1. INTRODUCTION

1.1 Background

Tuberculosis (TB) is the world's most serious public health problem particularly in developing and under developing countries. It is a disease of great antiquity and contributing to more morbidity and mortality than any other bacterial infection (Grange et al. 1998). It causes ill-health among millions of people each year and ranks as the second leading cause of death from an infectious disease worldwide, after the human immunodeficiency virus (HIV) (WHO 2012a). Infection approximately one-third of the world's population is infected from it (Miller and Schieffelbein 1998). So, TB remains one of the deadliest threats to public health.

TB is an infectious disease caused by the bacillus *Mycobacterium tuberculosis* that spreads to others via aerosol route. Cellular immune responses control *M. tuberculosis* infection in most healthy individuals, resulting in less than 10% of infected persons developing active TB (Comstock 1982). There were almost 9 million new cases and 1.4 million TB deaths (9, 90,000 among HIV-negative people and 4, 30,000 HIV-associated TB deaths) in 2011 (WHO 2012b). The World Health Organization (WHO) declared TB a global public health emergency in 1993. Starting in the mid-1990s, efforts to improve TB care and control intensified at national and international levels, WHO developed the Directly Observed Treatment Short course (DOTS) strategy (WHO 2012a).

TB typically affects the lungs (pulmonary TB) but can affect other sites as well (extra-pulmonary TB) (Kumar et al. 2007). The disease is spread in the air when people who are sick with pulmonary TB expel bacteria, for example by coughing. In general, a relatively small proportion of people infected with *Mycobacterium tuberculosis* will develop TB disease; however, the probability of developing TB is much higher among people infected with the human-immunodeficiency virus (HIV). TB is also more common among men than women, and affects mostly adults in the economically productive age groups (WHO 2012a). Without treatment, mortality rates are high. In studies of the natural history of the disease

among sputum smear-positive and HIV-negative cases of pulmonary TB, around 70% died within 10 years; among culture-positive (but smear-negative) cases 20% died within 10 years (Tiemersma 2011).

The most common method for diagnosing TB worldwide is sputum smear microscopy (WHO 2002a), in which bacteria are observed in sputum samples examined under a microscope. Following recent developments in TB diagnostics, the use of rapid molecular tests for the diagnosis of TB and drug-resistant TB is increasing. In countries with more developed laboratory capacity, cases of TB are also diagnosed via culture methods which is the current reference standard (WHO 2002a). Treatment for new cases of drug-susceptible TB consists of a 6-month regimen of four first-line drugs: isoniazid, rifampicin, pyrazinamide, and ethambutol represented by the letter "H", "R", "Z" and "E" respectively (WHO 2012a).

Treatment for multidrug resistant TB (MDR-TB), defined as resistance to isoniazid and rifampicin (the two most powerful anti-TB drugs) is longer, and requires more expensive and toxic drugs (WHO 2011). For the patients with MDR-TB, the current regimens recommended by WHO, last for 20 months (Tiemersma et al. 2011). Due to high mobility, TB mainly attacks the most economically productive age group of the society (people aged 15-45 years) and hence the community injury that it causes extends far beyond individual disease and death (Sbarbaro 2001). TB is a disease of poverty; virtually all TB deaths occur in the developing world, affecting mostly the vulnerable such as the poorest and malnourished. TB, if not treated each person with active TB infects an average 10 to 15 people every year (WHO 2012a). According to recent data from WHO, there were almost 9 million new cases in 2011 and 1.4 million TB deaths, 80% of them in 22 countries (WHO 2012b). Ten percent of the TB infected people; who are immunosuppressive e.g. with autoimmune disease, HIV/AIDS etc, develop active TB disease as HIV weakens the cellular immunity. *M. tuberculosis* owes its virulence due to its ability to survive within the macrophage rather than the production of toxic substance (Grange et al. 1998).

Nepal, one of the developing countries of SAARC region, has an elevated risk of TB infection, estimated to be 2.0% in rural and 4.5% in urban areas (WHO 1997). TB is a major

public health problem in Nepal. About 45 percent of the total population is infected with TB, of which 60 percent are adult. Every year, 40,000 people develop active TB, of whom 20,000 have infectious pulmonary disease. These 20,000 are able to spread the disease to others (DoHS 2010/11). Treatment by Directly Observed Treatment Short course (DOTS) has reduced the number of deaths; however 5,000-7,000 people still die per year from TB (DoHS 2007/8). Expansion of this cost effective and highly successful treatment strategy has proven its efficacy in reducing the mortality and morbidity in Nepal.

DOTS is the strategy for improving treatment outcome to control TB by giving drugs to the patients under the direct observation of health workers. DOTS has been found 100% effective to cure TB (WHO 2002a). DOTS have been successfully implemented throughout the country since April 2001. The treatment success rate stands at 90 percent and case finding rate of 73 percent. At the national level 36,951 TB patients have been registered of whom 15,000 infectious (sputum smear positive new cases) and are being treated under the DOTS strategy in NTP during the FY 2067/68 (2010/2011) (DoHS 2010/11).

1.2 OBJEVTIVES

General objective

- To find out the situation of TB in Jutpani VDC, Chitwan, Nepal.

Specific objectives

- To determine the prevalence of TB in Jutpani VDC, Chitwan, Nepal.
- To find out the status of TB transmission among the family member of TB patients under DOTS treatment.
- To determine the relapse rate of TB case in the Jutpani VDC.
- To determine the Knowledge, Attitude and Preventive Practices (KAP) of DOTS patients in Jutpani VDC.

2. LITERATURE REVIEW

2.1 TUBERCULOSIS (TB)

TB is a great contagious bacterial disease. It is a chronic granulomatous disease that has become a major public health concern worldwide as it is recognized as the leading cause of death among the infectious diseases. It is the disease, which most commonly affects the lungs. Because of the serious health threat posed by TB, the WHO declared it a global emergency' in 1993 (Cheesbrough 2003). However, in healthy people, infection with *M. tuberculosis* often causes no symptoms, since the person's immune system acts to "wall off" the bacteria (Kumar et al. 2007).

2.1.1 History

- In Ayurveda texts, Charak Samhita which was written in 5,000 BC describes TB as *Rajayakshma* and *Rograj* meaning "The King of disease"(Sukla and Tripathi 2005). Details about the etiology, patho-physiology and the treatment are also well described.
- TB was present in Egypt from early dynastic lines, perhaps as early as 3700 BC. In the past TB has been referred to as the "white plague" and by John Bunyan as "the captain of all these men of death" (Grange et al. 1998).
- TB is assumed to exist in this world far back since Neolithic period of human being. Many evidences since that period support this fact by skulls and bones of Neolithic period, joint of mummified bodies of ancient Egypt and 21th dynasty of Egypt. It is evident that it was indicated as early as 5,000 BC, man suffered from it. After that it was described in other way as in Ayurveda literature '*Charak* and *Susruta samhiha*', Chinese literature 'Laoping' and fire worshiper of Persia (Miller and Schieffelbein 1998).
- TB was well recognized by the time of Hippocrates (377-400 BC), who gave an excellent clinical description of the disease, called "pthisis", a Greek word that mean,

"to consume to spit" and "to waste away" (Grange 1998, Miller and Schieffelbein 1998).

- The Dutch Physician, Franciscus Sylvius (1614-1672) deduced from autopsies that TB characterized by the formation of nodules, which he named "tubercles".
- Richard Mortan (1637-1698) described the signs and symptoms of pulmonary tuberculosis (PTB).
- Pierre Desalt (1675-1740) observed the transmission of TB via sputum.
- Gaspard Laurent Bayle (1774-1816) introduced the term “Tuberculosis” and the relation between pulmonary TB and extra pulmonary TB (Miller and Schieffelbein 1998).
- Rene Theodore Laennec (1781-1826) a French clinician, who himself was a consumptive and suffered from the TB. In 1819 he invented the Stethescope and described the details about the tubercular lesions (Miller and Schieffelbein 1998). Modern knowledge of TB started from his work.
- Jean-Antoine-Villemin (1827-1892), a French military surgeon, established the transmissible nature of TB and published the result of a series of studies in which he convincingly demonstrated that TB could be produced in rabbits by inoculating them with the tuberculous materials from man or cattle (Webb 1936).
- In 1874, Armauer Hansen identified a rod-shaped bacillus (*Bacillus leprae)* in a tissue biopsy from a lepromatous leprosy patient and suggested that it was the aetiological agent of leprosy (Hansen 1880).
- Robert Koch discovered the *M. tuberculosis* organism in 24 March 1882 and succeeded in culturing it on inspissated serum and identified a rod-shaped bacillus (*Bacterium tuberculosis*) as the causative agent of TB and formulated Koch's postulates for establishing a causal relationship between a suspected pathogen and a given disease (Koch 1882, Zopf 1883).
- The acid fast nature of the organism was discovered by Ehrlich in 1885 and the present method of acid-fast staining was developed by Ziehl and subsequently modified by Neelsen and hence the named Ziehl Neelsen staining technique (Kumar et al. 2007). The word "tuberculosis" means "a small clump". Several names have

been used to refer to TB in the year gone by; acute progressive TB has been referred "tabes pulmonali" (Lehmann and Neumann 1896).

- In 1884, Coni found chicken tubercle bacillus (Zopf 1883).
- In 1890 Magucci isolated the avian bacillus (Zopf 1883).
- Latter in 1898, Theobald Smith distinguished the human and bovine strain of *M. tuberculosis* (Zopf 1883).
- In 1896, these species were subsequently renamed *Mycobacterium leprae* and *Mycobacterium tuberculosis*, respectively, and placed in the genus *Mycobacterium* ('fungus bacterium', named to reflect the mould-like pellicle formed by *M. tuberculosis* on liquid medium) (Lehmann and Neumann 1896).
- At the beginning of this 21st century, *M. tuberculosis* was the only species of *Mycobacterium* routinely isolated from, and associated with, human disease. As other species of *Mycobacterium* were recognized as causes of human disease, they were often simply categorized as non-tuberculosis *Mycobacterium* without further speciation.

2.1.2 Etiological agent: Mycobacteria

The *Mycobacterium* genus is the only genus in the family Mycobacteriaceae. The name *Mycobacterium* (Greek Mykes, fungus; bacterium, small rod), meaning ' Fungus – like bacterium' is derived from the mould like appearance of *M. tuberculosis* when growing in liquid media (Watt et al. 1996). The genus *Mycobacterium* consists of more than 55 well defined species, including the causative agents of TB, leprosy, and chronic hypertrophic enteritis (John's disease) of cattle (Hasleton 1996). Of these 14 are known to cause disease in humans (Forbes and Sahm 2002). *Mycobacteria* were among the first bacteria to be ascribed to specific diseases. *Mycobacteria* are rod- shaped, aerobic bacteria that do not form spores. They are acid and alcohol fast, meaning that once stained by an aniline dye, such as carbol fuchsin, they resist decolorization with acid and alcohol. Therefore *Mycobacteria* are often called “acid-fast bacilli” (AFB). The acid and alcohol fastness is due to the presence of thick, complex, lipid rich, and waxy cell wall component called mycolic acid. The degree of acid

fastness is different for different species due to variation of lipid percent (40%-60%) in the species(Forbes and Sahm 2002).

In addition to mycolic acid (Principle constituent) layer, *Mycobacterium* possess peptidoglycan (innermost) layer, Arabinogalactan (external to peptidoglycan) layer and mycosides layer (forming species or strain species surface lipid). The organisms are poor gram positive and either straight or slightly curved rods but coccobacillary, filamentous and branched forms may also occur. They usually measure 1-4μm by 0.3-0.6μm. The morphology varies from species to species (Hasleton 1996). *Mycobacteria* have a cell wall with high lipid content that includes waxes having characteristics mycolic acid with long branched chains (Forbes and Sahm 2002).

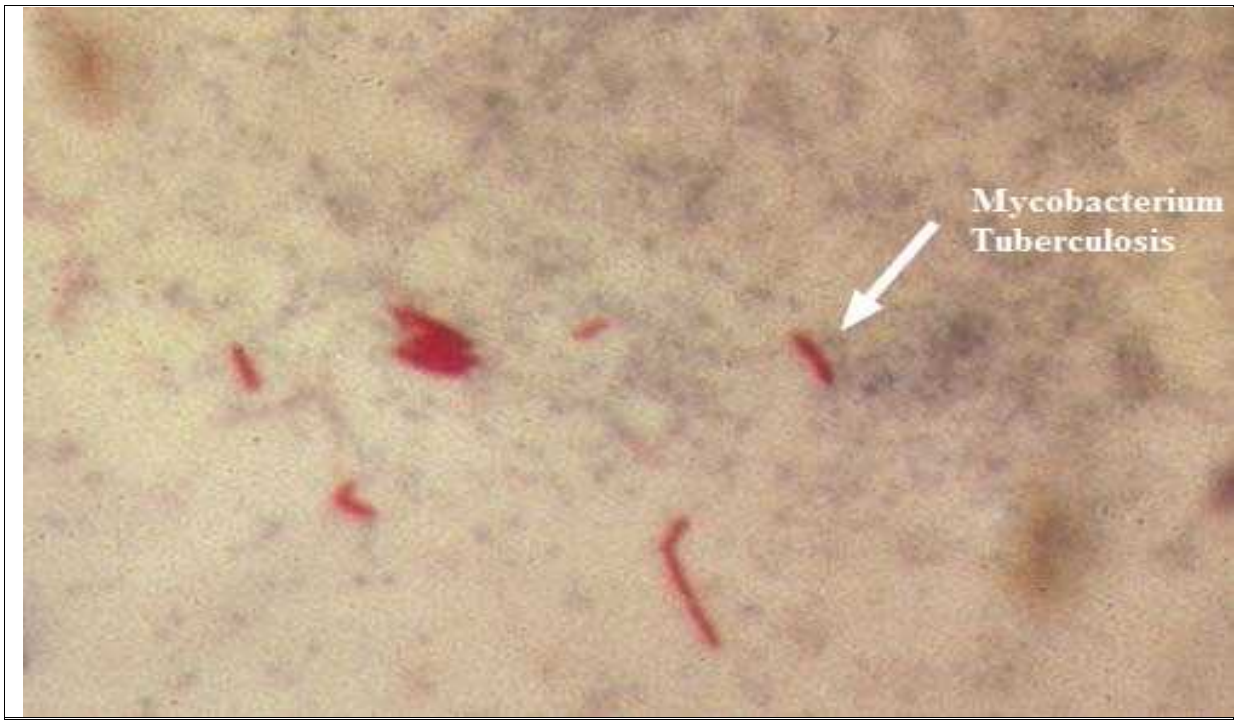
Mycobacteria of clinical interest are divided into those associated with the *M. tuberculosis* complex or MTC (*M. tuberculosis, M. bovis, M. africanum* and *M. micro*ti) and other mycobacteria that may be associated with human disease or "atypical" 'anonymous', 'non-tuberculous', tuberculoid, opportunist and *Mycobacteria* other than TB (MOTT). Many MOTTs are found in the environment but they cannot colonize in man and cause clinical infection (Watt et al. 1996).

2.1.2.1 *Mycobacterium tuberculosis*

2.1.2.1.1 Morphology and colony characteristics

TB is a chronic granulomatous disease affecting humans and many other mammals. It is caused by four very closely related species: *M. tuberculosis* (the human tubercle bacillus), *M. bovis* (the bovine tubercle bacillus), *M. microti* (the vole tubercle bacillus) and *M. africanum* (Grange et al. 1998). Koch first described the tubercle bacillus in 1882 now known as *M. tuberculosis.* Most human TB is caused by *M. tuberculosis*, but some cases are due to *M. bovis*, which is the principal cause of TB in cattle and many other mammals *M. tuberculosis*, the cause of TB, which is one of 55 recognized species of *Mycobacterium*, is classified in the family Mycobactericeae of the order Actinomycetales. *M. tuberculosis* is a small (1 to 4um long), slender, slightly curved, rod shaped bacterium of about 3 X 0.3 μm in size (Good and

Shinnick 1998). Photograph1 showed the M. tuberculosis viewed in 100x through microscope.



