PREVALANCE OF PULMONARY TUBERCULOSIS IN PATIENTS VISITING NATIONAL TUBERCULOSIS CENTRE, THIMI, BHAKTAPUR



Entry 23

M.Sc. Zoo Dept. Papas tology

Signature ... Anon

6/4/2021

Ashik Husain

T.U. Registration No.: 5-2-0022-0097-2013

T.U. Examination Roll. No.: 556

Batch: 2074

A thesis submitted in partial fulfillment of the requirements for the award of the degree of Master of Science in Zoology with special paper Parasitology

Submitted to

Central Department of Zoology

Institute of Science and Technology

Tribhuvan University Kirtipur, Kathmandu Nepal April, 2021

DECLARATION

I hereby declare that the work presented in this thesis has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author or institution.

Ashik Husain

T.U. Registration No.: 5-2-0022-0097-2013

T.U. Examination Roll No.: 556

Batch: 2074

Date: _____6 th _April 2021

01-4331896



CENTRAL DEPARTMENT OF ZOOLOGY

Kirtipur, Kathmandu, Nepal.

Central Department of St. V. Kirtiput

RECOMMENDATION

This is to recommend that the thesis entitled "Prevalence of Pulmonary Tuberculosis in Patients Visiting National Tuberculosis Centre, Thimi, Bhaktapur" has been carried out by Ashik Husain for the partial fulfillment of Master's Degree of Science in Zoology with special paper Parasitology. This is his original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

Supervisor

Janak Raj Subedi

Assistant Professor

Central Department of Zoology Tribhuvan University Kirtipur, Kathmandu, Nepal

Date: 6th April 2021

1 01-4331896



CENTRAL DEPARTMENT OF ZOOLOGY

TRIBHUVAN UNIVERSITY

Ref.No.:

LETTER OF APPROVAL

On the recommendation of supervisor " Assistant Professor Janak Raj Subedi" this thesis submitted by Ashik Husain entitled " Prevalence of Pulmonary Tuberculosis in Patients Visiting National Tuberculosis Centre, Thimi, Bhaktapur " is approved for the examination in partial fulfillment of the requirements for Master's Degree of Science in Zoology with special paper Parasitology.

> Prof. Dr. Tej Bahadur Thapa Head of Department Central Department of Zoology

> > Tribhuvan University Kirtipur, Kathmandu, Nepal

Date: 6th 1.981/2021



TRIBHUVAN UNIVERSITY

01-4331896

CENTRAL DEPARTMENT OF ZOOLOGY

Kirtipur, Kathmandu, Nepal.

Ref.No.:

Central Departments

CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Ashik Husain entitled "Prevalence of Pulmonary Tuberculosis in patients Visiting National Tuberculosis Centre, Thimi, Bhaktapur "has been approved as a partial fulfillment for the requirements of Master's Degree of Science in Zoology with special paper Parasitology.

EVALUATION COMMITTEE

(Supervisor)

Assist. Prof. Janak Raj Subedi

Central Department of Zoology

Tribhuvan University

Kirtipur, Kathmandu, Nepal

(Head of Department)

Prof. Dr. Tej Bahadur Thapa

Central Department of Zoology

Tribhuvan University

Kirtipur, Kathmandu, Nepal

External Examiner

Internal Examiner

Date: 5th July QQ1

ACKNOWLEDGEMENTS

I would like to express my heartfelt gratitude to my supervisor Assistant Professor

Janak Raj Subedi, Central Department of Zoology, T.U. for his supervision,

guidance and invaluable suggestion throughout my study.

I am thankful to our honorable Head of Department Prof. Dr. Tej Bahadur Thapa,

Central Department of Zoology, and T.U. for providing me such an opportunity to

carry out this dissertation work.

I am indebted to Mr. Gokarna Raj Ghimire, head of National Tuberculosis

laboratory reference, National Tuberculosis Centre for his constant help during

questionnaire with patients and for providing laboratory facilities for the

preparation of my present dissertation. I also acknowledge to all the teachers and

staffs of Central Department of Zoology for their continuous aspiration and

motivation. I am equally grateful to Lab technicians for their help.

