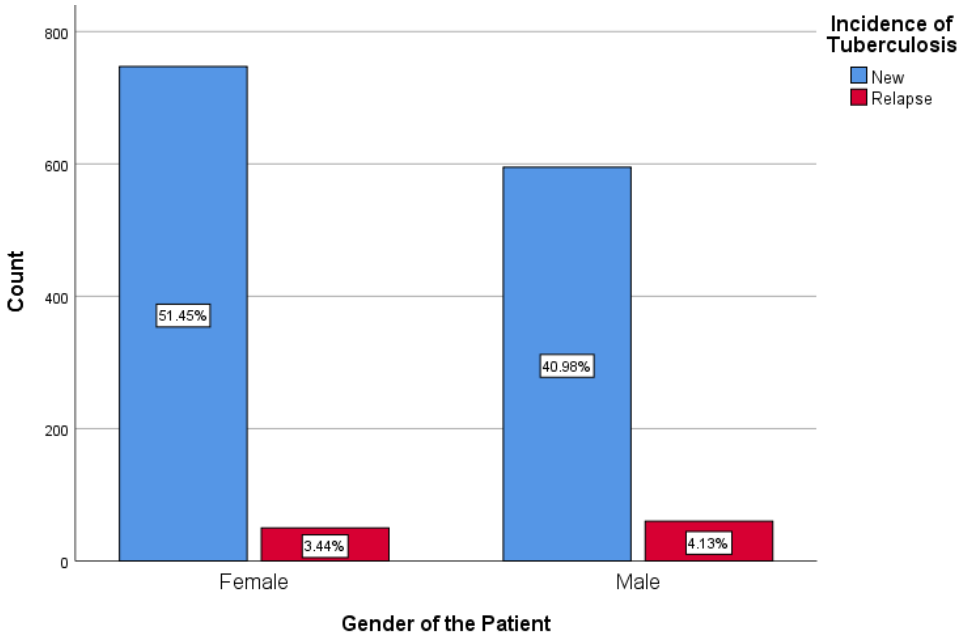

FIGURE 4.5: Graph shows the relapse rate of tuberculosis in males and females. Statistical analysis was done by chi square and p value was found to be 0.039. In the figure x-axis indicates gender and y-axis indicates the rate of occurrence.

high in females as compared to males (figure 4.5); it may be due to presence in close contact with patients or due to the less immunity or may any other factor responsible for it. The P value observed from the Chi-Square is about 0.039 which indicates the strong association between gender and the recurrence of the Tuberculosis.

4.3.4 Association of Gender with Smear Results

The small amount of patient specimen (sputum) is placed on the slide either direct or processed specimen for the microscopic examination is called as Smear and it results play an important role in diagnosis of tuberculosis; identify the presence of *Mycobacterium tuberculosis* in the sputum results as smear positive or the absence of *Mycobacterium tuberculosis* in the specimen results as smear negative. Out of 1452 patients, only 333 patients show smear positive results while rest of them show smear negative results. Only 153 females show smear positive results while 644 shows negative results; on the other hand 180 males show smear positive results while only 475 show smear negative results as shown in table 4.7.

TABLE 4.7: Association of Smear results with Gender

	Before treatment Smear results	
Gender	Smear Negative	Smear positive
Female	644	153
Male	475	180
Total	1119	333
	1452	