Photograph 1: Acid-Fast Bacilli as seen in sputum smear by Ziehl-Neelsen stain method

It is a non-spore forming, non-encapsulated, non-motile, slow growing, and obligate aerobe, with a generation time 15 to 20 hours. In sputum and other clinical specimens they mainly occur singly or in small clumps on microscopic examination and in liquid cultures, human tubercle bacilli often grow as twisted rope like colonies termed serpentine cords (Kumar et al. 2007).

2.1.2.1.2 Cultural characteristics

Mycobacteria are obligate aerobes and slow growers (average generation time 18 hours) (Good and Shinnick 1998). Colonies of *M. tuberculosis* in culture are rough and characteristically buff colored (non-pigmented) i.e. gives luxuriant growth i.e. eugonic growth on glycerol pyruvate medium. Colonies of human tubercle bacilli (*M. tuberculosis*) generally appear on egg media i.e. Lowenstein-Jensen (L-J) medium after 2-3 weeks at 35-

37^0C (Good and Shinnick 1998). Most disease associated mycobacteria require up to 8 weeks on complex media enriched with eggs. Colonies first appears as small 1 to 3 mm, dry, friable colonies that are rough, warty, granular and off-white (buff) colored. After several weeks, these increase in size (as 5-8mm) typical colonies have a flat irregular margin and a 'cauliflower' center (Grange et al. 1998). Colonies are easily detached from the medium's surface but are difficult to emulsify. In laboratory, specialized culture media such as Lowenstein Jensen (L-J) media or Middle brook 7H10 or 7H11 agars are used for the mycobacterial culture because *M. tuberculosis* will grow slowly, do not produce yellow pigment and fail to grow on egg media containing p-nitro benzoic acid (500 mg/l). They even fail to grow at 25 and 41^0C (Grange et al. 1998). In biochemical test, *M. tuberculosis* are niacin positive and nitrate reduction is also positive (Forbes and Sahm 2002). Characteristically, strains of *M. tuberculosis* form cords when growing on solid medium. When colonies are suspended in liquid or when they are grown in liquid culture, the cording characteristic can be seen clearly in stained preparations (Good and Shinnick 1998).

2.1.2.1.3 Susceptibility to chemical and physical agents

Mycobacteria are as susceptible as other non-spore-forming bacteria to heat and to some other physical and chemical agents, although some early work on the heat susceptibility of mycobacteria may have suggested otherwise (Corper and Cohn 1937). Mycobacteria are generally resistant to acids and alkalis, and this feature is used to advantage in isolation procedures. As much as 2% sodium hydroxide 2% sulphuric acid or 2.5% oxalic acid can be used to kill contaminants in specimens prior to culture. However, killing activity of acids and alkalis increases with increasing temperature of exposure, and resistance varies greatly among different species. Turbercle bacilli are also resistant to quaternary ammonium compounds and, indeed, cetylpyridinium chloride has been used for decontamination of clinical specimens prior to culture (Kent and Kubica 1985).

With respect to chemical disinfectants, mycobacteria are suspectible to a variety of chemical agents including: alcohols (ethyl and isopropyl although the latter is not as active as the former), chlorine, glutaraldehyde, iodophores, phenolic compounds, ethylene oxide,

formaldehyde and hydrogen peroxide. Derivatives of phenol, in which a functional group replaces one of the hydrogen atoms on the aromatic ring, are effective and safe to use (Marsik and Denys 1995).

Mycobacteria are resistant to drying and can survive for long periods on inanimate objects if protected from ultraviolet (UV) light, which is highly tuberculocidal. Tubercle bacilli can remain suspended as a stable aerosol in air for many hours. The persistence of tubercle bacilli on surfaces and in air prompted development of methods for removal including treatment of the air and surfaces with UV light (Riley 1957, 1961).

2.1.2.1.4 Mycobacteria other than tuberculosis (MOTT)

Human disease can also be caused by species of mycobacteria other than *M. tuberculosis* (MOTT), also known as atypical mycobacteria. These organisms are widespread in nature and have been frequently found in environment habitants that may colonize and occasionally cause infection in humans and animals. MOTT have been isolated from a variety of sources, including soil, dust, water, milk, animals and birds. Infection caused by these organisms is called mycobacteriosis. They are becoming more prevalent with the increasing prevalence of immunocompromised hosts, particularly in relation to the AIDS pandemic and in patients with preexisting lung disease (Haslett et al. 1999). MOTT are still a rare cause of disease in sub-Saharan Africa. The large majority of patients in Africa who are diagnosed and treated for TB, even those infected with HIV, have disease caused by *M. tuberculosis* (Jamison et al. 2006). The most commonly encountered MOTT that is isolated in clinical specimens and rare cause of disease are *Mycobacterium kansasii, Mycobacterium xenopi, Mycobacterium malmosense,* MAIS complex *Mycobacterium avium, Mycobacterium intracellulare, Mycobacterium scrofulaceum* (Haslett et al. 1999).

2.1.2.2 Pathogenesis of TB

M. tuberculosis is the classical representative of an intracellular pathogen. Thus the organism owes its virulence due to its ability to survive with in macrophage rather than the production

of toxic substance (Grange et al. 1998). The immune response to the bacillus is of the cell-mediated type which, depending on the type of T helper cells involved, may either lead to protective immunity and resolution of the disease or to tissue-destroying hypersensitivity reactions and progression of the disease process. The nature of the immune responses following infection changes with time so that human TB is divisible into primary and post-primary forms with quite different pathological features (Kumar et al. 2007).

a. Primary Pulmonary Tuberculosis (PTB)

The first infection of the lung with the tubercle bacillus is known as primary pulmonary TB and usually includes the draining lymph nodes in addition to the initial lesion. The great majority of Primary TB infection is usually asymptomatic, at least in young adults and adolescents (Seaton et al. 2000). The site of initial infection is usually the lung, following the inhalation of bacilli. These bacilli are engulfed by alveolar macrophages in which they replicate to form the initial lesion called as *'Gohn focus'*. Some bacilli are carried in phagocytic cells to the hilar lymph nodes where additional foci of infection develop. The Ghon focus together with the enlarged hilar lymph nodes form the *'Primary complex'*. In addition, bacilli are seeded by further lymphatic and haematogenous dissemination in many organs and tissues including other parts of the lung. When the bacilli enter the mouth, the primary complexes involve the tonsil and cervical nodes or the intestine, often the ileocaecal region, and the mesenteric lymph nodes (Issaelbacher et al. 1992).

Within about 10 days of infection, clones of antigen-specific T lymphocytes are produced. These release lymphokines which activate macrophages and cause them to form a compact cluster, or granuloma, around the foci of infection. These activated macrophages are termed epithelioid cells from their microscopical resemblance to epithelial cells. Some of them fuse to form multinucleate giant cells. Such hypersensitivity reaction leading to giant cell formation and epitheloid cell formation is called as *'Granulomatous reaction'* (Kumar et al. 2007)

In a minority of cases one of the infective foci progresses and gives rise to the serious manifestations of primary disease, including progressive primary lesions. If a focus ruptures into a blood vessel, bacilli are disseminated throughout the body with the formation of numerous granulomata. This, from the miller seed-like appearance of the lesions, is known as *'miliary tuberculosis'* (Grange et al. 1998)

b. Post primary tuberculosis

Post primary TB is by far the most important type of TB, partly because it is most frequent and partly because smear positive sputum is the main source of infection responsible for the persistence of disease in the community. This form of TB may arise in one of three ways (Seaton et al. 2000);

- Direct progression of a primary lesion.
- Reactivation of a quiescent primary of post-primary lesion.
- Exogenous re-infection

It is generally a disease of the adults due to endogenous reactivation or exogenous re-infection in a patient who had infection in the past and has retained a degree of acquired immunity. Reactivation may occur spontaneously or after any immune-compromised state. In post primary TB, dissemination of bacilli to lymph node and other organ is unusual. Instead, the infection spread through the bronchial tree so that secondary lesion develops in lower lobes of the lung, trachea, larynx and mouth, and swallowed bacilli cause intestinal lesions; secondary lesions may also develop in the bladder and epididymis in cases of renal TB (Grange et al. 1998).

2.1.3 Epidemiology

2.1.3.1 Global aspect of TB burden

In 2011, there were an estimated 8.7 million incident cases of TB (range, 8.3 million–9.0 million) globally, equivalent to 125 cases per 100 000 population and most of the estimated number of cases in 2011 occurred in Asia (59%) and Africa (26%); 1 smaller proportions of

cases occurred in the Eastern Mediterranean Region (7.7%), the European Region (4.3%) and the Region of the Americas (3%) (WHO 2012b). Most new cases were in adults aged 15 to 49 years (5.4 million; 172/100 000). Among WHO regions, the African Region (essentially sub-Saharan Africa) had by far the highest annual incidence rates (290/100 000), while the South-East Asian Region had the largest number of cases (3.0 million). Half the new cases (4.3 million) were in the top 5 countries, all in Asia. Of 15 countries with the highest incidence rates per capita 13 were in Africa (Corbett et al. 2011).

The five countries with the largest number of incident cases in 2011 were India (2.0 million–2.5 million), China (0.9 million–1.1 million), South Africa (0.4 million–0.6 million), Indonesia (0.4 million–0.5 million) and Pakistan (0.3 million–0.5 million). India and China alone accounted for 26% and 12% of global cases, respectively (WHO 2012b). Of the 8.7 million incident cases in 2011 1.0 million–1.2 million (12–14%) were among people living with HIV, with a best estimate of 1.1 million (13%). The proportion of TB cases co-infected with HIV was highest in countries in the African Region; overall, 39% of TB cases were estimated to be co-infected with HIV in this region, which accounted for 79% of TB cases among people living with HIV worldwide (WHO 2012b). Globally, incidence rates were relatively stable from 1990 up to around 2001 (WHO 2002d), and then started to fall. Between 2010 and 2011 (WHO 12b), the rate of decline was 2.2%; if this trend is sustained, MDG Target will be achieved. The absolute number of incident cases is also falling, albeit slowly, as the decline in the incidence rate (per 100 000 population) exceeds the rate of growth in the world's population (WHO 2012b).

The global burden of TB is growing. The total number of new TB cases increased at a rate of 1.8% per year between 1997 and 2000, and incidence rates per capita (all ages) at a rate of 0.4% per year. Case numbers increased much more quickly in the former Soviet Union (6.0% per year) and in the WHO African Region (6.4% per year) (WHO 2011).

An estimated 9 million new cases of TB occurred in 2004 at the rate of 140/ 100000 population, of which 3.9 million (62/100000 pop) were smear positive and 741000 were in adults infected with the human immunodeficiency virus (HIV). 14.6 million were estimated to be prevalent TB cases at the rate of 229/100000 pop, of which 6.1 million were smear

positive (95/100000 pop). More than 80% of all new TB patients in 2004 were in the Africa, South East Asia and Western Pacific Region. An estimated 1.7 million people (27/100000 pop) died from TB in 2004, including those co infected with HIV (248000) (Corbett et al. 2003, WHO 2002, 2006).

A total of 183 countries and territories were implementing the DOTS strategy during 2004 (WHO 2006). By the end of 2004, 83% of the World's population lived in countries, or parts of countries, covered by DOTS. At the end of 2004, DOTS expansion was completed in nine High Burden Countries and nearing completion in five others. Pakistan reported full DOTS coverage by the end of 2005, and coverage has increased considerably in Afghanistan, Brazil, India and the Russian Federation. DOTS programs notified 4.4 million new and relapsed TB cases in 2004 of which 2.1 million were new smear positive. In total 21.5 million TB patients, and 10.7 million new smear positive patients, were treated in DOTS programs over the 10 years 1995-2004 (STC 2006). There are estimated 17 million cases of active TB globally. Every year, about 9 million people develop active TB and 2 million die of the disease; 84% of all TB sufferers live in developing countries. Most are poor people aged between 15 to 54 years of age. Between 2000 to 2020, nearly 1 billion additional people will be infected with TB 200 million will become sick and 35 million will die of the disease (Stephen et al. 2006).

2.1.3.2 TB burden within SAARC Countries

The SAARC region, which contributes 22% of global population, bears 29% of global TB burden and approximately 2.5 million of all forms of new TB cases occur per year (out of them 1.1 million are sputum positive) and 0.6 million deaths per year (WHO 2012a). Despite the establishment of national TB programmes for over 3 decades along with the existence of cost-effective TB control strategies, TB remains a prevention and control challenge within SAARC region. In 1993, the urgency of the global TB epidemic prompted WHO to declare

TB a global emergency and urged each National TB control programme (NTP) to work towards 2 objectives by the year 2000, these being 1) to treat successfully 85 percent of detected smear positive TB cases and 2) to detect 70 percent of all such cases by implementing DOTS strategy, an effective approach to TB control.

Almost 50% of the adult population of this region have already been infected with *M. tuberculosis* and are at high risk of developing TB and almost 95% of TB cases and 98% of deaths occur within developing countries, where 75% of cases are within the economically most productive age group (15-49 years) (WHO 2011). On an average, 3-4 months of work time are lost if an adult is ill with TB.

2.1.3.3 TB burden in Nepal

Nepal is a land-locked country of about 26.6 million in 2011 with the population growing at a projected rate of 1.4% (WHO 2011). It is predominantly rural despite an increasingly rapid rate of urbanization from 14% in 2001 to 17% in 2011. Life expectancy at birth continues to increase for both males and females; increasing from 55.0 years for males and 53.5 years for females in 1991 to 67 years and 68 years for males and females respectively in 2009 (WHO 2011).

In the context of Nepal, TB is a well-known disease, recognized and feared for centuries. It has long been important disease in Nepal, reflected in language, culture and history of services. About 45 percent of the total population is infected with TB, of which 60 percent are adult. Every year, 40,000 people develop active TB, of whom 20,000 have infectious pulmonary disease. These 20,000 are able to spread the disease to others (DoHS 2010/11). Treatment by Directly Observed Treatment Short course (DOTS) has reduced the number of deaths; however 5,000-7,000 people still die per year from TB. Expansion of this cost effective and highly successful treatment strategy has proven its efficacy in reducing the mortality and morbidity in Nepal. DOTS have been successfully implemented throughout the country since April 2001. The treatment success rate stands at 90 percent and case finding rate of 73 percent. At the national level 36,951 TB patients

have been registered of whom 15,000 infectious (sputum smear positive new cases) and are being treated under the DOTS strategy in NTP during the FY 2067/68 (2010/2011) (DoHS 2010/11). The male to female ratio is 2:1 possibly because Nepali men are more frequently exposed to infection than women are, and/or women, due to cultural influences, have less access to health care services than men (DoHS 2010/2011).

2.1.4 Extra pulmonary tuberculosis

TB is a disease that can affect any organ and tissue of the body but much less common than pulmonary TB (Kumar et al. 2007). Extra pulmonary TB is most commonly found in the mediastinal lymph nodes, larynx, cervical lymph nodes, pleurae, meninges, central nervous system, spine, bones, joints, kidneys, pericardium, intestine, peritoneum and skin. Extra pulmonary TB occurs more frequently among persons who are infected with HIV, but pulmonary TB remains the most common type of TB in this group worldwide (WHO 2003). Extra pulmonary tuberculosis can be classified as Severe Extra-PTB (e.g., Miliary TB, Meningitis TB, Genitourinary TB, Abdominal TB, Pericarditis TB, Peritionitis TB, Bilateral or Extensive plural effusion TB, Spinal TB) and less severe Extra-PTB (e.g., Lymph node, Pleural effusion (unilateral), Bone (excluding spine), Peripheral joint and Skin TB).