Ashik Husain

T.U. Registration No.: 5-2-0022-0097-2013

T.U. Examination Roll No.: 556

Batch: 2074

V

C	ONTENTS	Pages
De	eclaration	i
Re	commendation	ii
Le	tter of Approval	iii
Ce	rtificate of Acceptance	iv
Ac	knowledgement	V
Ta	ble of contents	vi
Lis	st of Tables	viii
Lis	st of Figures	ix
Lis	st of Photograph	X
Lis	st of abbreviations	xi
At	ostract	xii
1.	INTRODUCTION	1-3
	1.1 Background	1
	1.2 Objectives	3
	1.3 Rationale of the Study	3
2.	LITERATURE REVIEW	4-8
3.	MATERIALS AND METHODS	9-11
	3.1 Clinical setting	9
	3.2 Study Design	10
	3.3 Materials	10
	3.3.1 Apparatus Required	10
	3.3.2 Chemical Required	10
	3.4 Methods	10
	3.4.1 General Screening of TB suspected Patients	10
	3.4.2 Questionnaire Survey	11
	3.4.3 Data collection and Analysis	11
4.	RESULTS	12-15
	4.1 Prevalence of TB in suspected patients visiting NTC	12
	4.2 Age and Sex wise Prevalence of TB	12
	4.3 Knowledge regarding TB	14
5.	DISCUSSION	16-19

6.	CONCLUSION AND RECOMMENDATION	20
	6.1 Conclusion	20
	6.2 Recommendation	20
7.	REFRENCES	21-31
	APPENDICES	32-33

LIST OF TABLES

Tables	Title of Table	pages
Table 1.	Knowledge about Tuberculosis (Multiple response).	12

LIST OF FIGURES

Figure	Title	page
Fig 1.	Site of laboratory setting	8
Fig 2.	General prevalence of TB	11
Fig 2.	Sex wise distribution of Positive Cases	12
Fig 3.	Age wise Distribution of positive Cases	12

LIST OF PHOTOGRAPH

Photograph	Title of photograph	Pages
1	Staining with auramine	33
2	Destaining with sulphuric acid	33
3	Counterstaining with methylene blue	33
4	Mycobacterium under fluorescent microscopy	33

LIST OF ABBERVIATIONS

Abbreviated form	Details of abbreviations
AFB	Acid Fast Bacillus
BCG	Bacillus Calmette Guerin
CDC	Centre for Disease Control and Prevention
DM	Diabetes Mellitus
DOHS	Department of Health and Science
DOTS	Directly Observed Treatment Course
HIV	Human Immuno Deficiency Virus
LTBI	Lateral Tuberculosis Infection
NTC	National Tuberculosis Centre
PHC	Primary Health Care
PTB	Pulmonary Tuberculosis
ТВ	Tuberculosis
WHO	World Health Organization
ZN	Zeihl Nelson

ABSTRACT

Tuberculosis is a main public health problem all over the world. On an average one third of the world's population is infected with Mycobacterium tuberculosis and problem of being in a state of existence disease. Tuberculosis remains a major threat in developing country like Nepal. Most of the cases occur in south-east Asia and Africa. This study mainly aims to find prevalence in terms of age, sex and to assess the knowledge tuberculosis. The study was analytical and hospital based conducted from December 2019 to February 2020 in National Tuberculosis Centre in Bhaktapur. During the study period a total of 765 sputum samples were collected and examined in the laboratory of National Tuberculosis Centre using fluorescence microscopy to detect the *Mycobacterium* and to assess the knowledge a total of 100 suspected patients were interviewed with closed ended questionnaire with multiple responses. Among the suspected patients, 73 (7%) showed positive with acid fast bacillus (AFB) and males (62%) were found to be more infected than female (38%) which was statistically insignificant (χ^2 =0.903, P> 0.05). The highest prevalence was found in age groups 51-60 (22.64%) which was statistically significant (F=39.6, P<0.05) between ages. There is mixed response showing good knowledge in affected parts and symptoms with statistically significant association (F=12.594, P<0.05) in the views of male and female. In general there is inadequate knowledge in terms of causative agent, preventive measures and treatment duration. Effective strategies must be implemented to bring equality to seek health behavior in males and females are required. Strategy with major focus on cultural and social bias must be integrated to bring more people aware of the disease.