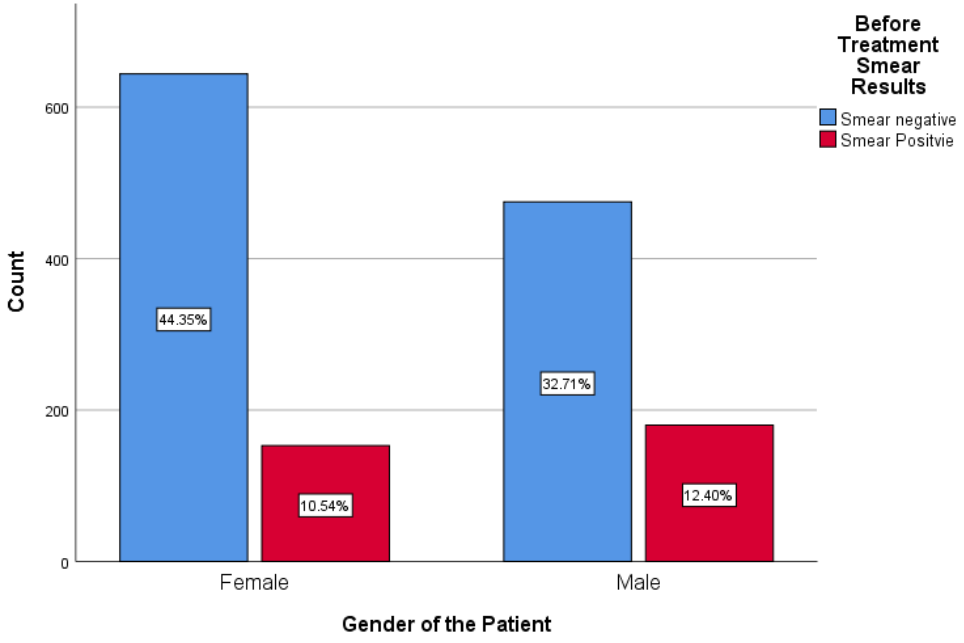

FIGURE 4.6: Graph shows association of Gender with the Smear results. Statistical analysis was done by chi square and p value was found to be 0.001. In the figure x-axis indicates gender and y-axis indicates the rate of smear results.

Males show high smear positive results as compared to the females on the other hand females show high smear negative results on testing (figure 4.6). There are the two main reasons responsible for it; first either patient is suffering from any other co-infection so that suppressed immune system allow *Mycobacterium tuberculosis* to escape from the host defense system and that's why cannot detect its presence in the clinical specimen and had received anti-tuberculosis drugs before that's why results smear negative; the second thing which is responsible for it is environmental factors i.e. smoking, air pollution, alcoholism etc. or the resources

available for diagnosis of *Mycobacterium tuberculosis*. The P value for the association among the gender and the smear result is about 0.001 which shows that there is strong association between them.

4.3.5 Association of Age with Categories of Tuberculosis

On the basis of the development of drug- resistance Tuberculosis is divided into two main Categories i.e. Category I and Category II. Those who have never received the anti-Tuberculosis drugs before and don't have drug resistant tuberculosis can be easily treated with the first line of anti-tuberculosis drugs are categorize into Category 1 and those who have received anti-Tuberculosis treatment before and have developed drug resistance in them are categorize into Category II Tuberculosis and can be treated with the second line of anti-tuberculosis drugs. Out of 1452 total studies subjects, patients are divided into different age group so that it is identified that the patient with age between 40-60 has more Category II Tuberculosis as compared to the patients with the age between 20-40 and 60-80. While on the other hand the occurrence of the Category I tuberculosis is higher as compared to the Category II Tuberculosis as shown in table 4.8.

Table 4.8: Association of age with the categories of Tuberculosis

Age of the Population (years)	Categories of Tuberculosis	
	Category I	Category II
1-20	135	10
20-40	385	47
40-60	401	58
60-80	303	53
80-100	30	3

The graph (figure 4.7) shows that the rate occurrence of category I and the category II tuberculosis is higher in the age group 40-60 while the risk of occurrence of the category II tuberculosis are very minimal at the age of 80-100 and then 1-20 due