2.1.5 Severity of disease

Bacillary load as reported by the microscopy examination, the radiological extent of pulmonary disease and the anatomical site of disease determine disease severity. A pulmonary TB case is classified as severe if parenchymal involvement is extensive. Meningitis TB, Miliary TB, Pericarditis TB, Peritonitis TB, Bilateral or extensive pleurisy TB, Spinal TB with neurological complication, intestinal and genitourinary TB are severe forms of extra-pulmonary TB. The extra-pulmonary TB are classified as less severe in lymph node, unilateral and non-extensive pleurisy, bone (excluding spine), peripheral joint, and skin TB.

2.1.6 Mode of transmission

TB is an airborne disease. When a patient with pulmonary TB coughs, sneezes, spits or talks, very small droplets containing TB bacteria are released into air. These droplets, which float in the air, if inhaled by another person, may cause infection in his/her lungs. Every person who inhales the droplets will not develop TB disease unless his immunity status is poor. It is estimated that only 10% of infected people will develop the disease. Extra pulmonary TB is virtually never infectious. Transmission generally occurs indoors, where droplets foci can stay in the air for a long time. Ventilation removes droplets foci. Direct sunlight quickly kills TB bacteria, but they can survive in dark for several hours (STC 2006).

Many reports emphasize the importance of droplet nuclei in the transmission of TB. Riley's study confirms that, general, prolonged contact with a highly infectious case was necessary before infection was acquired. At the other extreme, infection may be acquired by single exposure, e.g. in laboratories or postmortem room. Transmission to and from the HIV infected patient is more likely, particularly where cough inducing procedures such as sputum induction or pentamidine nevulization are being employed, recommended precaution should be taken (Seaton et al. 2000). Three types of contacts were identified in the transmission of TB. They are household contact, community contact and biomedical contact. Recent reports of studies using DNA finger printing suggest that person-to-person transmission may account for as many as one-third of new cases of TB in large urban population (Dhungana 2002). It has been reported that high prevalence of TB infection and tuberculin test conversion among close contact of PTB patients and PTB/HIV co-infected patients were less infectious than HIV negative PTB patients (Maharjan 2007).

2.1.7 Laboratory diagnosis of TB

The definitive diagnosis of TB in laboratory is based on the detection of acid-fast bacilli in clinical specimens by microscopy, cultural techniques or by Polymerase Chain Reaction (PCR). Numerous attempts have been made to develop serological tests for the disease with little success (Grange et al. 1998). The diagnosis is established when tubercle bacilli are

identified in the sputum, urine, body fluids, or tissues of the patients. However, developing countries like Nepal where routine use of sophisticated technique is troublesome, must rely on following conventional methods for diagnosis of TB in suspected patients:

- Sputum microscopy
- Sputum Culture
- Tuberculin Test
- CSF investigation
- Examination of lymph node aspirates
- Biopsy.

Specimens:

For the diagnosis of pulmonary TB, the most usual specimen is sputum but, if none is produced, bronchial washings, brushings, laryngeal swabs, and early morning gastric aspirates (to harvest any bacilli swallowed overnight) may be examined. Tissue biopsies are homogenized by grinding in Griffth's tubes for microscopy and culture. Cerebrospinal fluid, pleural fluid, urine and other fluids are centrifuged and the deposits are examined (Grange et al. 1998). Contaminated samples are rejected in the laboratory. Saliva sample is rejected during sputum sample collection. Blood mixed CSF samples are also not accepted for microbiological examination.

a. Sputum microscopy

In high prevalence countries, TB case detection is largely based on microscopic examination of sputum for Acid-Fast Bacilli (AFB). The technical guidelines of WHO and International Union Against TB and Lung Diseases (IUATLD) specify that this should be done by examination of three samples- the first spot, early morning and the second spot. It has been recommended that a minimum of 100 microscopic fields should be examined for maximum yield. A minimum of 10 AFB/100 fields is taken as the threshold for considering a result as positive, a definite case should have at least one such results confirmed by a second smear

examination, a suggestive chest radiograph or alternatively there should be one positive mycobacterial culture result.

In microscopic examination, two types of staining methods are widely used in laboratory to detect AFB in clinical specimens. The staining characteristics of *M. tuberculosis* allow its rapid identification in clinical specimens. The specificity of stains for AFB typically is 99% or more and the sensitivity ranges from about 25% to about 75%. About 95% of infectious cases thus be detected by AFB microscopy. The main value of AFB-microscopy for diagnosis lies in its speed and extremely high specificity, while the main disadvantage is its low sensitivity. A high proportion (75%) of pulmonary cases positive in culture is also positive on smear. But, microscopy cannot distinguish between live and dead AFB, so that some patients excreting non-viable bacilli at the end of treatment may be roughly considered as failure-cases (Duen 2001).

AFB microscopy by Z-N staining method

Z-N staining method is widely used carbol fuchsin method to detect AFB in smears by microscopic examination of specimens. This method is a modification of Ehrlich's (1882) original method. It is also called 'hot stain' method because carbol fuchsin is heated for the better penetration in cell wall of *Mycobacterium*. In Z-N stained smears, AFB typically appears as purple to red slightly curved rods (1-10um X 0.2-0.6 um) that occasionally are beaded or banded but also may appear coccoid or filamentous.

Fluorescence microscopy

This is the screening procedure recommended for those laboratories that posses a fluorescent (ultraviolet) microscope (Bleed et al. 2000). With the flurochrome stain, such as auramine rodamine stain, Mycobacteria fluoresces with rodamine stain, with a bright orange color and can be easily seen on low power microscopy, increasing the sensitivity of the smear. This fluorescence method allows large numbers of specimens to be examined rapidly. When

prepared smear is stained with fluorescent auramine-rhodamine, tubercle bacilli can be seen under usual high (100X) magnification.

b) Sputum culture

Sputum culture is the definitive diagnosis of TB by isolating the causative organisms in pure culture. So, culture remains the "gold standard" for diagnosis of TB (WHO 2003). Diagnosis of TB by culture method is more sensitive than AFB staining method and can reliably find mycobacteria when they are present in a concentration of about 10^3 organisms/ml of specimen. Depending on the decontamination method and the type of culture medium used, as few as ten viable tubercle bacilli can be detected.

Among the different types of media, the routinely used media are Lowenstein Jensen (LJ) and Ogawa. Liquid media are used for sensitivity test, biochemical test and preparation of antigens and vaccines. To prevent overgrowth by contaminants, a cocktail of antibiotics such as PANTA (polymixin, amphotericin, Nalidixic acid, Trimethoprim and Azlocillin) are added to the liquid media (Dhungana 2004).

c) Tuberculin test

The intracutaneous tuberculin skin test is indirect test method for diagnosis of TB and reliable means of recognizing prior mycobacterial infection. It is useful for identifying persons infected with *M. tuberculosis* complex (MTBC), but does not differentiate active disease from infection. Persons infected with MTBC develop a hypersensitivity reaction to proteins of the bacilli, which comprise the skin test reagent-PPD (Purified Protein Derivative) (Bleed et al. 2000). The preferred method of skin testing is Mantoux test, performed by intracutaneous injection of 0.1ml of intermediate strength (5 tuberculin units) PPD-S.

d) CSF investigation

The patients suspected of tubercle meningitis are generally processed for CSF investigation. Tubercle meningitis is common disseminated TB in AIDS. In AIDS patients suspected of tubercle meningitis; AFB staining and culture of CSF is highly appreciable (Bleed et al. 2000). Centrifugation of the CSF followed by microscopic examination of the deposit and culture of that deposit increases the chance of detection of tubercle meningitis.

e) Examination of lymph node aspirates

Persistent generalized lymphadenopathy (PGL) is the first symptom (if present, otherwise asymptomatic) to appear in the progression of HIV infection. Thus early detection of suspected lymph node TB can be made by lymph node aspiration (Dhungana 2004).

f) Biopsy

It is the invasive technique and is used in situation where tubercle bacilli are not frequently shed into body fluids. Isolated involvement of the pleura or other tissues that doesn't communicate externally may be diagnosed through examination of tissue (Rijal 2005). In miliary TB, multiple organs may be seeded with tubercle bacilli. Although organs like lungs and kidneys are frequently involved, in such setting sputum and urine specimens are found to have tubercle bacilli in 20-25% of cases. Tissue examination may be very helpful in such situation. Transbronchial biopsy has been reported to be diagnostic or highly suggestive of TB up to 85% of patients with miliary changes on the chest X-ray in negative sputum study (Rijal 2005). Liver and bone marrow biopsy are important for diagnosis of miliary TB up to 40-90%.

g) New methods for tuberculosis diagnosis

With the recent advances in the laboratory diagnosis of TB; techniques are more directed towards the development of rapid culture identification and drug susceptibility system for use

in TB specialist laboratories. Serological test for the rapid diagnosis of TB that are based on the recognition of serum IgG antibody to selected mycobacterial antigens and that use ELISA techniques have been developed and appear with sensitivity similar to that of sputum microscopy (Bleed et al. 2000). Serology has its greatest application in children and in patients with Extra-Pulmonary tuberculosis where sputum is not available. Gene amplification by Polymerease Chain Reaction (PCR) has been used with great sensitivity and specificity to identify mycobacterial DNA. This technique offers great promise for rapid diagnosis. In reference laboratories with sufficient instrumentation, high-performance chromatographic techniques are capable of rapidly identifying mycobacteria by their characteristic lipids (Issaelbacher et al. 1992). Other newer techniques that have been applied for the diagnosis of TB are as follows:

Bactec 460 TB rapid radiometric Culture System

Bactec 9000 MB System

Septi-Check AFB System

Mycobacteria Growth Indicator Tube (MGIT)

ESP Culture System II and Chromatographic Analysis

Among these different techniques developed, PCR is used extensively for the diagnosis of TB (Forbes and Hicks 1993). PCR enables the amplification of specific sequences of target nucleic acids. It is not only simple and fast, but also very sensitive and specific to amplify even a single molecule of DNA. With the increased incidence of TB and the advent of MDR-TB strains, the demand of PCR is high in developing countries. The PCR microplate hybridization assay was also sensitive enough to detect as little as 1 pg of DNA which is equivalent to approximately three bacilli. Recently, a commercial PCR amplification kit for the detection and identification of *M. tuberculosis* complex bacteria has become available. The target for the PCR is the 16S rRNA sequence. The detection system is based on hybridization with *M. tuberculosis* complex specific capture probe in a microplate format.

h) Radiological diagnosis

For the diagnosis of TB, radiological methods have been the best options for the physicians. However, TB is difficult to diagnose with certainty on an X-ray alone. X-rays are expensive, unreliable as patients are often treated for TB when they do not have it. But X-ray is sometimes needed for difficult individual problems in particular for HIV infection. So, the chest radiograph is an important tool for both diagnosis and evaluation of TB. Multi-nodular infiltration in apical posterior segments of the upper lobes and superior segments of the lower lobes is the most typical lesion of PTB.

Cavitation is frequently present and is usually accompanied by substantial amounts of infiltration in the same pulmonary segments. Laminagrams are very helpful in recognizing satellite nodular lesion, which are characteristics of TB and not usually seen in carcinoma (Issaelbacher et al. 1992). Radiology suffers mainly of a lack of specificity. The place of radiology will thus be restricted to the second-line of diagnosis, in hospitals and used by medical officers for cases that stay negative on repeated smear microscopy (Duen 2001).

i) Clinical diagnosis

Clinical signs and symptoms can be regarded as the basis for the diagnosis of TB. A careful history and physical examination often suggest the diagnosis of PTB before any laboratory test is ordered. Symptoms suggestive of Pulmonary TB (Issaelbacher et al. 1992) are as follows:

a) Persistent cough with expectoration for 2 weeks,
b) Rise of body temperature in the evening,
c) Chest pain,
d) Weight loss,
e) Loss of appetite,
f) Haemoptysis (coughing up of blood in sputum),
g) Lethargy, spontaneous pneumothorax.

h) Chronic cough for 2 weeks or more (usually with haemoptysis) with or without fever and i) chest pain is the principal respiratory symptoms of PTB.

With the progression of PTB, the normal pulmonary architecture is lost. Fibrosis, volume loss and upward contraction are typical. Rales that are accentuated or heard only pottussively are characteristics of apical disease. Amphoric breath sounds may be present with extensive cavitations (Issaelbacher et al. 1992).

The signs and symptoms of Extra-pulmonary tuberculosis depend on the organ involved. Swelling in the neck with or without discharge is symptom of lymph node TB. Symptoms like Headache, fever, drowsiness, confusing and neck rigidity may be suggestive of TB meningitis. Back pain, fever and in some cases swelling of the backbone are symptoms of spinal TB (Issaelbacher et al. 1992).

2.1.8 Identification tests

Identification tests are mainly done to confirm whether the isolate is a tubercle bacilli or mycobacteria other than TB (MOTT). *M. tuberculosis* can be differentiated from MOTT by the growth rate, pigmentation and some biochemical tests. *M. tuberculosis* grow slowly, produce rough, tough and buff colored (not yellow) colonies, reduce nitrite to nitrate, gives niacin test negative and do not produce catalase at 68^0C. Thus a series of biochemical tests in combination with the observation of growth rate and pigmentation characters of *Mycobacterium* aid to encounter *Mycobacterium* species.

2.1.8.1 Growth rate

Observation of growth rate not only helps to separate *M. tuberculosis* from MOTT but also helps to distinguish different members of MOTT. For e.g. *M. avium* complex (slow grower) can be distinguished from *M. fortuitum-chelonae* complex (Rapid Growers). On the basis of growth rate *Mycobacterium* are classified as:

- Slow grower (>7 days)
- Rapid grower (2-3 days)

2.1.8.2 Pigmentation

Presumptive identification of certain pigment producing mycobacteria can be done by the color of their colonies. For e.g. *M. tuberculosis* produce buff colored colonies, *M. kansasii* produce bright yellow or orange colors after 2 weeks of ambient light color whereas MAC (*Mycobacterium* avium complex) do not produce color.

2.1.8.3 Niacin test

Niacin (Nicotinic acid) plays a vital role in the oxidation-reduction reaction that occurs during metabolic process in all mycobacteria. Although all mycobacteria produce niacin, comparative studies have shown that, because of a blocked metabolic pathway, *M. tuberculosis* accumulates the largest amount of nicotinic acid and its detection useful for its definitive diagnosis. Niacin negative *M. tuberculosis* strains are very rare, while very few other mycobacterial species yield positive niacin tests.

2.1.8.4 Nitrate reduction test

With combination of niacin test, nitrate reduction test can be used to differentiate *M. tuberculosis* from other mycobacteria as *M. tuberculosis* is one of the strongest reducers of nitrate among the mycobacteria. Culture isolates to be tested for nitrate reduction should be four weeks old and have abundant growth on Lowenstein-Jensen egg medium are recommended (Dhungana 2004).