1. INTRODUCTION

1.1 Background

Tuberculosis (TB) is the world's most serious public health problem particularly in developing and under developing countries (Park 2005). It is a disease of great antiquity and contributing to more morbidity and mortality than any other bacterial infection. It primarily affects lungs, however it also infects bone, lymph, spine and brain (Nugussie et al. 2017). The tuberculosis is mainly transmitted as droplet nuclei each containing 1-10 bacillus. These droplet become aerosolized by coughing and sneezing which have half-life of 6 hours (Loudon et al. 1969). The transmission is strengthened by immunoresistance of host, environmental stress, source and biosocial factors (Lee 2016).

Despite of availability of effective TB control measures, low and middle income countries continue to be affected dramatically. It is estimated that one third of the world population is suffering from TB. However only 5-10% usually develop the symptomatic disease due to latent nature of the *M. bacterium* (O'Garra et al. 2013). In 2017 alone, it is estimated that 10 million people have developed TB worldwide (WHO 2018). It is estimated that 95% of total death of TB cases and 98 % of all TB cases occur in south-East Asia and Africa. Globally although the incidence rate is falling, but not as much meeting with 2020 objective. Further, the decrease rate is 2.3 percent between 2018 and 2019 (WHO 2020).

Like many other developing countries, Nepal is also burdened by TB disease with poor control strategy and ignorance of the affected population. About 45% of the population is infected by TB both active and latent case (NTC 2013). For controlling the disease National TB Control Programme in 1965 and adopted DOTS strategy in 1996 (Shrestha et al. 2010). In Nepal most people rely on numbers of institution before they are properly diagnosed with TB. This has also posed a threat to control scheme, early detection and early treatment (Marahatta & Karki 2016). Another study state that people in Nepal are delayed more than 5 weeks for diagnosis (Mahato et al. 2015). The situation has become more serious due to poor adherence to the treatment course leading to drug resistant to first line drugs like rifampicin (Law et al. 2017). Every day the about 15 people lose their life to this

disease and every year 151 per 100,000 develop active TB (WHO 2020). A survey conducted in 2018/19 also revealed that the annual reduction of incidence rate is significantly higher than global with 3% (WHO 2020). The Directly Observed Treatment Course (DOTS) in Nepal was implemented from 2001, which has significant role in controlling over the disease jointly with government and nongovernment organization (DoHS 2020). Consequently, Nepal has higher treatment outcomes of 75% than India and Pakistan to (Khatri & Thapa 2018).

Discovered by Robert Koch, *M. tuberculosis* if an anaerobic, acidophilic, intracellular bacterium with very slow growth rate (Boskovska et al. 2018). The disease primarily affects lungs and causes pulmonary tuberculosis. It can also affect intestine, meninges, bones and joints, lymph glands, skin and other tissues of the body (Kumar et al. 2007). The main site of infection begins when alveolar macrophages ingest the bacterium. When macrophages die, the bacterium released and spread to other parts through lymphatic system (Houben et al. 2006). However the tissue damage is not due to the pathogen itself, rather it is caused due to reaction of the host to the presence of the bacterium (Alam & Roy 2019). It is non-motile, gram-positive bacteria, measuring about 2-4μm in length and 0.2 - 0.6μm in breadth, has no capsule and spores. The cell wall envelope of the Mycobacterium consists of mycolic acid responsible for protection against host's dangerous chemicals and evasion from immunostimulatry response from macrophages (Takayama et al. 2005).

Patients with pulmonary TB may be asymptomatic(latent) or symptomatic with range of symptoms such as weight loss, chest pain, fatigue, night sweat, headache, fever, bloody sputum and tiredness (Roy et al. 2019). When an infected person sneezes, micro droplet of about 40,000 are released into air (Cole and Cook 1998). People with closeness with the infected are at high risk of getting the pathogen. The probability of transmission depends on various factors like droplet, effectiveness of ventilation and exposure to strain of the pathogen (Kumar et al. 2007).