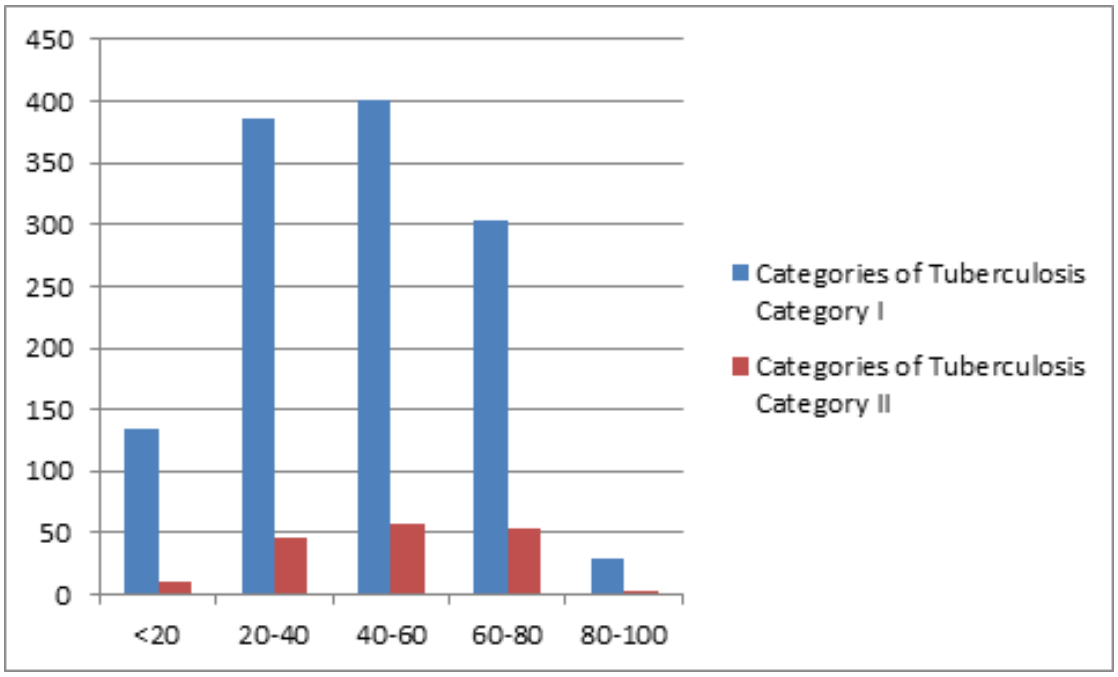

FIGURE 4.7: Graph shows the association of age with the categories of Tuberculosis. Statistical analysis was done by chi square and p value was found to be 1.00. In the figure x-axis indicates age groups and y-axis indicates the categories of tuberculosis

to the less development of drug resistance in these age groups. The chances of occurrence of category I is higher as compared to category II tuberculosis. The P value for the above mention factors is 1.00 which indicated that there is very low association between the age and the categories of tuberculosis.

4.3.6 Association of Age with Disease Recurrence

In the case of Tuberculosis it is defined that the rate of recurrence of Tuberculosis are expected after the 6 months of post-therapy. Age plays an important role in the recurrence of Tuberculosis; as the recurrence is the second episode of Tuberculosis after the completion of the first episode of Tuberculosis. The reason behind the recurrence may be either due to the development of drug resistance or due the presence of any other co-infection which suppressed the immune system so it cannot completely cure for the second episode of Tuberculosis. The whole data is divided into different age groups so that it is confirmed that the rate of recurrence

is higher in the age between 20-40 and those who rely in the age group between 60-80 have higher risk of recurrence of Tuberculosis as shown in table 4.9.

TABLE 4.9: Association of Recurrence of Tuberculosis with respect to the Age of the Population

Age of the Population (years)	Incidence of Tuberculosis	
	New	Relapse
1-20	98	7
20-40	385	36
40-60	384	28
60-80	387	32
80-100	67	3