2.1.8.5 Catalase test

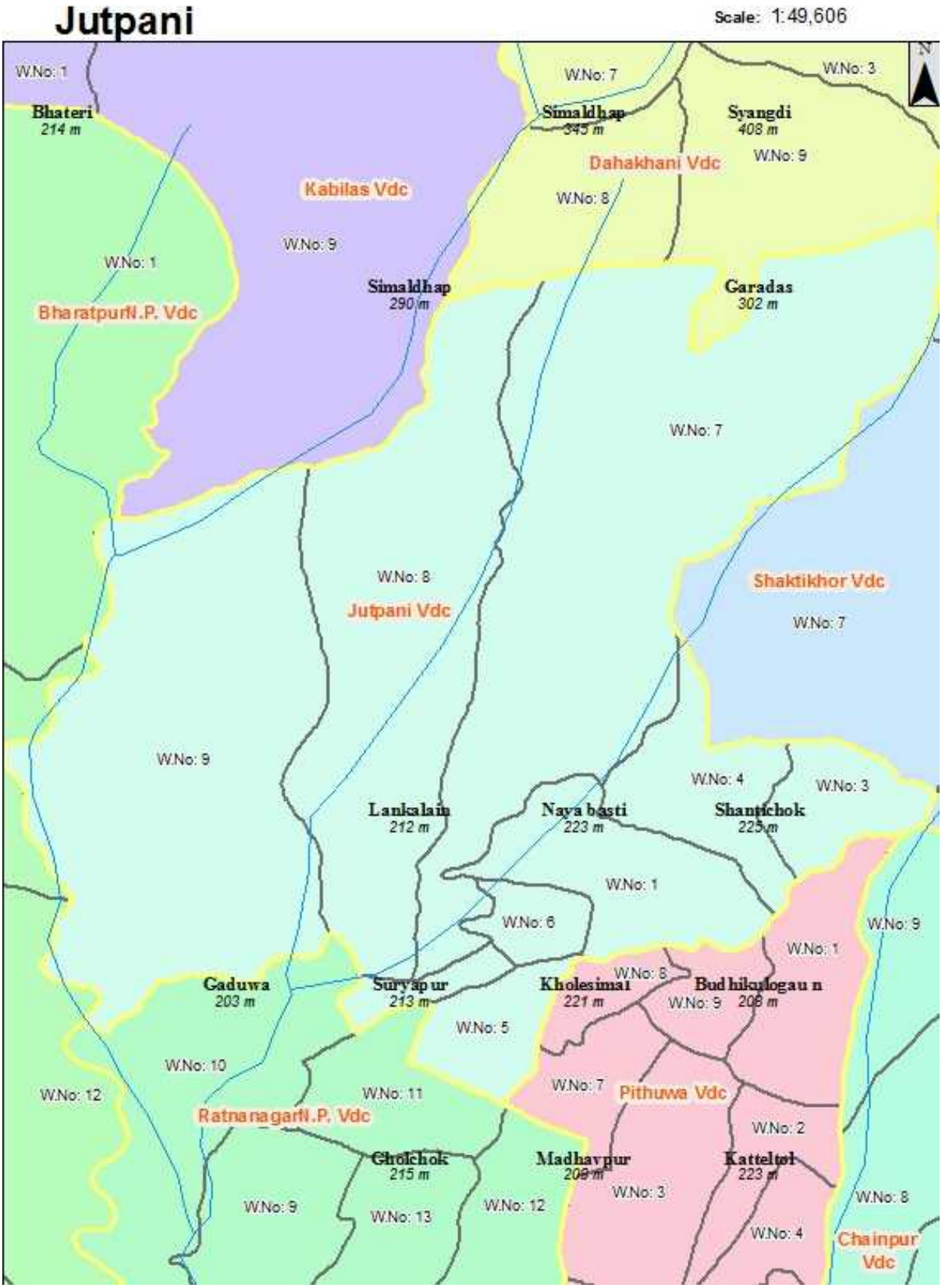
All mycobacteria posses catalase enzymes except for certain isoniazid resistant mutants of *M. tuberculosis* and *M. bovis*. As *M. tuberculosis* loses catalase activity at 68^{o}C, a performance of catalase test at this temperature is done for its identification.

3. MATERIALS AND METHOD

3.1 Study Area

Jutpani village development committee (VDC) lies in Chitwan District in the Narayani Zone of southern Nepal. Among the 40 VDC of Chitwan district, it is one of the famous VDC for tourism. It has the total population of 17,310 (GoN 2011). It is situated at latitude of 27.70 deg. and longitude: 84.42 deg. (Photograph 2 and 3). There are altogether 12 sputum examination centers in the Chitwan district. Jutpani PHC is one of them. The present study area, "Jutpani VDC" lies in among Pithuwa and Shaktikhor VDC in its east, Bharatpur Municipality in its west, Padampur VDC in its north and Ratnanagar municipality in its south. Jutpani Primary Health Center (PHC) is located in Jutpani VDC. It has been running in the official building of Jutpani VDC since last four years.

Photograph 2: Jutpani VDC in the map of Nepal. Source: Google Map



Photograph 3: Map of Jutpani VDC Source: Google Map

3.2 Materials and Chemicals used for the study

1. Equipments

Bunsen burner	Microscope with oil immersion

2. Glass wares and materials

Bamboo sticks	Bottles for reagent
Dropper	Test tube stand
Plastic containers for sample collection	Staining Slides
Forceps	

3. Chemicals

3% Acid alcohol	4% Sodium hydroxide
Basic fuchsin	Ethanol
Hydrochloric acid	Immersion oil
Lysol	Malachite Green
Methylene blue	Phenol

4. For Safety

Discard pans	Disinfectant
Disposable masks	Protective clothing
Single use gloves	

5. Miscellaneous

Cotton wool	Diamond pencil
Marker	Slide drier
Staining racks	Staining Pan and bridge

3.3 Methodology

This study was carried out in 'Laboratory' of Jutpani Primary Health Center (PHC) from July 2012 to December 2012. All TB suspected patients of Jutpani VDC visiting to the PHC with the clinical history of two or more week's continuous cough, fever, and marked weight loss were included in this study. Data analysis was done using SPSS Program (Version-16), Microsoft Excel and chi-square test as statistical tool. The study was designed to assess the situation of TB in the study area.

The study was divided into four phases as shown below:

i.) General screening of TB suspected patients visiting the PHC.

ii.) Family screening of the PTB patients of Jutpani VDC currently undergoing DOTS

iii.) Screening of DOTS completed cases of PTB in the fiscal year 2067/68 and 2068/69.

iv.) Questionnaire survey to assess the Knowledge, Attitude and Preventive practices (KAP) of the PTB patients towards TB.

3.3.1 General screening of TB suspected patients

All the TB suspected patients with the clinical history of two or more week's continuous cough, fever, and marked weight loss visiting to Jutpani PHC from July 2012 to December 2012 were included in this study. Sputum samples of three consecutive days were collected from all clinically suspected patients of TB. The samples were examined for Acid Fast Bacilli (AFB) after staining by Z-N method in the laboratory of Jutpani Primary health center (PHC). TB was diagnosed by the microscopic examination of acid-fast bacilli.

3.3.2 Family screening of TB patient under DOTS treatment

For this purpose, all the PTB patients currently undergoing DOTS treatment were counseled individually and sputum samples of three consecutive days of their all family members were collected and examined in the laboratory of Jutpani PHC for AFB. All family members of PTB patients of Jutpani VDC were included. The children below five years were excluded for this study.

3.3.3 Screening of DOTS completed cases

Sputum samples of three consecutive days were collected from the DOTS completed cases of the fiscal year 2067/68 and 2068/69. The collected samples were examined for AFB in the laboratory of Jutpani PHC.

3.3.4 Questionnaire survey

To assess the Knowledge, Attitude and Preventive practices (KAP) of the TB patients, a questionnaire survey of the PTB patient currently undergoing DOTS treatment as well as DOTS completed PTB patients was done. Only smear positive TB patients were included in the questionnaire survey. Smear negative TB and extra pulmonary TB patients were excluded from this KAP study.

3.4 Diagnosis of PTB

All the patients who were suspected to be infected by TB were subjected for sputum examination. TB was diagnosed by the microscopic examination of acid-fast bacilli by Ziehl-Neelsen staining method. As it is known that, TB is a disease of lungs. It mainly affects lungs than other organs. Acid-fast bacilli are usually detected in expectorated sputum specimens from the patient with active TB. Cases of extra-pulmonary TB were excluded from this study since it was difficult to obtain samples and in most cases, it is self-limited and not frequently transmitted by tubercle bacilli. Moreover it is not frequently diagnosed in DOTS center due to the unavailability of diagnostic tools in the PHC. So, only PTB cases were enrolled in this study.

3.4.1 Specimen collection (sputum sample)

Those patients who were suspected of PTB infection were requested for the sputum sample in the laboratory. Patients were counseled for sputum collection according to the standard methods (WHO 2000). During the collection of sputum sample, patients were instructed to inhale deeply 2-3 times and coughed up deeply from the chest and spitted closer to mouth. It

was made sure that the collected sputum sample is of good quality i.e., thick and purulent and avoid of saliva. About 5 ml of sputum sample (but not saliva) was collected in plastic universal container. Each patient was requested for three consecutive sputum samples in the early morning on three days.

3.4.2 Acceptance or rejection of sputum sample

To eliminate the wrong evaluation, quality control of the sputum was done for possible case; physical examination of sputum sample was done for the detection of presence or absence of mucopurulent portion of sputum. Specimen without muco-purulent portion was rejected if another sampling was possible.

3.4.3 Microscopic examination of sputum

3.4.3.1 Smear preparation and heat fixation

Collected sputum sample was opened carefully and if splitted outside the container, before processing it was decontaminated carefully following standard protocol. A small portion of the mucopurulent material was selected and separated from the remainder with the help of wooden stick and transferred to the slide. Mucopurulent part was separated evenly on a clean slide to a size approximately 1x2 cm. Smear was dried at room temperature completely inside the safety cabinet and was heat fixed by passing through the flame 3-4 times (but shouldn't be over heated).

3.4.3.2 Staining of fixed smears by Ziehl-Neelsen (Z-N) method

1. Heat fixed smear slides were marked with the laboratory serial number and were placed on the staining rack with smeared slide facing upward.
2. The smear was flooded with Carbol Fuchsin stain and heated from below with spirit cotton until the vapor just begins to rise. It was noted that, carbol fuchsin was not allowed to boil or the slide to dry.

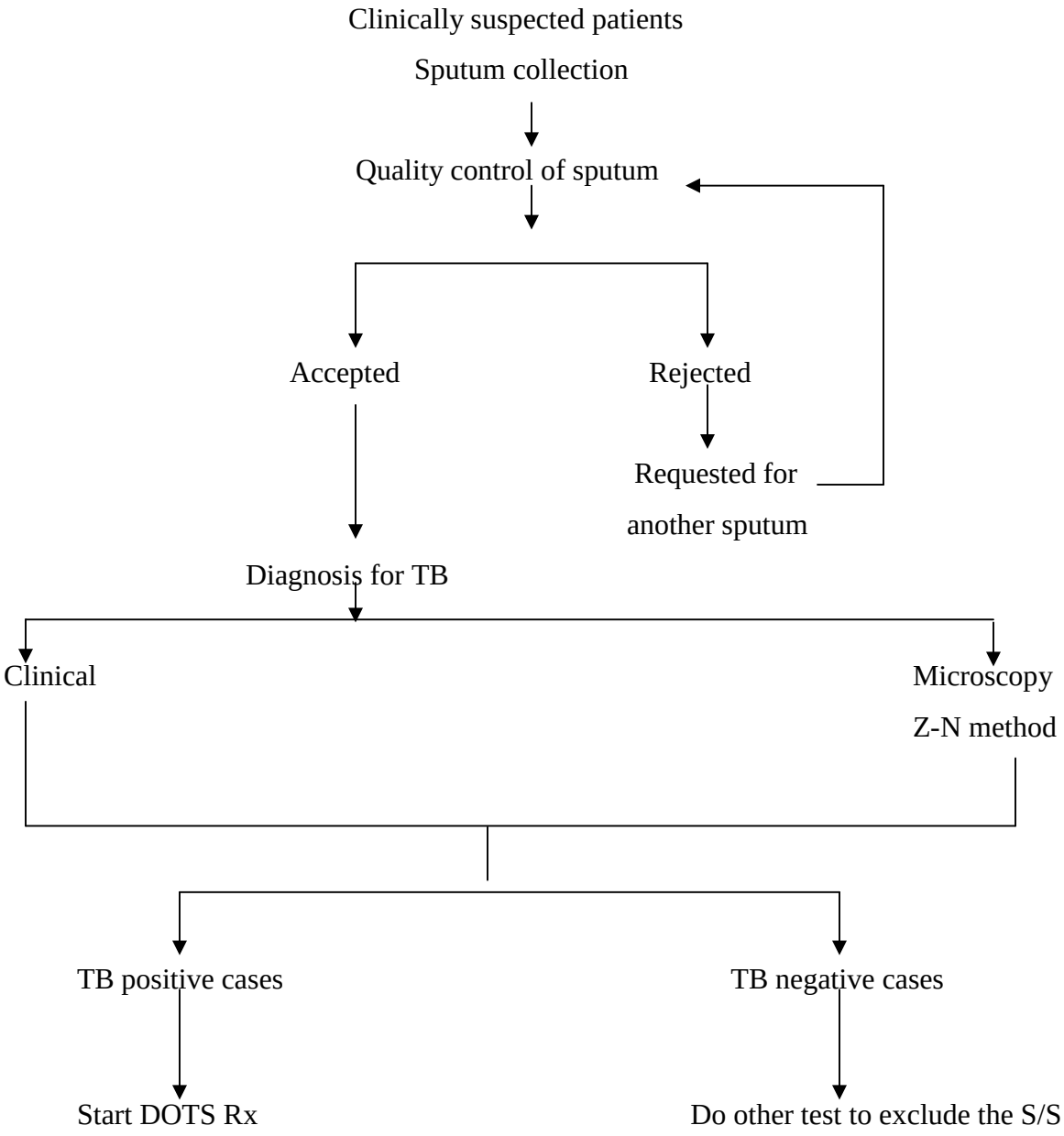
3. Heated Carbol Fuchsin was allowed to remain on the slide for 5-7 minutes and then, the slides were gently rinsed with tap water to remove excess stain and slides were tilted to drain.
4. The smear was covered with 3% acid alcohol for 2-4 minutes or until the smear was sufficiently decolorized i.e. pale pink.
5. Smear was washed off with tap water and tilted to drain.
6. The smear was covered with malachite green (0.5%) or methylene blue for 1-2 minutes.
7. The smear was washed off by tap water and tipped to drain off the water.
8. Backside of the slide was wiped out by cotton and placed at the draining rack.

3.4.3.3 Observation of stained smear

The dried slides were examined microscopically using 40X lens to select suitable area of the slide and then examined using oil immersion objective i.e. 100X. The interpretation of the AFB stain of microscopic examination was done according to WHO/IUATLD protocol.

Photograph 4: Investigator observing Z-N stained slide in microscope

3.5 Flowchart of the methodology



4. RESULTS

4.1.1 Age and sex wise prevalence of PTB

Out of 200 suspected TB cases, 18 (9%) of them were found smear positive and diagnosed as PTB by smear microscopy. Out of 18 smear positive cases, 18 (9%) smear positive patients, 10 (55.56%) were male and 8 (44.44%) were female. The highest prevalence of TB (38.89%) was found in the age group of (30-40) years followed by the age group (20-30) year which was 22.22% of the total smear positivity. Two age groups (10-20) years and (50-60) years were found to have same prevalence of TB (5.89%) of total TB positivity. Age and sex wise prevalence of TB is shown in the Table 1.

Table 1: Age and sex wise prevalence of PTB

Age in years	Male Patients		Female patients		Total slide number	Total positive slides	**Slide Positivity Rate (%)**
	Total slide	Positive slides	Total slide	Positive slides			
<10	4	0	2	0	6	0	**0**
10-20	17	2	17	0	34	2	**5.89**
20-30	13	1	27	3	40	4	**10.00**
30-40	13	3	23	4	36	7	**19.44**
40-50	12	1	13	0	25	1	**4.00**
50-60	20	1	14	1	34	2	**5.89**
60-70	9	1	6	0	15	1	**6.67**
70-80	4	0	2	0	6	0	**0**
80-90	4	1	0	0	4	1	**25**
Total	**96**	**10**	**104**	**8**	**200**	**18**	**9 %**

Source: field survey 2012

4.1.2 Month wise prevalence of PTB patients

Among 18 smear positive cases, the highest prevalence of TB was found in the month of *Asar* (6/18) which was 33.33%, followed by the same prevalence of 22.22% in the month of *Shrawan* and *Ashwin* (4/18). Two months; *Kartik* and *Mansir* had the least prevalence of TB (1/18). In all six months males had the high prevalence of TB than in females except in the month of *Bhadra* where females had three times greater prevalence than in males. Month wise prevalence of TB is shown in the table 2.