Initially the treatment of the disease include mostly supportive and isolation with major five drugs isoniazid; rifampin; pyrazinamide; streptomycin; and ethambutol (Alam & Roy 2019). An effective method is immunization of Bacillus Calmette Guerin (BCG) produced from weakened *Mycobacterium*. Along with well-

established risk factors of TB such as HIV, there are other important factors including diabetes, alcohol, immunosuppressant drugs, air pollution, tobacco and smoking (Amare et al. 2013). Recently, others factor are being included in the group including kidney dialysis, silicosis, organ transplantation (Ai et al. 2016).

One of the crucial factor of TB suffering and mortality comes from the fact lack of knowledge awareness of TB. Also for better treatment result, compliance of the patient is must for which knowledge is essential (Rami et al. 2015). Still there are many stigmas and discrimination related to TB which is due to lack of awareness of the disease (Priyanka & Dahal 2016). Patients with high negative attitudes has been associated with the social stigmas and which effect early diagnosis and treatment (Sagir et al. 2018, Salleh et al. 2018). Accordingly, patients with positive attitude has sense of self- preventive motifs and protect others from infection (Ardi et al. 2018).

1.2 Objectives

1.2.1 General objective

To find out the prevalence of TB among the suspected patients

1.2.2 Specific objective

> To assess the knowledge of patient about tuberculosis

1.3 Rationale of the study

The Tuberculosis holds a root of the poor country like Nepal. Proper knowledge about causative agent, mode of infection, treatment and preventive measures would form a basic framework for the reduction for the disease, hence it is an requisite to conduct such type of research in order to assess the knowledge, attitude and perceptive towards the disease. This research is mainly outlined to find the susceptibility of sex for disease and to know the knowledge regarding TB in the suspected patients.

2. LITERATURE REVIEW

Tuberculosis is a worldwide public health problem; despite the fact that the causative organism was discovered more than 100 years ago, and highly effective drugs and vaccine are available, making Tuberculosis preventable and curable diseases (Park 2005). Any stressor, such as poor nutrition, that compromises immune competence may contribute to the conversion from dormant to active TB and may inhibit recovered (Heywood and Marks 1993). Globally there were 9.6 million TB cases reported in 2014 however from 2000 to 2014 there has been gradual reduction of the TB cases by 18% (WHO 2014). Most of the cases were estimated to be from Asian and African countries with 58% and 28 % respectively in 2014.

Tuberculosis in Asian countries

South Asian Association of Regional Cooperation (SAARC) countries has an estimate of 21% of global population and is a favorable place of TB epidemiology as being mostly developing countries (SAARC 2019). The SAARC countries accounts for 36 % of global TB burden with India being 26% of global TB burden and china 9%. A total of 2.6 million Cases were notified in the 2017 with 77% success rate (WHO 2017). In Indonesia, the prevalence of lateral Tuberculosis infection (LTBI) was found to be 23.6 % and was higher in cases of comorbidity in health care workers (Erawati & Andriany 2020). In Sri lanka, comparative study showed higher prevalence of TB in urban areas (13.9%) than rural areas (2.2%) (Wijesinghe et al. 2013). The prevalence rate was 1.6% in a study in Philippines with positivity rates more in case of male 9.6% than female 6.8% (Afroz et al., Chadha et al. 2012, Belizario et al. 2014). One major cause of TB mortality was bacterial co-infection in positive patients in Philippines (Shimazaki et al. 2018). In a study conducted in central India, it was observed that prevalence increases with the increase in age and also higher in male than female (Bhat et al. 2009). There is disparate prevalence is different region of china probably due to disproportionate access to health care (Mijiti et al. 2016). The notification rate was 20 times more than national report in the household contacts in china (Jia et al. 2014). Zaman et al. (2006) in Matlab found 14% positivity with being male more being infected than female. In malnourished children higher TB rate was observed with 7% in sputum microscopy in Bangladesh (Chisti et al. 2014). In Bangladesh, out of 3579 reported cases, 1.54% were AFB positive with male 50.9% and females 49.1 % (Jasmine et al. 2018). In south India the overall prevalence was 228 per 100,000 smear positive with highest in the group more than 35 years (Dhanaraj et al. 2015). In similar study, the prevalence was found 101.4 per 100,000 (Sharma et al. 2015). In a study conducted in Bhutan, females were found to be more infected with PTB than males which contradict with other findings (Zangpo et al. 2019).