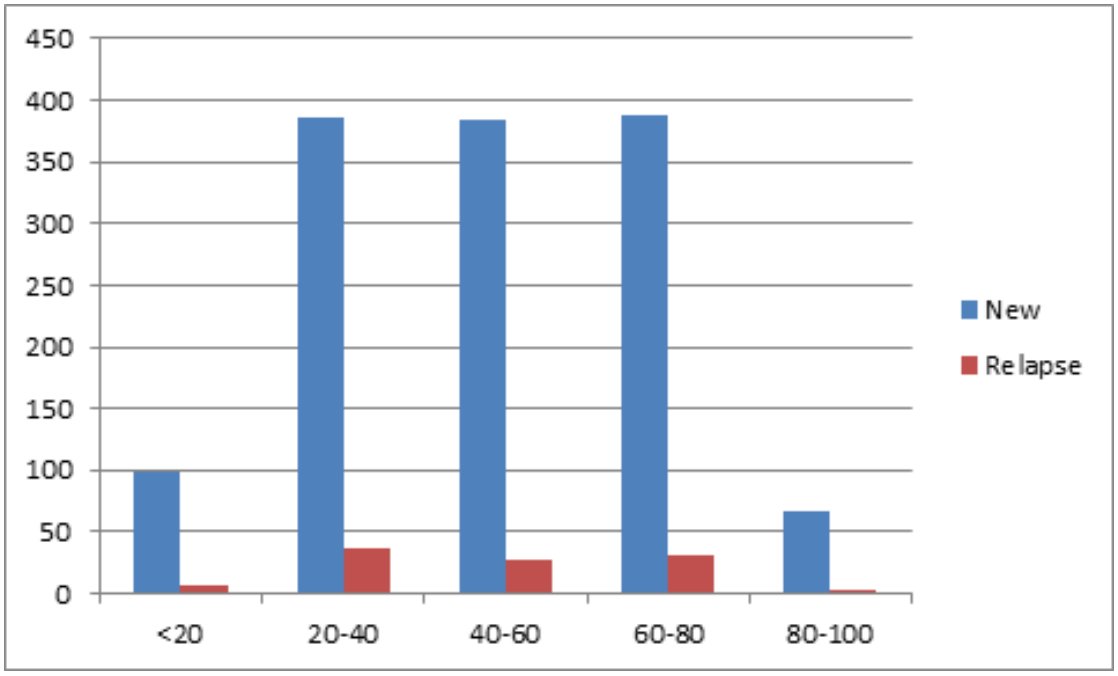

FIGURE 4.8: Graph shows association of recurrence of tuberculosis with respect to the age of population. Statistical analysis was done by chi square and p value was found to be 0.09. In the figure x-axis indicates age groups and y-axis indicates the recurrence rate of tuberculosis

The graph (figure 4.8) shows that relapse rate is higher in the age group of 20-40 because in this age group people are more vulnerable to get infection with the increase of exposure to the environment, and there is higher risk of relapse of tuberculosis to the overage people with age group of 60 to 80. There are

very few data available for the relapse of tuberculosis in the children may be due to the less occurrence of tuberculosis in children and the main reason is that children do not produce sputum while coughing that's why tuberculosis cannot be detected in the children but the chances of transmission from the children is same as from adults this is also the reason to identify tuberculosis bacteria in children by same diagnostic techniques used for the diagnosis in adults. The P value for the association among age and the relapse tuberculosis is 0.09 which indicated that there is no strong association between these two factors.

4.3.7 Association of Age with Smear Results

The clinical specimen (Sputum) which is taken from the patient is directly observed on the slide for the microscopic examination is known as smear and it is the best microscopic examination which is used to diagnose the presence of Mycobacterium Tuberculosis. The whole data is divided into different groups on the basis of age to identify those age groups which shows smear positive results and those who show smear negative results on testing. It is identified that the age group of 20-40 shows more smear positive results while the age group of 40-60 shows more smear negative results on testing as shown in Table 4.10.

TABLE 4.10: Association of age with the smear results

Age of the Population (years)	Before Treatment Smear Results	
	Smear Negative	Smear positive
1-20	103	42
20-40	323	109
40-60	353	107
60-80	292	64
80-100	24	9

From the analysis of the whole data it is identified that the smear positive results are higher in the age group of 20-40 while minimal in the age group of 80-100 (figure