Table 2: Month wise prevalence of TB positive patients

Month	Male Patients		Female patients		Total Slide Number	Total positive slide	**Slide positivity Rate (%)**
	Total slide	Positive slide	Total slide	Positive slide			
Aasad	15	4	11	2	26	6	**23.07**
Shrawan	11	1	7	2	18	4	**22.22**
Bhadra	22	2	39	0	61	2	**3.28**
Ashoj	22	1	23	3	45	4	**8.89**
Kartik	10	1	9	0	19	1	**5.26**
Mansir	16	1	15	0	31	1	**3.23**
Total	**96**	**10**	**104**	**8**	**200**	**18**	

Source: field survey 2012

4.1.3 Ward and Sex wise prevalence of PTB patients

The highest prevalence of TB (27.78%) was found in the ward number four (5/18) of Jutpani VDC, followed by the ward number one (4/18) which is 22.22% of total TB positivity. Ward wise prevalence of TB is shown in the table 3.

Table 3: Ward and Sex wise prevalence of total TB positive patients

Ward No.	Male Patients		Female patients		Total slide number	Total positive slides	**Slide positivity Rate (%)**
	Total slide	Positive slides	Total slide	Positive slides			
One	17	2	24	2	41	4	**9.75**
Two	6	0	6	0	12	0	**0.00**
Three	8	1	4	0	12	1	**8.33**
Four	28	3	34	2	62	5	**8.06**
Five	4	1	7	1	11	2	**18.18**
Six	1	0	4	1	5	1	**20.00**
Seven	2	0	4	1	6	1	**16.67**
Eight	19	2	14	1	33	3	**9.09**
Nine	11	1	7	0	18	1	**5.55**
Total		**10**		**8**	**200**	**18**	**9%**

Source: field survey 2012

4.1.4 Month wise Prevalence of New and Follow up cases of TB

Among 200 TB suspected patients, 142 were new cases and 58 were follow up cases of TB under DOTS treatment of various months. Out of 142 new patients 18 (12.68%) of them were new smear positive cases and out of 58 follow up patients, 5 (8.62%) were diagnosed as follow up smear positive TB by smear microscopy as shown in table. 4.

Table No. 4: Prevalence of New and Follow up case of TB

Month of visit	**New case**			**Follow up case**		
	Total	Positive	% positivity	Total	Positive	% positivity
Aasad	15	6	33.33	11	0	0
Shrawan	14	4	22.22	4	0	0
Bhadra	52	2	11.11	9	3	60
Ashoj	32	4	22.22	13	1	20
Kartik	11	1	5.56	8	1	20
Mansir	18	1	5.56	13	0	0
Total	**142**	**18**		**58**	**5**	

4.1.5 Slide positivity in follow up patients of TB under DOTS treatment

Out of 58 follow up cases, 4 patients (2 males and 2 females) were found to be positive in the second month of DOTS treatment which is (80%) of total follow up positivity. Only one patient (20%) was found to be positive in third month of follow up sputum examination. In fifth and sixth month of DOTS treatment, sputum of all TB patients was negative. Slide positivity rate of each month of follow up shown in the table no. 5.

Table No. 5: Slide positivity of follow up cases

Follow up Month	Male Patients		Female patients		Total Number of Slide	Total Number of Positive slide	**Slide positivity Rate (%)**
	Total slide	Positive slides	Total slide	Positive slides			
Second	13	2	11	2	24	4	**80**
Third	3	1	1	0	4	1	**20**
Fifth	9	0	8	0	17	0	**0**
Sixth	8	0	5	0	13	0	**0**
Total	**33**	**3**	**25**	**2**	**58**	**5**	

Source: field survey 2012

4.2 Family members screening of current TB patients under DOTS

During the study, screening of all family member of the TB patient currently undergoing DOTS treatment was done by smear microscopy. Altogether there were total 75 members (33 male and 42 female). All the family member of the patients under DOTS was found negative as shown in the table no. 6.

Table 6: Family member screening of current TB patients under DOTS

Ward no.	Total Number of DOTS patients	Family Members of the DOTS patients Undergoing Treatment				Result of screening of DOTS's family member	
		Male	AFB Positivity	Female	AFB Positivity	Total Family member	Total AFB Positivity
One	4	7	Negative	9	Negative	16	0
Two	2	4	Negative	3	Negative	7	0
Three	0	0	0	0	0	0	0
Four	5	10	Negative	17	Negative	27	0
Five	1	2	Negative	4	Negative	6	0
Six	0	0	0	0	0	0	0
Seven	0	0	0	0	0	0	0
Eight	2	4	Negative	4	Negative	8	0
Nine	2	6	Negative	5	Negative	11	0
Total	**16**	**33**	**Negative**	**42**	**Negative**	**75**	**0**

Source: field survey 2012

4.3 Screening of DOTS completed case in last two years

There were 27 DOTS completed smear positive TB case (PTB) in 2067 and 12 in 2068 till the starting time of this study. During the study period, three consecutive day's sputum of all DOTS completed cases from were collected and examined for AFB. All sputum samples were found to be negative. Among the 5 relapsed cases of TB, 40% (2/5) were form ward number one and 20% (1/5) of relapsed cases were from the ward number four, seven and eight. Relapsed cases of TB were found more in male than in female. 20% of female (1/5) and 80% (4/5) of male were relapsed in last two years as shown in table 7.

Table No. 7: Screening of PTB in DOTS completed cases of fiscal year 2067/68 and 2068/69

Ward Number	Cases of TB in the Fiscal Year 2067/68				Cases of TB in the Fiscal Year 2068/69				Screening for AFB	Percent (%) of TB Cases	**Total No. of Relapse Cases**
	Male TB Patients		Female Patients		Male TB Patients		Female Patients				
	N	R	N	R	N	R	N	R			
One	2	0	1	0	1	2	0	0	Negative	15.4	**2**
Two	1	0	0	0	0	0	1	0	Negative	5.1	**0**
Three	2	0	3	0	1	0	0	0	Negative	15.4	**0**
Four	3	1	0	0	1	0	1	0	Negative	15.4	**1**
Five	1	0	0	0	0	0	0	0	Negative	2.6	**0**
Six	1	0	1	0	0	0	1	0	Negative	7.7	**0**
Seven	0	0	0	1	0	0	0	0	Negative	2.6	**1**
Eight	2	0	0	0	0	1	1	0	Negative	10.3	**1**
Nine	4	0	3	0	2	0	1	0	Negative	25.6	**0**
Total	**16**	**1**	**9**	**1**	**5**	**3**	**4**	**0**		**100%**	**5**
G.Total	17		10		8		4				

Note: N= New smear positive PTB, R= Relapse case of TB **Source:** field survey 2012

4.4 Result based of questionnaire survey for assessing KAP

This sub-section shows the views of TB patients under DOTS about the TB regarding knowledge, symptoms, mode of transmission, treatment, prevention, side effect of the anti tubercular drugs etc.

4.4.1 Age and Gender Distribution of PTB patients in Jutpani VDC

Out of 57 patients, 37 (64.9%) were male and 20 (35.1%) were female. Only 9 (15.8%) of TB patients were unmarried and remaining 48 (84.2%) were married. Maximum PTB patients were married and male TB patients were more than female TB patients as shown in the fig. 1.

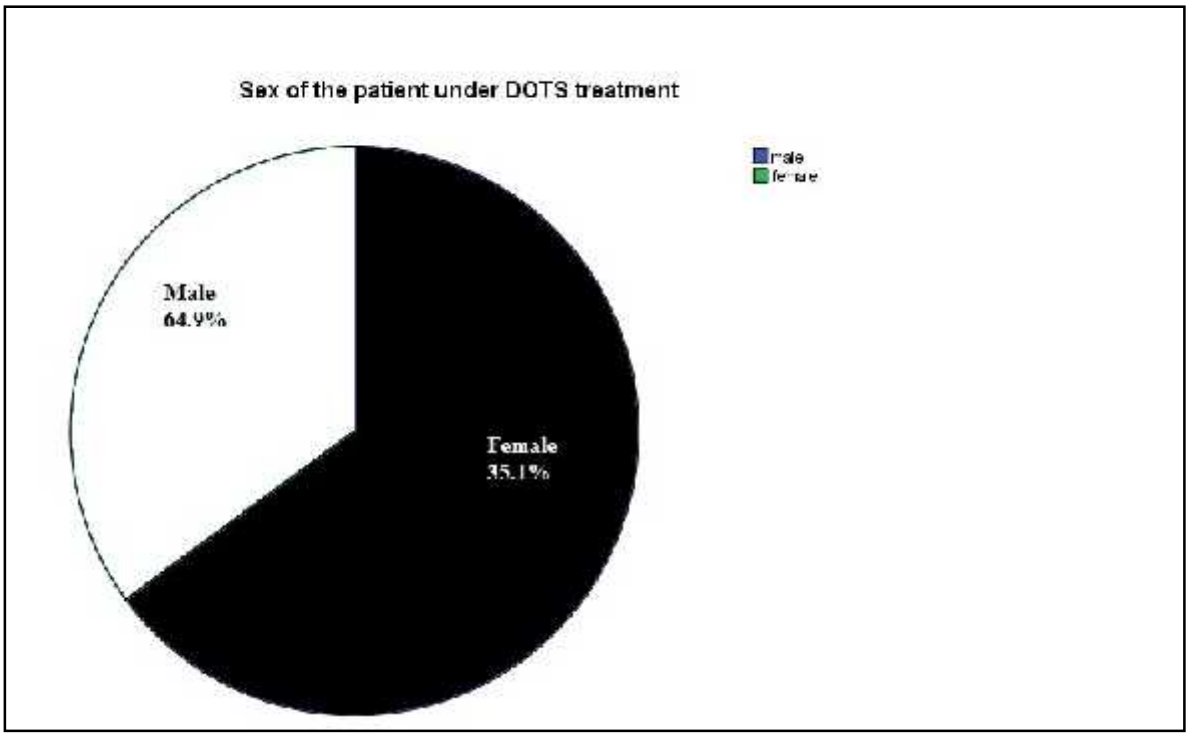


Figure No. 1: Sex of the TB patient under DOTS treatment

Among the studied population, highest percentage of population (19.3%) was in the age group 50-60 followed by 40-50 year age group (17.5%) and third predominant population was from 30-40 year age group with (15.8%). Statistical analysis showed the mean age as 47.28 years and median age as 46 years as shown in fig. 2.

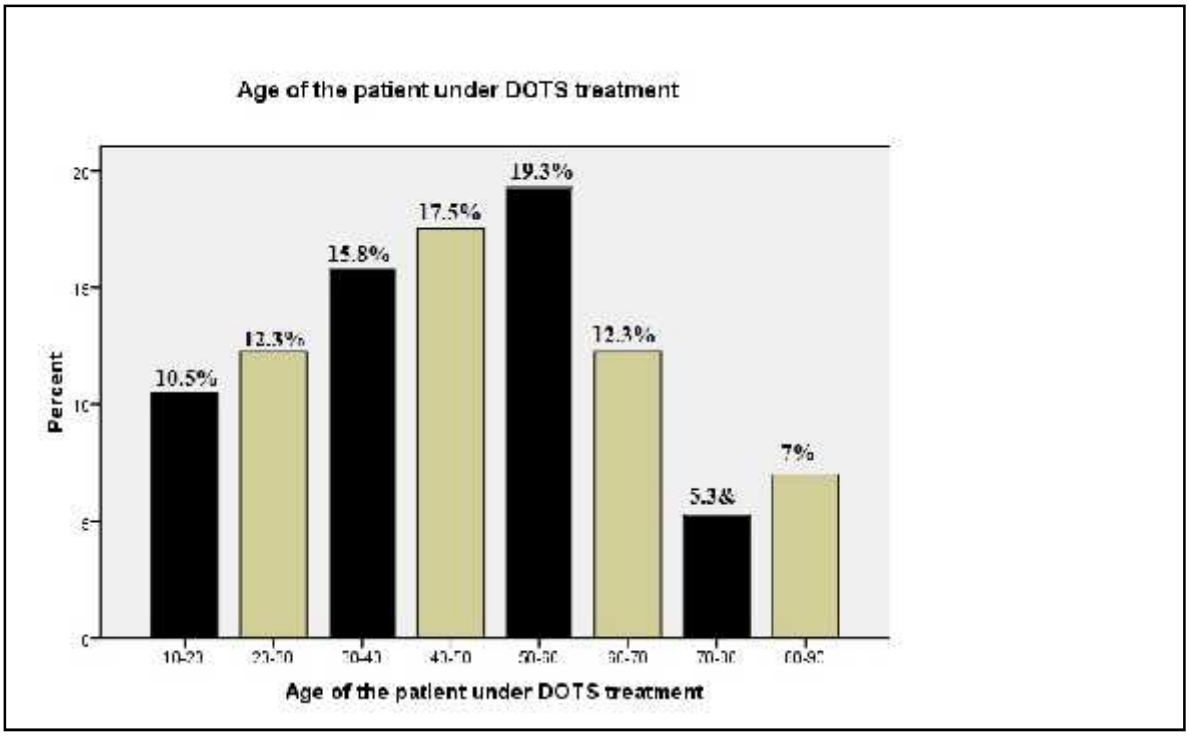


Figure No. 2: Age distribution of the TB patient under DOTS treatment

4.4.2 Educational status of PTB patients

Out of 57 pulmonary positive TB patients of Jutpani VDC 16 (28.1%) were illiterate, 5 (8.8%) were in primary education, 6 (10.5%) were in lower secondary level 12 (21.1%) secondary level 12 (21.1%) were in were in bachelor level 2 (3.5%) were in master level and 4 (7%) were educated informally. Among the studied population, highest percentage of population (28.1%) was illiterate and 21.1% of TB patients were studied up bachelor level education as shown in fig. 3.

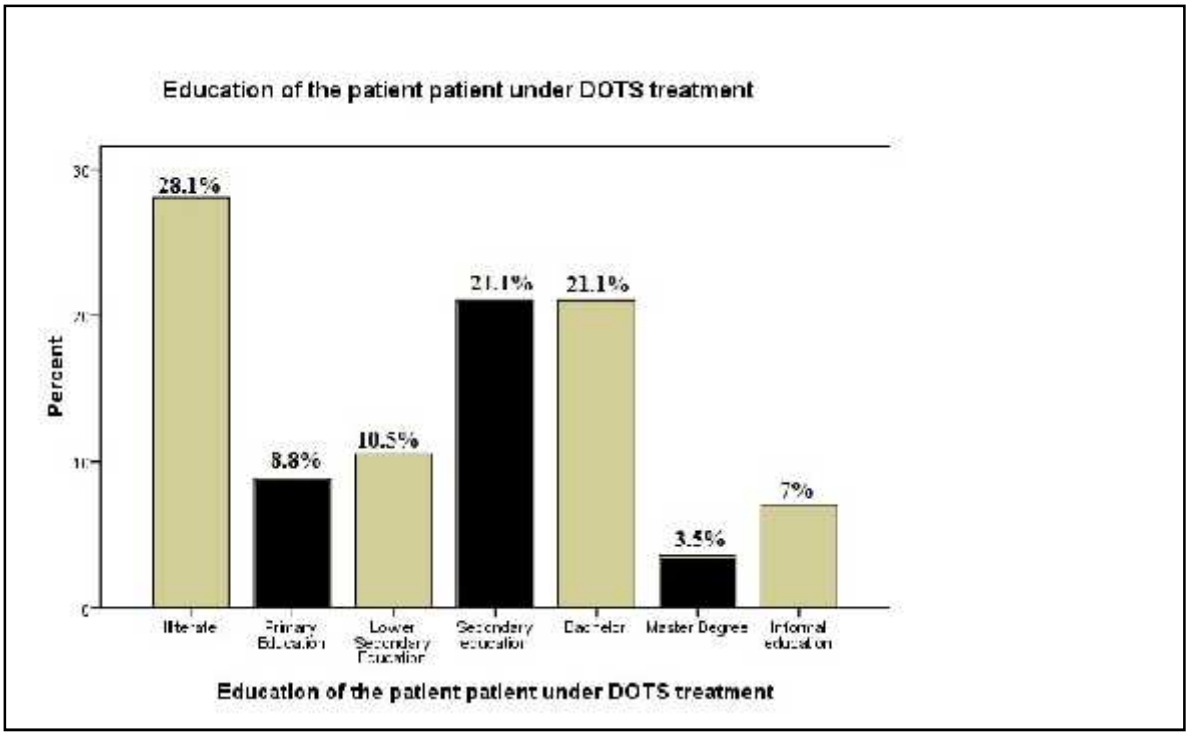


Figure No. 3: Educational status of the TB patient under DOTS treatment

4.4.3 Occupational status of TB patients

Out of 57 TB infected patients, majority of them farmer 28 (49.1%) 12 (21.1%) were student, 5 (8.8%) were teacher, 4 (7%) were businessman and having private sector job, 3 (5.3%) were labor and 1 (1.8%) were having government job as shown in fig. 4.