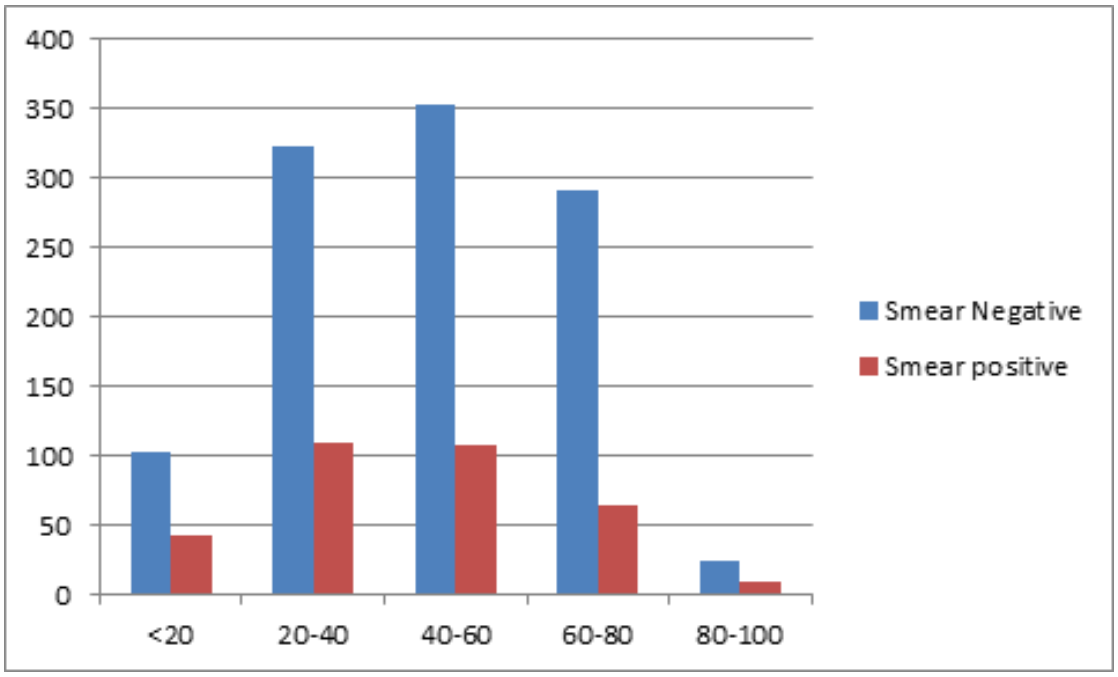

FIGURE 4.9: Graph shows association of age with the smear results. Statistical analysis was done by chi square and p value was found to be 0.021. In the figure x-axis indicates age groups and y-axis indicates the sputum smear results—

4.9) may be it is due the presence of any other co-infections which compromise the immune system and due to the previous history of tuberculosis (received anti-tuberculosis before) that's why it is not detected in the smear results. On the other hand smear negative results are more acquired by the patients of age group40-60 it means they persist Tuberculosis bacteria in them but not detected by its presence in the smear it also indicates that although they have Tuberculosis either pulmonary or extra pulmonary tuberculosis but results smear negative. 0.021 P value is calculated for the association among age and smear results which indicates that they strong association with each other.

4.3.8 Association of Age with Site of Tuberculosis

On the basis of site of occurrence of Tuberculosis, it is categorize into two main groups i.e. pulmonary Tuberculosis and extra-pulmonary Tuberculosis. The Tuberculosis which attacks lungs is known as Pulmonary Tuberculosis; if it invades into different organs i.e. liver, kidney, brain etc. it is known as extra-pulmonary

Tuberculosis. Age plays an important role in the site of occurrence of Tuberculosis. The overall data of about 1452 is divided into different age groups so it is observed that the chances of extra-pulmonary Tuberculosis is higher in age group of 20-40 while the chances of getting pulmonary Tuberculosis is higher in age group of 40-60 as shown in Table 4.11.

TABLE 4.11: Association of age with disease site of Tuberculosis

Age of the Population (years)	Disease Site of Tuberculosis	
	Extra pulmonary tuberculosis	Pulmonary Tuberculosis
1-20	17	128
20-40	57	375
40-60	39	421
60-80	23	333
80-100	2	31

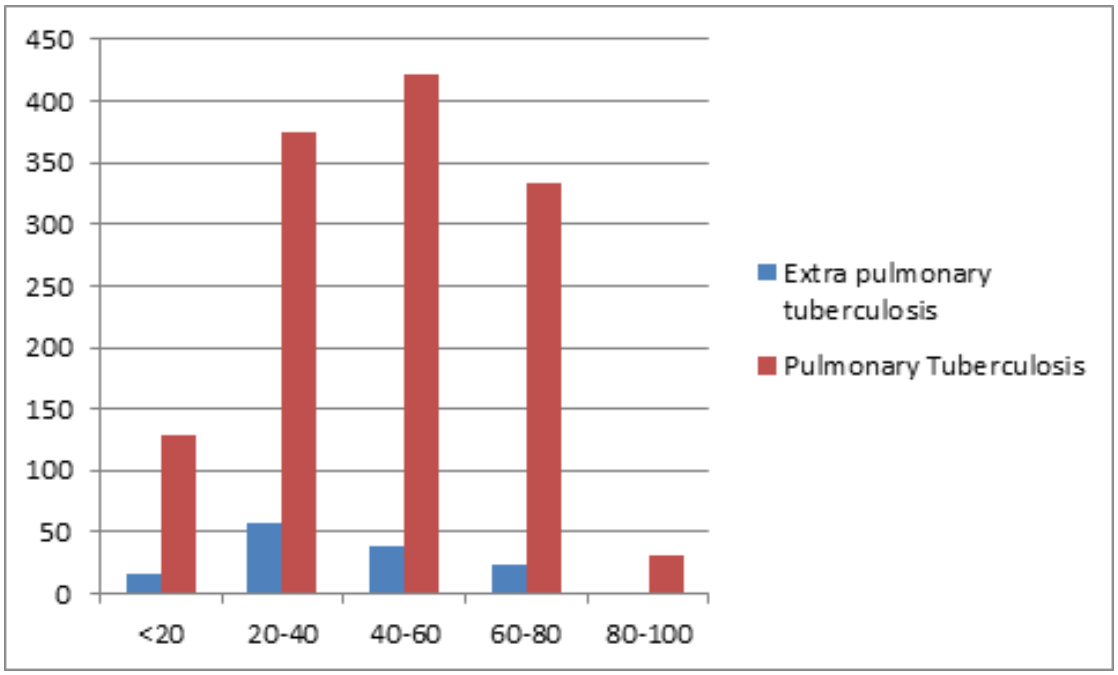

FIGURE 4.10: Graph shows association between the age and the type of tuberculosis Statistical analysis was done by chi square and p value was found to be 0.018. In the figure x-axis indicates age groups and y-axis indicates the site of occurrence of tuberculosis

The graph (figure 4.10) shows the strong association of the age with the occurrence of tuberculosis; as it is shown in the graph that the age group of 40-60 are more

vulnerable for getting the tuberculosis disease while the age group of 20-40 have higher risk for getting the infection because in these age groups people are more vulnerable for getting disease due to the environment exposure i.e. work place, community, home, friends etc. or sometime due to the suppressed immune system as well as in this age group the occurrence of extra pulmonary tuberculosis is higher as compared to any other age groups. The P value which is calculated to find out the association between the age and the site of occurrence of tuberculosis is about 0.018 which indicates that they are strongly associated with each other.

4.3.9 Association of Smear Results with Recurrence

There is very strong association between the smear results and the recurrence of Tuberculosis as the presence of Tuberculosis bacteria in the smear considered as positive result and its absence in the smear considered as smear negative results. Out of 1452 samples, 110 relapse cases has been identified in which 87 samples show smear negative results and only 23 samples show smear positive results. On the other hand 1342 new cases of Tuberculosis has been identified in which only 310 shows smear positive results while rest of them show smear negative results as shown in Table 4.12.

Table 4.12: Association of Recurrence with Smear Results

			Recurrence of Tuberculosis		Total
			New	Relapse	
Before Treatment Smear Results	Smear Negative	Count	1032	87	1119
	Smear Positive	Count	310	23	333
Total		Count	1342	110	1452

The graph (figure 4.11) indicates that new cases of Tuberculosis shows more smear negative results as compared to the relapse cases; there are the several reasons