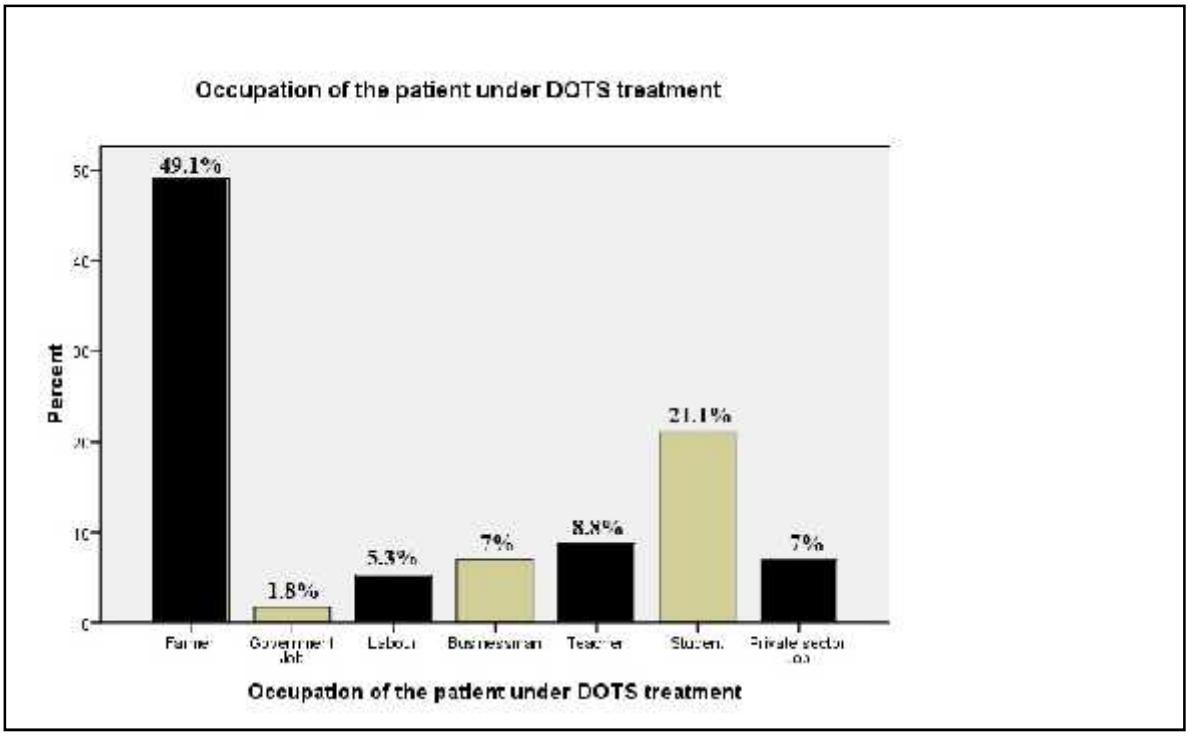


Figure No. 4: Occupational status of the TB patient under DOTS treatment

4.4.4 General knowledge of TB

Out of 57 TB patients under DOTS, 54 (94.7%) had knowledge about the TB while rest only 3 (5.3%) were unknown about it. It is shown in the fig.5.

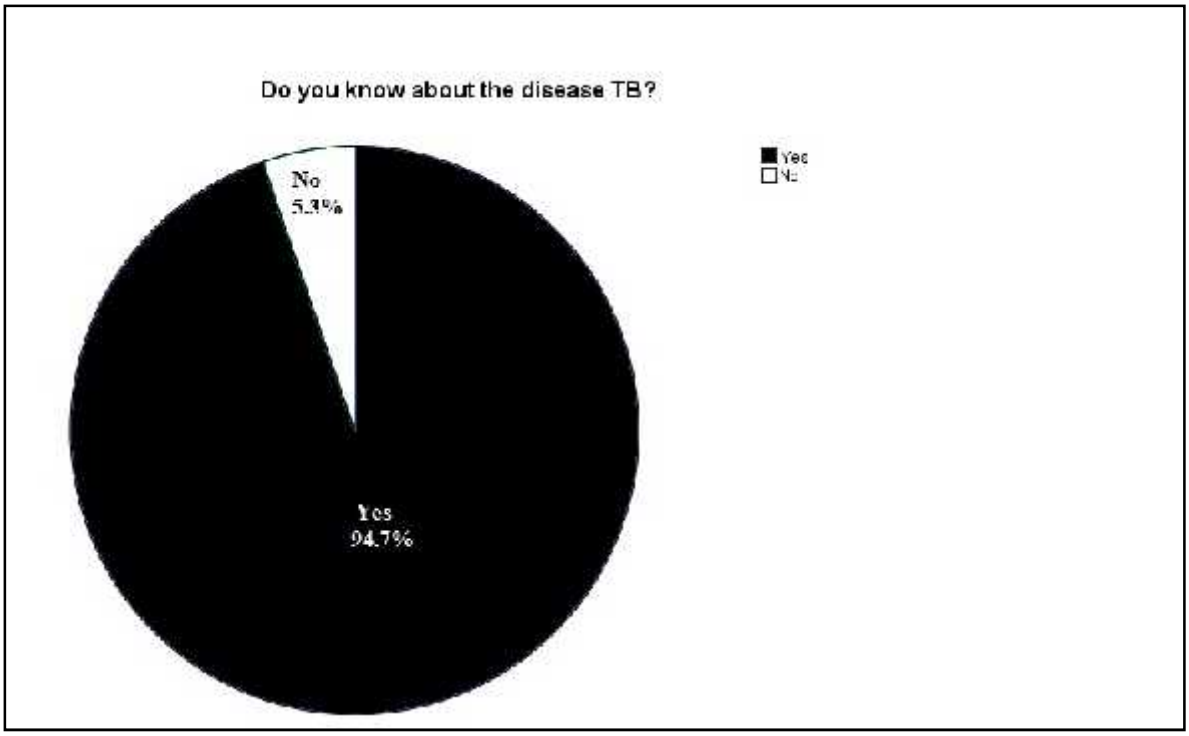


Fig. 5: Knowledge about TB in the patients under DOTS treatment

4.4.5 Knowledge about clinical features/Sign and Symptoms of TB

Out of 57 pulmonary positive TB patients, 46 (80.7%) believed that blood in sputum, 41 (71.9%) believed evening rise in fever, 16 (28.1%) believed weakness and loss of weight and 12 (21.1%) believed continue cough for more than 3 weeks were the symptoms of TB.

Table 8: Knowledge about clinical features of TB.

What are the Clinical features of TB?	**Frequency**	**Percent (%)**
Evening rise of temperature	41	71.9
Weakness/weight loss	16	28.1
Continue cough more than 3 weeks	12	21.1
Headache	2	3.5
Loss of appetite	9	15.8
Blood in sputum	46	80.7
Lose stool	4	7.0

4.4.6 Nature of TB

Among 57 TB patients of Jutpani VDC, 47 (82.5%) believed that TB is a communicable disease and 10 (17.5%) believed that it is non-communicable disease as shown is figure 8.

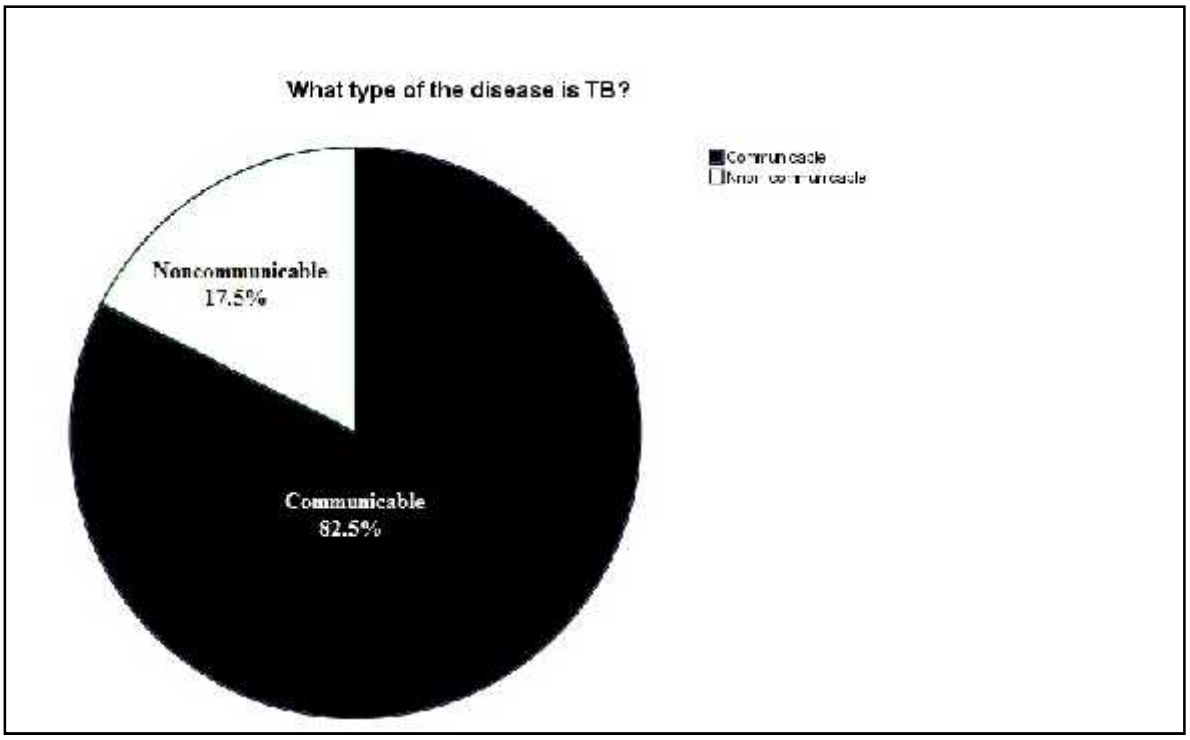


Fig.6: Knowledge about the transmission of TB in the patients under DOTS treatment

4.4.7 Route of transmission of TB

Out of 57 patients, 33 (57.9%) knew that TB is transmitted during coughing, 32 (56.1%) knew that it is transmitted by sharing common things with TB patient and 14 (24.6%) believed that TB is transmitted from person to person by smoking. Only 3 (5.3%) believed TB is air borne disease as shown in the table 9.

Table 9: Knowledge about the route of TB transmission

How does this disease transmitted?	Frequency	Percent (%)
During coughing	33	57.9
Sharing common things	32	56.1
Air borne	3	5.3
Smoking	14	24.6
Consuming alcohol	7	12.3

Source: field survey 2012

4.4.8 Prevention of TB

Out of 57 TB patients, 45 (78.9%) believed that TB can be prevented 10 (17.5%) believed that it can't be prevented and 2 (3.5%) were unknown about it as shown in figure 6.

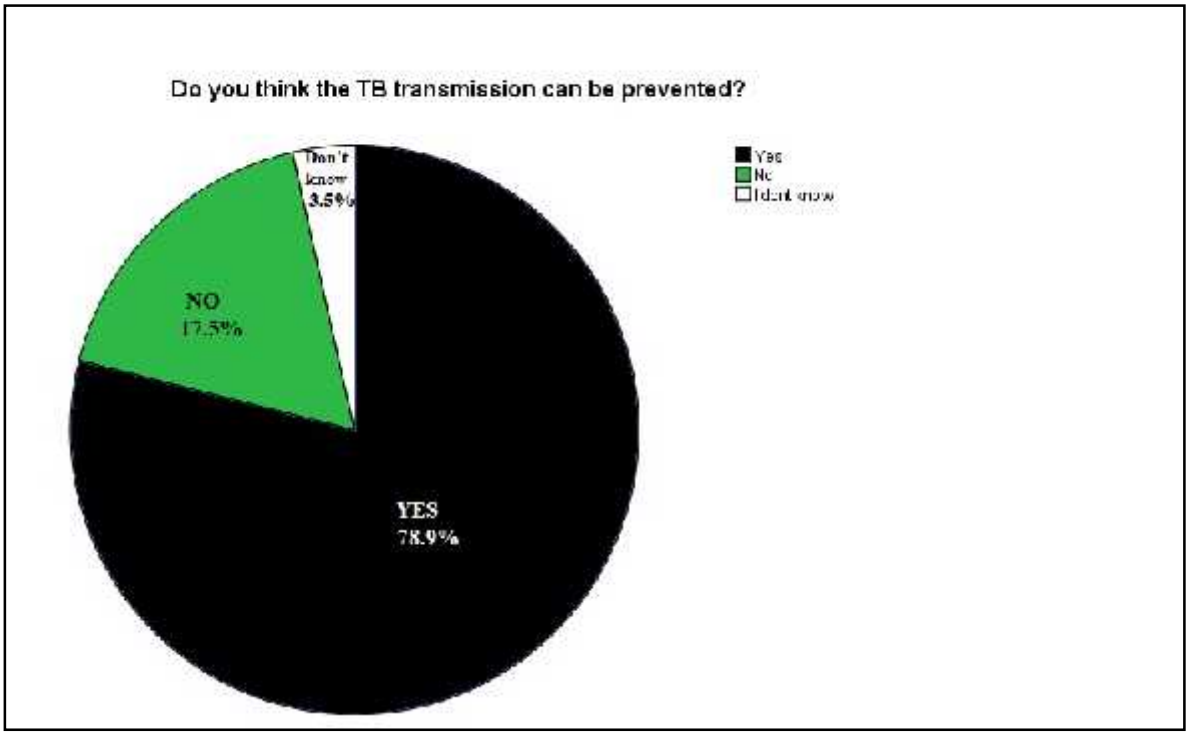


Fig.7: Knowledge about the prevention of TB in the patients under DOTS treatment

4.4.9 Preventive measures of TB

Out of 57 patients, majority of them, 30 (52.6%) believed that TB can be prevented by avoiding the personal contact with the TB patient and few of them 25 (43.9%) believed that It can be prevented from transmission by providing public awareness about the TB and 8 (14%) believed that TB can be prevented by avoiding alcohol. Only 7 (12.3%) believed that personal protection like using mask can prevent TB transmission as shown in table 10.

Table 10: Knowledge about the mode of prevention in the patients under DOTS.

What are the preventive measures of TB?	**Frequency**	**Percent (%)**
Avoiding smoking	20	35.1
Avoid alcohol	8	14.0
Providing the awareness about TB	25	43.9
Avoiding personal contact to the TB patient	30	52.6
Personal protection like using mask etc	7	12.3

4.4.10 Cause of TB

Out of 57 patient, 41 (71.9%) believed that consuming excessive alcohol is the cause of TB and 40 (70.1%) believed that smoking is the cause TB. Only 10 (17.5%) peoples knew *Mycobacterium tuberculosis* is the causative organism of TB. 15 (26.3%) believed that, consuming excessive *surti, bidi, surti* etc is the cause TB as shown in table 11.