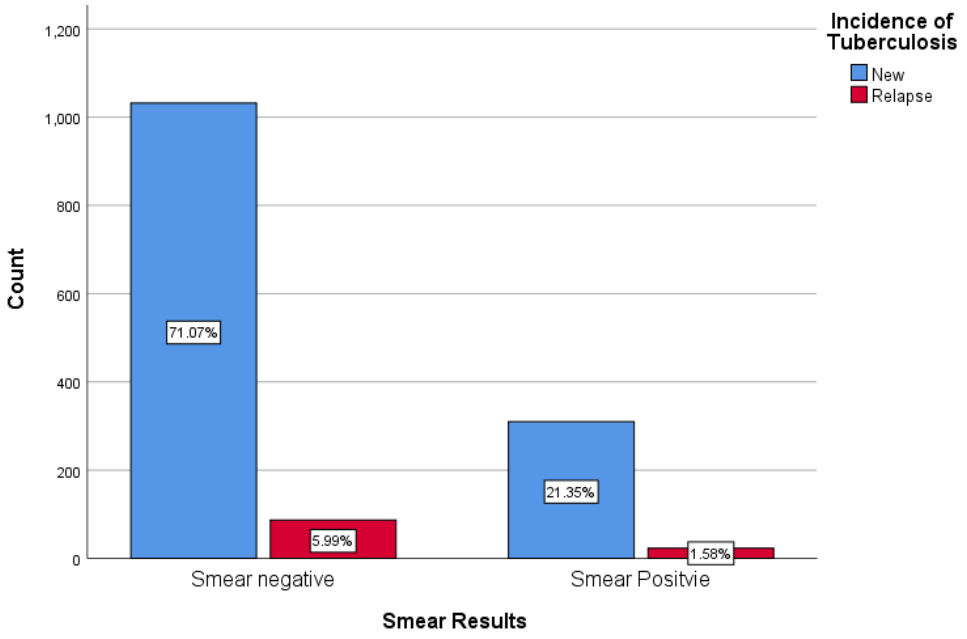

FIGURE 4.11: Graph shows relationship between the recurrence and the sputum smear results. Statistical analysis was done by chi square and p value was found to be 0.5. In the figure x-axis indicates sputum smear results and y-axis indicates recurrence of tuberculosis.

which are responsible for it either individual get infection but not in active form don't show any signs and symptoms of active Tuberculosis i.e. cough, fever etc. or cannot be detected in the smear (microscopic examination). On the other hand there are very few cases of recurrent Tuberculosis which shows smear positive results. The calculated P value for smear results and recurrence of Tuberculosis is about 0.5 which shows that the association between the above factors is not very strong or not very weak.

4.4 Cumulative Impact of Various Variables

Gender plays an important role in the onset, development, resistant and the site of any disease. In case of Tuberculosis Gender have drastic effects on the occurrence of Tuberculosis along with the presence of different factors i.e. Categories of Tuberculosis, Site of occurrence of Tuberculosis and the recurrent rate of Tuberculosis. Out of 797 females 706 have Category I Tuberculosis and only 90 have Category II Tuberculosis , while 68 females have extra-pulmonary Tuberculosis

TABLE 4.13: Cumulative Impact of Gender on Different Factors

		Categories of TB		Disease Site of TB		Incidence of TB	
Sex	Total Number	Category I	Category II	Extra pulmonary TB	Pulmonary TB	New	Relapse
Female	797	706	90	68	729	747	50
Male	655	569	86	70	585	595	60
Total	1452	1275	176	138	1314	1342	110
		1452		1452		1452	

and 729 have Pulmonary Tuberculosis, and there is only 50 cases of relapse cases is identified in females where 747 new cases of Tuberculosis has been identified. On the other hand out of 655 males 569 have Category I Tuberculosis and 86 have Category II Tuberculosis in which 70 is suffering from extra-pulmonary Tuberculosis and 585 have Pulmonary Tuberculosis and about 60 recurrence cases has been identified in males and 595 new cases of Tuberculosis has been identified as shown in table 4.13.