Table 11: Knowledge about the cause of TB in the patients under DOTS treatment

What is/are the cause of TB?	**Frequency**	**Percent (%)**
Mycobacterium tuberculosis	10	17.5
Smoking	40	70.2
Alcohol	41	71.9
Surti, *Bidi* etc	15	26.3
Polluted water/environment/food	7	12.3

4.4.11 Side effect of the anti-tubercular drugs

Out of the 57 patients of TB, 32 (56.1%) felt the side effects of anti-tubercular drugs and 25 (43.9%) didn't feel the side effect of the drugs as shown in the figure 7.

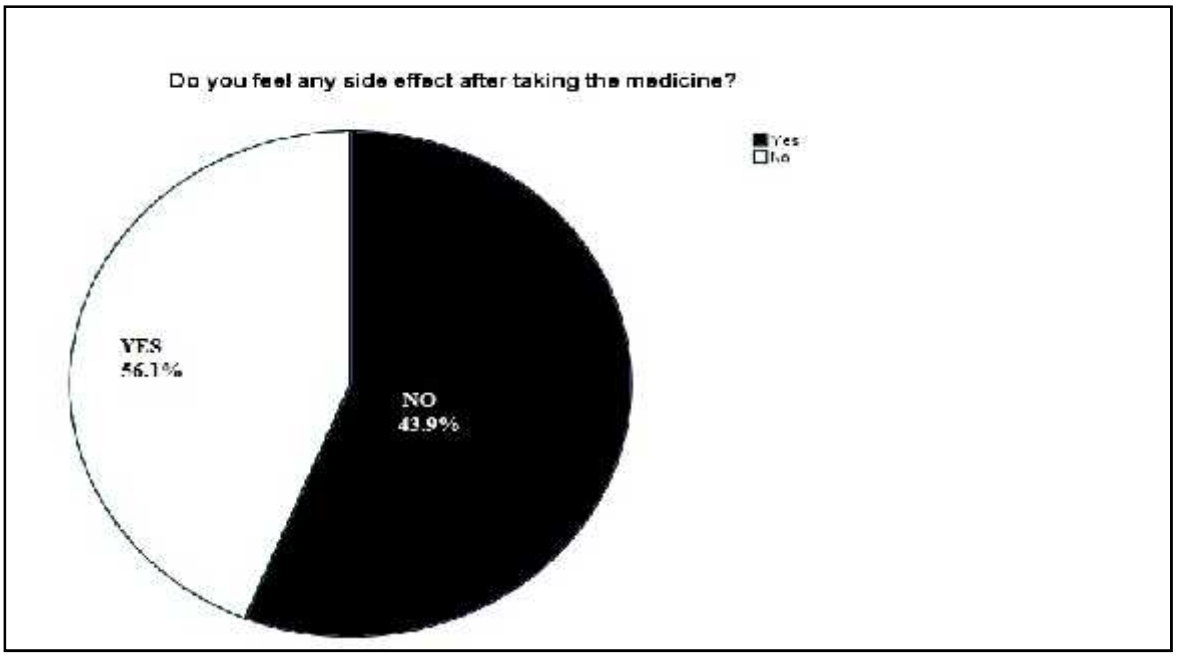


Fig. 8: Knowledge about the side effects of TB drugs in patients under DOTS treatment

Out of the 32 (56.1%) patients asked regarding the side effects of ATD 24 (42.1%) told that nausea/vomiting 17 (29.8%) told abdominal discomfort, 8 (14%) told weakness, 7 (12.3%) told loss of appetite and reduced vision and 4 (7%) told reduced vision were the side effects. The details about their views regarding the side effects of the anti-tubercular drugs are listed in the table 12.

Table 12: Side effects of the anti tubercular drugs in the patients under DOTS

What are the side effects of the medicine?	Frequency	Percent (%)
Loss of Appetite	7	12.3
Headache	6	10.5
Abdominal discomfort	17	29.8
Nausea/vomiting	24	42.1
Reduced vision	7	12.3
Reduced hearing	4	7.0
Weakness	8	14.0

4.5 Attitude of TB Patients

Structured questionnaires were filled up to all 57 TB patients by interview method. They responded differently in different attitude related questions. Among the 57 respondent, majority of them 36 (63.2%) were strongly agree while 8 (14%) of them disagree for that TB is communicable disease. Only 1 (1.8%) were strongly agree that TB is due to past life's bad action while 19 (33.33%) were disagree. Majority of the respondent 41 (71.9%) agree that TB can be prevented but only 2 (3.5%) strongly disagree. More than half of the respondents 33 (57.6%) agreed to the statement that 'if one family member is infected, other family members will also develop TB.

Majority of patients believed that alcohol (71.9%) and smoking (70.2%) is cause of TB. Also 7 (12.3%) of patients strongly believed that after completing medicine smoking, alcohol etc could be taken. 38 (66.7%) of patient agreed that probability of TB is equal in smoker and non-smoker. And 31 (54.4%) patients disagree that after completing the full course of medicine smoking, alcohol etc could be taken. In this study 23 (40.4%) of patient disagree that TB was a disease of poverty while only 4 (7%) of patient strongly agree and 8 (14%) strongly disagree that TB was a disease of poverty. In our study, more than half of the respondents 33 (57.6%) agreed to the statement that TB can be re-occurred even if you have completed the full course of medicine as listed in the table no. 13.

Table No. 13: Attitude of DOTS patients towards TB

S. N.	Attitude of DOTS Patients	Strongly Agree	Per cent	Agree	Per cent	Disagree	Per cent	Strongly Disagree	Per Cent
1	TB is communicable disease	36	63.2	13	22.8	8	14.0		
2	TB is due to past life's bad action *(purba janma ko paap)*	1	1.8	19	33.3	19	33.3	18	31.6
3	TB can be prevented	4	7.0	41	71.9	10	17.5	2	3.5
4	*Dhami/jhakri* can also treat TB	2	3.5	13	22.8	22	38.6	20	35.1
5	Regularly taking medicine is must for the complete treatment of TB.	31	54.4	26	45.6	_	_	_	_
6	One never dies from TB	1	1.8	28	49.1	23	40.4	5	8.8
7	We should not discontinue the medicine even if the symptom is relived	11	19.3	35	61.4	10	17.5	1	1.8
8	We should not discontinue medicine even if s/s is relieved?	15	26.3	35	61.4	6	10.5	1	1.8
9	After completing the full course of medicine, smoking, alcohol etc can be taken	7	12.3	10	17.5	31	54.4	9	15.8
10	Probability of TB is equal to smoker and non-smoker			38	66.7	16	28.1	3	5.3
11	Lungs is the only organ affecting TB	6	10.5	17	29.8	19	33.3	15	26.3
12	If one family member is affected by TB, other family member or your friends may get it.	15	26.3	33	57.9	8	14.0	1	1.8
13	TB can be re-occurred even if you have completed the full course of medicine.	18	31.6	33	57.9	5	8.8	1	1.8
14	TB is a disease of poor people	4	7.0	22	38.6	23	40.4	8	14.0
15	DOTS is very effective for TB patients.	39	68.4	15	26.3	_	_	3	5.3

Source: field survey 2012

4.6 Secondary data of TB

During the study period, secondary data of Jutpani VDC was collected from the PHC and analyzed by using SPSS (version 16.00).

4.6.1 Ward wise prevalence of all types of TB in the year 2067/68 and 2068/69

Out of 51 TB cases in the fiscal year 2067/68, highest prevalence of TB 27.45% (14/51) was found in the ward number nine of Jutpani VDC following the ward number four 13.72% (7/51). But in the fiscal year 2068/69, ward number 8 had highest prevalence as 29.78% (14/47) followed by the ward number four 17.02% (8/47). In both fiscal years, ward number five had the least (3/51=5.88%) in fiscal year 2067/68 and (1/47=2.13%) in the fiscal year 2068/69. The ward wise prevalence of TB is shown in table no.14.

Table 14: Ward wise prevalence of different types of TB in the year 2067/68 and 2068/69

Ward No. of TB patient	Cases of TB in the Fiscal Year 2067/68				Cases of TB in the Fiscal Year 2068/69			
	Pulmonary Positive (P+ve)	Pulmonary Positive (P-ve)	Extra Pulmonary (EP)	Total TB Case	Pulmonary Positive (P+ve)	Pulmonary Positive (P-ve)	Extra Pulmonary (EP)	**Total TB Cases**
One	3	1	1	5	5	1	1	**7**
Two	2	1	0	3	1	1	0	**2**
Three	4	1	1	6	3	1	1	**5**
Four	5	1	1	7	7	0	1	**8**
Five	2	1	0	3	0	1	0	**1**
Six	4	0	0	4	1	1	1	**3**
Seven	2	2	1	5	2	0	0	**2**
Eight	2	2	0	4	7	4	3	**14**
Nine	10	4	0	14	4	0	1	**5**
Total	**24**	**13**	**4**	**51**	**30**	**9**	**8**	**47**

Source: Secondary data collected from Jutpani PHC during the study period.

4.6.2 Month wise prevalence of smear positive, negative & Extra-pulmonary TB

From the secondary data of the last two year, prevalence of TB cases in the fiscal year 2067/68 was 51. Among them 66.67% (34/51) was pulmonary positive 25.49% (13/51) pulmonary negative and 7.84% (4/51) was extra-pulmonary. Highest prevalence of TB was seen in the month of *Baishakh* 11.74% (6/51) followed by the month of *Shrawan* and *kartik* 9.8% (5/51). Similarly in the fiscal year 2068/69, total prevalence of TB cases was 47. Among them, 63.82% (30/47) were pulmonary positive TB 19.15% (9/47) were pulmonary negative and remaining 17.02% (8/47) were extra pulmonary TB. Month wise prevalence of each type of TB in respective fiscal year of 2067/68 and 2068/69 is shown in the table no.15.

Table 15: Month wise prevalence of TB in the fiscal year 2067.68 and 2068.69

Month	Cases of TB in the Fiscal Year 2067/68				Cases of TB in the Fiscal Year 2068/69			
	Pulmon Positive (P+ve)	Pulmonary Positive (P-ve)	Extra Pulmonary (EP)	**Total TB Case**	Pulmonary Positive (P+ve)	Pulmonary Positive (P-ve)	Extra Pulmonary (EP)	**Total TB Cases**
16 Asar	2	1	0	**3**	2	1	1	**4**
Shrawan	5	0	0	**5**	2	1	0	**3**
Bhadra	2	1	0	**3**	0	0	0	**0**
Ashwain	2	1	1	**4**	4	0	0	**4**
Kartik	3	1	1	**5**	2	3	3	**8**
Mansir	4	1	1	**6**	1	1	0	**2**
Paush	3	1	0	**4**	1	2	0	**3**
Margh	1	3	0	**4**	1	0	1	**2**
Faglgun	2	0	0	**2**	1	0	3	**4**
Chaitra	0	0	0	**0**	1	1	0	**2**
Baisakh	5	2	1	**8**	7	0	0	**7**
Jestha	2	2	0	**4**	6	0	0	**6**
Asar 15	3	0	0	**3**	2	0	0	**2**
Total	**34**	**13**	**4**	**51**	**30**	**9**	**8**	**47**

Source: Secondary data collected from Jutpani PHC during the study period.

4.6.3 VDC wise Total TB cases in Jutpani PHC in fiscal year 2068/69

In the fiscal year 2068/69, Jutpani VDC (47) had the highest prevalence of all types of TB followed by Padampur VDC (28) as shown in the table 16.

Table No. 16: VDC wise Total TB cases in Jutpani PHC in fiscal year 2068/69

Months	Jutpani	Padampur	Pithuwa	Shaktokhor	Dahakhani	Siddhi
Shrwan	3	6	0	6	1	0
Bhadra	0	1	0	0	0	0
Ashoj	4	0	1	2	0	0
Kartik	8	1	1	1	1	1
Mansir	2	2	0	2	0	0
Poush	3	0	0	2	0	1
Magh	2	2	0	0	0	0
Falgun	4	3	1	4	0	0
Chaitra	2	4	0	2	2	0
Baisakh	7	3	2	1	1	1
Jestha	6	6	1	5	0	0
Asar	6	0	1	1	0	0
Total	**47**	**28**	**7**	**26**	**5**	**3**

Source: Secondary data collected from Jutpani PHC during the study period.

4.6.4 VDC wise total TB cases in the fiscal year 2067/68

In the fiscal year 2067/68, also highest prevalence of all types of TB was found in the Jutpani VDC (51 cases of TB) followed by Padampur VDC (24 cases of TB) as shown in the table 17.

Table No.17: VDC wise total TB cases in the fiscal year 2067/68

Months	**Jutpani**	**Padampur**	**Pithuwa**	**Shaktokhor**	**Dahakhani**	**Siddhi**
Shrwan	5	1	1	3	1	1
Bhadra	3	1	1	2	0	0
Ashoj	4	1	0	1	0	0
Kartik	5	3	1	2	0	0
Mansir	6	3	1	0	0	0
Poush	4	1	2	0	0	1
Magh	4	1	0	0	0	1
Falgun	2	0	0	0	0	0
Chaitra	0	4	1	1	1	0
Baisakh	8	4	1	2	0	0
Jestha	4	2	0	0	1	1
Asar	6	3	0	0	0	0
Total	**51**	**24**	**8**	**11**	**3**	**4**

Source: Secondary data collected from Jutpani PHC during the study period.

5. DISCUSSION

TB remains a major global public health problem (WHO 2002). It was estimated that about one-third of the world's population was infected with *Mycobacterium tuberculosis* (Sudre et al. 1992). With a population of about 30 million, Nepal has an estimated incidence of 163 and prevalence of 238 per 1, 00,000 populations (all types of TB) in 2010, according to a World Bank report published in 2012. The notification rate of all forms of TB was 117 and new smear-positive case was 52 per 1, 00,000 in Nepal (WHO 2011). Nepal was a high-burden country for TB. About 45% of the total population was infected with TB and an estimated 20,000 new infectious cases of TB are reported each year (NTC 2000/2001).

In this study, it was intended to estimate prevalence of pulmonary TB, since detection of other forms of TB was technically and operationally not feasible in the DOTS centre at PHCC level. Because extra-pulmonary TB in most cases, are self-limited and not frequently transmitted by tubercle bacilli; it was not frequently diagnosed in DOTS center and in most of the TB health centers.

The main objective of this study was to determine the prevalence of pulmonary TB among the suspected patients visiting Jutpani Primary Health Care Center (PHCC). During the study period, a total of 600 sputum samples were collected from 200 TB suspected patients and examined in the Laboratory of Jutpani PHCC. After staining the sputum smear by Z-N staining method and examining under light microscope 18 (9%) samples were found to be positive for acid fast bacilli (AFB). That means prevalence of TB was found to be 8% among 200 Tb suspected patients. Similar study carried out by Smith (1996) found that out of 1630 samples, 78 (4.8%) were smear positive for AFB which was less than the present study.

Age wise observation of the smear positivity showed that the highest prevalence of TB 38.89% (7/18) was found to be in the age group of 30-40 years followed by 20-30 years where prevalence of TB patients was found to be 22.22% (4/18). Age group of 10-20 and 50-60 years has been found to have same TB prevalence i.e. 11.11% (2/18). This showed that

the majority of TB patients were in the productive age group which was in accordance with the TB report of South-East Asia Regional Report on TB published by WHO (2012) which stated that most cases continue to occur in the most productive age-group of 25-54 years, with males being disproportionately affected.

A similar study conducted by Dhungana (2002) at United Mission Hospital Tansen (UMHT) and Dhungana (2004) in TUTH had found the highest prevalence of TB among age group of 20-30 years. The highest prevalence of TB among this age group might be due to the exposure of young people to different environment during their work and activities that would make their health more prone to infection by TB organisms. Sudre et al. (1992) described that in developing countries; about 70% of TB patients were under 50 years age group which was similar to the present study. In this age group many person smoke and drink excessively and travels different places for many purposes which increases the susceptibility for TB. Highest prevalence of TB in the age group 30-40 was because people of this age group are exposed to the outer environment as well as due to high work load and wide range of mobility. So peoples of this age group are more prone to the infection with TB organisms. Due to the higher prevalence of TB infection in 30-40 years (38.89%) and in 20-30 years (22.22%), it harms adversely not only for the diseased person but also for their family members and ultimately to the society and then to country. Majority of the affected population was of productive age group. So, it was greatly affecting the economic and social status of country.

Sex wise prevalence of TB showed that out of 18 smear positive cases, 10 (55.54%) male and 8 (44.46%) female. WHO (2012) also showed the similar result in the South-East Asian region. Similar type of study was conducted in collaboration with WHO in the Kolin district, South Africa, to determine the epidemiological situation of TB during the period of 1965-1972. In that study, among 504 persons, 379 (75%) peoples were diagnosed smear positive. Out of these 379 cases 220 (58%) were male and 159 (42%) were female. The majority of new cases were found to be in the middle age group and elderly person which was similar to the present study. Similar study done by Smith et al. in 1994, also found the higher prevalence of TB infection in males than in females from tuberculin survey carried out in

Gorkha district, Nepal. Smith (1996) also reported that in most countries of the world, in Nepal also, there was higher incidence of TB in men than in women. A study done by Shrestha (1989) in histo-pathological specimen at TUTH, Kathmandu, also found the similar result (47% of male and 53% female). A similar study done by Tamrakar (2002) in Ramechhap District Nepal, Sharma (2008) in Gorkha District Nepal and Joshi (2004) in Patan hospital also found the similar result. It was possibly because Nepali men are more frequently exposed to infection than women are, and/or women, due to cultural influences, have less access to health care services than men (NTP 2010).

Female/male ratio of less than one was observed among the TB suspects undergoing sputum examination in all countries except in Pakistan where it was more than one (STC 2006). Significantly higher sputum positivity among male TB suspects reported in India, Nepal, Sri-Lanka and Bangladesh (STC 2002). With the objective to assess the gender differences in TB suspects undergoing smear microscopy and smear positivity, the project conducted by STC (2001), 61% in male and 39% in female with overall female/male ratio of 0.6 which was similar to that of the present result which showed the ratio of female/male 0.8. The reason behind this gender differentiation in TB infection might be due to exposure of male to the external environment more than female. Exposure to the external environment might be during their daily activities as according to our social rules and norms, males are supposed to do their job outside the home while females are more restricted at home. From the occupational status of TB infected patients also, higher percentage of patients has been actively involving in jobs that need to get exposed to outer environment to greater extent and need to spend more time out of home. When male members of family gets ill, they have quick and easy access to the clinics/doctors and visit the health centers independently whereas female members of family has to depend on other male or senior members of family to have access to health centers as well as they can't freely express their health problems. According to NTC (2000), women visit to modern health care facilities less than men because of social pressure or stigma like to most developing countries of world. TB can be regarded as a symptom of poverty, instigated by unequal distribution of resources. However, poverty itself within a society was not distributed equally among its social classes and between the two sexes. Estimates show that 70% of the world's poor are women. Poverty and

genders are two key factors implicated in a women's vulnerability to TB. Hence, it could be assumed that women's poor health seeking behavior perhaps be a possible reason for low case detection in TB. Nakanishi and Shrestha (1990) conducted a study in Sunsari and Morang district in 125 healthy subjects, in collaboration of Nepal National Planning Commission (NPC) and Unicef found that immunoglobulin M (IgM) and immunoglobulin G (IgG) concentration found in significant higher in female than in male. The higher concentration of these antibodies in female has protective value against the infection. Hence low prevalence of TB among female than in male was observed. In another study done by Onozoki (2003), among 754 patients from the two districts of 'Dhading' and 'Chitwan reported 454 (60%) were new smear positive PTB and out of smear positive new cases of PTB, 354 (70%) were males and 134 (30%) were females, the mean age was 36.5 years. This finding of present study was similar to the various studies done in different part of the country and out of the country.

Prevalence TB was highest in the month of *Asar* 33.33% (6/18) followed by the month of *Shrawan* and *Bhadra* which had the similar prevalence of 22.22% (4/18) and lowest in the month of *kartik* and *mansir* 5.56% (1/18). The reason of high prevalence of TB in the month of Asar might be due to high incidence of cough and cold which might help in the transmission of PTB during coughing in the month due to seasonal change. More over a study done by Dhital (2007) in CMCTH found that the month of *Asar*, *Shrawan* and *Bhadra* had more incidence of upper respiratory tract infection than other months of the year which support the finding of the present study. Secondary data of the same VDC in the fiscal year 2067/68 also showed the similar result of high prevalence of pulmonary TB 14.7% (5/34) in the month of *Shrawan* followed by 11.74% (4/34) in the month of *mansir*. (Table No. 7). Table no. 16 and 17 showed that among the six VDCs under Jutpani PHC, the highest prevalence of TB was found in Jutpani VDC followed by Padampur VDC in the both fiscal year 2067/68 and 2068/69. Present study suggested that ward wise prevalence was highest in the ward number no. four and lowest in ward no. two. Out of total 18 positive cases, 5 (27.78%) were from ward number four of Jutpani VDC and 0 (0%) were from ward number two. Second highest prevalence of TB was found in ward number one (22.22%) followed by ward number eight (16.67%). Similar data were obtained from the secondary data collected

from Jutpani PHCC during the study. Ward wise prevalence of pulmonary TB in the fiscal year 2068/69 showed that ward number four and eight had the highest prevalence (23.33%) of PTB followed by ward number one (16.67%). But in the fiscal year 2067/68 ward number nine had the highest (41.67%) prevalence of TB followed by ward number four (20.8%) as shown in table 6. From the table no. one, three and seven, it was cleared that out of 18 smear positive cases three were relapsed cases (16.67%). Among these relapse cases two were from ward no. one and one from ward no. eight of the Jutpani VDC.

Present study revealed that, the highest prevalence of TB were found among illiterate people (28.1%) followed by lower secondary level education (21.1%). This showed that educational status of the person affects the TB occurrence and transmission. Similarly highest prevalence of PTB was found among farmer (49.1%) followed by people in service (22.80%). This showed that occupation of the individuals also plays a major role in the prevalence of TB. Lifson et al. (1999) stated that the risk of TB was greater in areas of residence characterized by crowding, poverty and lower education. In another study done National TB Center also found that majorities of the respondents (67.8%) were employed in the agriculture sector as farmers (NTC 2009).

Out of 57 TB patients interviewed, majority of them, 30 (52.6%) believed that TB can be prevented by avoiding the personal contact with the TB patient. A similar study conducted in Sindhupalchok district by Ministry of Health and Population, National TB Centre in collaboration with "Britain Nepal Medical Trust" in 2009, found that about 40% of the respondents in the study areas believed that the infection could be prevented by 'covering mouth and nose while coughing and sneezing'. But in present study showed that only 7 (12.3%) believed on personal protection like using mask could prevent TB transmission. It showed that the knowledge about TB prevention was poor among the TB patient of this VDC as compared to the people of Sidhupalchok district.

Out of 57 patient, 41 (71.9%) believed that consuming excessive alcohol was the cause of TB and 40 (70.1%) believed that smoking was the cause TB. Only 10 (17.5%) peoples knew *Mycobacterium tuberculosis* was the causative organism of TB. A study conducted by Yadav

et al. (2006) in Rajasthan reported that only 1.6% knew that TB was caused by bacteria, which was very less than the present study where 26.3 % have the knowledge about the causative organism of TB as *Mycobacterium tuberculosis*. From the secondary data since last two year, prevalence of TB in the fiscal year 2068/69 was 51. Among them 66.67% (34/51) was pulmonary positive 25.49% (13/51) pulmonary negative and 7.84% (4/51) was extra-pulmonary. Similarly in the fiscal year 2067/68, total prevalence of TB cases was 47. Among them, 63.82% (30/47) were pulmonary positive TB 19.15% (9/47) were pulmonary negative and remaining 17.02% (8/47) were extra pulmonary TB. But in present study of 6 months found that out of 22 all types of TB cases 18 (81.81%) were pulmonary positive, 3 (13.63%) were pulmonary negative and only 1 (4.5%) was extra-pulmonary. This showed that incidence of smear positive TB was highest among all forms of Tb and was increasing from last two years in the present study area.

Similar type of study was conducted in collaboration with WHO in the Kolin district South Africa to determine the epidemiological situation of TB during the period of 1965-1872. Among 504 persons, 379 (75%) peoples were diagnosed pulmonary positive. In another study done by Onozoki (2003), 754 patients from the two districts of 'Dhading' and 'Chitwan were enrolled. Among them 454 (60%) were new smear positive PTB. National data on type of TB as published by WHO is, 45% (15,000) new smear positive 29% (9,662) smear negative and 23% (7,484) Extra-pulmonary (WHO 2011). This showed that present study area had high prevalence of smear positive; smear negative and extra pulmonary TB of Nepal. A similar study conducted by Dhungana (2002) at UMHT, found that 88.11% of reported cases were PTB and others were extra-pulmonary TB. Higher number of PTB have been reported which might be due to poor quality of life, congested living in house and improper care of health in the developing countries like Nepal. In active TB, due to the congested living; tubercle bacilli from active TB patients can be transmitted to other healthy members in the family through the air or other means.

Regarding to the attitude, 36 (63.2%) of TB patients strongly agree that TB was a communicable disease. A similar study conducted by Sharma (2008) in Gorkha found that 256 (88%) believed that TB was an infectious disease. A study conducted by Joshi (2004) in

Patan Hospital found that 44 (75.86%) believed that TB was an infectious disease. This showed that people in Chitwan had poor knowledge about the infectious nature of TB. This might be one of the main reasons of high prevalence of TB in the present study population.

More than half of the respondents 33 (57.6%) agreed to the statement that 'if one family member was infected, other family members would also develop TB'. But in a similar a study in conducted in Sindhupalchok district by Ministry of Health and Population National TB Centre in collaboration with "The Britain Nepal Medical trust" in 2009, found over half of the respondents (52.6%) disagreed to the statement that 'if one family member was infected, other family members would also develop TB. This showed that knowledge of TB was more in Chitwan than in Sindhupalchok.

Majority of patients believed that alcohol (71.9%) and smoking (70.2%) was cause of TB. Also 7 (12.3%) of patients strongly believe that after completing medicine smoking, alcohol etc can be taken. 38 (66.7%) of patient agreed that probability of TB was equal in smoker and non-smoker. And 31 (54.4%) patients disagree that after completing the full course of medicine smoking, alcohol etc can be taken. Sharma (2008) found that out of 115 smokers, 7 patients had PTB. Similar study done by Subedi (1995) on "Tobacco smoking and its effects on lungs" among the 1336 patient attending in chest department of Tri-chandra Military Hospital showed that out of 885 (66.4%) smokers 431 (48,7%) had PTB and out of 451 (33.75%) non-smokers 186 (37.28%) had PTB. This clearly showed that TB was more prevalent in person having smoking habit. According to a study conducted by Karki (1995) found a positive correlation between alcohol consumption, smoking, surti, etc and respiratory tract infection.

In this study 23 (40.4%) of patient disagree that TB was a disease of poverty while only 4 (7%) of patient strongly agree and 8 (14%) strongly disagree that TB was a disease of poverty. But Lifson et al. (1999) stated that the risk of TB was greater in areas of residence characterized by crowding, poverty and lower education. In our study, more than half of the respondents 33 (57.6%) agreed to the statement that TB can be re-occurred even if you have completed the full course of medicine. That means they were aware of its relapse. But in a

similar a study in conducted in Sindhupalchok district by Ministry of Health and Population National Tuberculosis Centre in collaboration with the Britain Nepal Medical Trust in 2009, reported that majorities of respondents 115 (86.5%) disagreed to the statement that 'once infected, there was no chance of getting cure from TB'. This showed that the knowledge of relapse of TB was more in sindhupalchok district than in Jutpani VDC Chitwan.

John et al. (2001) conducted a study in Pakistan; enrolling 497 adults with new sputum-positive TB. 170 were assigned DOTS with direct observation of treatment by health workers; 165 were assigned DOTS with direct observation of treatment by family members; and 162 were assigned self-administered treatment. The trial was done at three sites that provide TB services strengthened according to WHO guidelines for the purposes of the research, with a standard daily short course drugs regimen (2 months of isoniazid, rifampicin, pyrazinamide, and ethambutol, followed by 6 months of isoniazid and ethambutol). The main outcome measures were cure, and cure or treatment completion. Analysis was by intention to treat. Findings Within the strengthened TB services, the health-worker DOTS, family-member DOTS, and self administered treatment strategies gave outcomes, with cure rates of 64%, 55%, and 62%, respectively, and cure or treatment-completed rates of 67%, 62%, and 65%, respectively which concludes the superiority of DOTS treatment. In present study, Majority of the patients (68.4%) strongly believed that that DOTS was very effective for TB treatment.

Maria et al. (2009) conducted a questionnaire-based survey to investigate the knowledge, attitudes and practices towards TB among patients. Out of the 62 respondents, 35 (57%) scored "good" in their overall knowledge on TB. Sixty-one percent of the respondents had acceptable attitudes and practices toward the disease. Ninety-six percent of the respondents knew that TBs was a highly-infectious disease, but was curable, while 13% believed that patients would die from non-adherence to the TB medication. But in our study 28 (49.1%) of patients agree that a person would die if he/she won't take medicine.

6. CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

A total of 600 sputum samples from 200 TB suspected patients, 225 sputum samples from 75 family members of TB patient currently under DOTS and 117 sputum samples from 39 DOTS completed TB patients were collected and examined in the Laboratory of Jutpani PHCC. Diagnosis was made after staining the sputum smear by Z-N staining method and examining under light microscope. Out of 200 TB suspected patients, 18 (9%) were found to be AFB positive. Among the 18 smear positive patients, 10 (55.56%) were male and 8 (44.46%) were female. Highest prevalence of TB infection (36.89%) was found to be in the age group of (30-40) year. Out of 18 smear positive cases, 3 (16.67%) were relapsed cases of TB. All relapsed cases were males and maximum relapsed cases were found in ward number one of the Jutpani VDC.

Similarly, for family member screening, three consecutive sputum samples were collected from the all family members (75) of the 16 PTB patients currently undergoing DOTS treatment and 39 DOTS completed PTB patient of Jutpani VDC and examined for AFB, which were found to be negative. Among the six VDCs under Jutpani PHC, the highest prevalence of TB was found in Jutpani VDC. Ward number four had the highest prevalence (27.78%) of PTB. Second highest prevalence was found in ward number one (22.22%) followed by ward number eight (16.67%).

A questionnaire survey of 57 PTB patients was done to assess their knowledge, attitude and preventive practice for TB. Out of 57 TB patients interviewed for the assessment of Knowledge, Attitude and Preventive practices, majority of the patients had acceptable attitudes but the knowledge regarding cause, transmission and prevention of the TB was not adequate. So there was still a need to strengthen the knowledge of TB through mass media to public level.

6.2 RECOMMENDATION

i. High prevalence of TB has been observed in Jutpani VDC, Chitwan. So NTP and TB control programmes need to launch awareness programme to the Jutpani VDC.

ii. Males and females are almost equally distributed within the population of our country. With such population distribution between sexes, the low detection of female TB cases remains a troubling public health issue demanding urgent focused study.

iii. Most of the TB infected peoples are illiterate. So community health education about TB should be provided in the area.

iv. Public awareness program related to sign and symptom, cause, treatment, prevention and control of TB should be included in mass media regularly by campaign and rally.

v. Public awareness programmes regarding the knowledge about the symptoms, treatment, prevention and control should be done regularly to educate the high people

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