Disease site of Tuberculosis have different impacts on the different factors i.e. on categories of Tuberculosis and the recurrent rate of Tuberculosis. Out of 138 extra-pulmonary Tuberculosis cases there is about 125 cases belongs to Category I while 13 belongs to category II Tuberculosis and only 131 new cases whereas only 7 relapse cases of extra-pulmonary Tuberculosis has been identified. On the other hand out of 1314 cases of pulmonary Tuberculosis,1150 belongs to Category I Tuberculosis only 163 belongs to Category II Tuberculosis, and about 1211 is new cases where as 103 relapse cases of pulmonary Tuberculosis has been identified as shown in table 4.14. Smear results play an important role in the identification of the presence of *Mycobacterium Tuberculosis* in the clinical specimen (sputum). Out of 1119 smear negative samples, 87 are relapse cases where as 1032 are the new cases of Tuberculosis; while out of 1119, 981 cases belongs to Category I Tuberculosis and only 137 cases belongs to Category II Tuberculosis, and there is only 111 cases of extra-pulmonary Tuberculosis whereas 1008 pulmonary Tuberculosis shows smear negative results. On the other hand out of 333 smear positive samples 310 are new case while 23 are relapse cases, and 39 cases out of 333 smear

positive results belongs to Category II Tuberculosis while 294 belongs to category II Tuberculosis, 27 are the extra-pulmonary Tuberculosis and about 306 are the pulmonary Tuberculosis which shows smear positive results as shown table 4.15.

TABLE 4.14: Cumulative Impact of Type of Disease on Different Factors

			Categories of TB		Incidence of TB		Total
			Cat I	Cat II	New	Relapse	
Disease Site of TB	Extra pulmonary TB	Count	125	13	131	7	138
		Expected Count	121.2	16.7	127.5	10.5	
	Pulmonary TB	Count	1150	163	1211	103	1314
		Expected Count	1153.8	159.3	1214.5	99.5	
Total		Count	1275	176	1342	110	1452
		Expected Count	1275.0	176.0	1342.0	110.0	1452.0

TABLE 4.15: Cumulative Impact of Smear Results on Different Factors

			Incidence of TB		Cat of TB		Disease site of TB		Total
Before Treat-ment SE Results			New	Relapse	Cat I	Cat II	EP TB	P TB	
	SE -ve	C	1032	87	981	137	111	1008	1119
		EC	1034	84.8	983	136	106	1013	
	SE +ve	C	310	23	294	39	27	306	333
		EC	309	25.2	292.4	40.4	31.6	301	
Total		C	1342	110	1275	176	138	1314	1452
		EC	1342	110.0	1275	176	138	1314	1452

Chapter 5

Conclusions and Recommendations

The total calculated samples were 1452 on which there were 797 females and 655 were males. From the total samples it was calculated that pulmonary tuberculosis as well as the new cases of tuberculosis have strong association with the smear positive test as compared to the extra-pulmonary tuberculosis or recurrence cases. From the total calculated samples, males have strong association with smear positive test while the females have weak association with the smear test. The occurrence of category I and category II pulmonary tuberculosis is higher as compared to the category I and category II extra pulmonary tuberculosis. The rate of occurrence of relapse and the occurrence of extra pulmonary tuberculosis is higher in males as compared to females. It is observed that the rate of relapse of pulmonary tuberculosis is higher as in case of extra pulmonary tuberculosis as well as pulmonary tuberculosis gives more smear positive results on testing as compared to extra pulmonary tuberculosis. On the other hand the age group of 20-40 show high risk of getting infection and the people with age group 40-60 have high occurrence of tuberculosis.

Tuberculosis is high burden disease now a days and it is necessary to take steps to eradicate the *Mycobacterium tuberculosis* from the environment. There is need to

focus on the risk factors which are responsible for recurrent tuberculosis to avoid mortality and morbidity rate. The development of drug-resistant tuberculosis is one of the major challenges faced by the Tuberculosis Management Programs; to know about the disease episode, treatment regimens and the treatment duration will help us to deal with the disease with proper regimens in order to cure as earlier as possible. Life style medications, early diagnosis, well equipped techniques and proper treatment regimens are the best future perspective to monitor and reduce the disease frequency.

Prediction of the frequency and the treatment outcomes of *Mycobacterium tuberculosis* play an important role in the countries where the prevalence rate of *Mycobacterium tuberculosis* is very high. This research will be helpful in future specially for those who are working with the strategies to eradicate the *Mycobacterium tuberculosis* from the environment. It will help to find out the occurrence rate of tuberculosis in patients which are more susceptible to *Mycobacterium tuberculosis*.

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