Tuberculosis in African Countries

Geographically, Africa is the second most affected region next to south east Asia (WHO, 2020). The TB was found to be very high with 43% prevalence in smokers than non-smokers 14% in Nigeria (Ekrakene & Igeleke 2010). In Ethiopia, due to poor ventilation of the prison, the prevalence was 19.4 % which was higher than national record (Ali et al. 2015). In another study conducted in Ethiopia showed higher prevalence in the age group 25–44 and 65–74 years group with 10% Acid Fast Bacillus (AFB) positive and also higher in urban areas (Derseh et al. 2017). In a survey conducted in Nigeria, number of females were more than males in 0-14 age group (Dim & Dim 2013). In another study 13% positivity results from sputum microscopy (Acquah et al. 2012). In HIV positive patients, the prevalence was found to be high with 29% (Linguissi et al. 2014). Similarly, a study in Ethiopia showed 10 % positivity out of 200 presumptive cases with male and female prevalence 60% and 40% respectively. This study also showed that more prevalence urban areas and rural areas (Nugussie et al. 2017). There is an increase in prevalence by 17.3 % up to 44 years with highest in 15 to 24 age groups (Muvunyi et al. 2010). Patients with uncontrolled Diabetes Mellitus (DM) showed 12 smear positive out of 60 stating higher prevalence of TB in DM patients (Ahmed et al. 2017).

Tuberculosis in European Countries

Since 1995, TB notification rate has been increasing by 2-4% annually in Bulgaria, Bosnia and Herzegovina (Walls & Shingadia 2007). On a global scale, European countries accounted for 2.5% of total global TB in 2019 (WHO 2020). In an asylum, a five year study showed 10,090 cases among 800,000 susceptible (Diel et al. 2016). In a study, 38.9% of health care workers were diagnosed with latent TB (Herzmann

et al. 2017). In 29 European countries, data from 2000 to 2010 concluded mortality rate higher for males than females (Al-Rahamneh et al. 2018). Prevalence of TB and Human Immuno Deficiency Virus (HIV) co-infection was higher in immigrants than nationals in France, Portugal, UK and Spain (Tavares et al. 2017).

Tuberculosis in American countries

According to WHO (2018), American countries accounted for 3% which has declined to 2.9% in 2020 (WHO 2020). In 2019, USA reported lowest number of incidence of TB showing only 2.7 per 100,000 cases with major population of being Asian immigrants (CDC 2020). In Canada there has been higher incidence rate of TB in foreign born than their own population by 33 times (Jensen et al. 2012). One study showed prevalence of both active and latent tuberculosis being 0.4% and 59% in prisoners of central- west Brazil (Estevan et al. 2013).

Tuberculosis in the context of Nation

Nepal being a land locked country between two countries with High TB burden, with open border with India has significant effect on the TB burden of the country (Adhikari et al. 2019). There has been annual reduction in incidence by 3% from the last decade which is higher than global case (NTC, 2020). The smear positive PTB prevalence conducted by Amgain et al 2013 found 9% in the suspected patients in Jutepani Primary Health Centre (PHC). In similar study by (Shrestha et al. 2013), the AFB positive samples was found to be 13.57% by Zeihl-Nelson (ZN) microscopy. The overall smear positivity was 13.1% based on ZN staining which was less than Immunochromatographic diagnosis which was 14.3% (Rai et al. 2007). A study revealed that 4.14% positive cases in Gurkha which is less than other relative studies (Sharma 2008).

In a sex- age wise prevalence, the males were slightly more infected with PTB than males (Manandhar & Krishna 2012) with highest in the age 30- 40 (Amgain & Maharjan 2013). Two similar study found contradicting results where highest prevalence was found in the age group 41-60 (Rai et al. 2007, Shrestha et al. 2013). The overall Prevalence was more than double for the male than females (Bhat et al. 2009). The development of Pulmonary TB was 1.4 fold greater in males than females(Raj et al. 2012). There was positive correlation between age and PTB.

Greater prevalence was found in the age group 55+ than 14 to 24 group (Datta et al. 2001). The smear positivity rate was 14.7% with greater number of individuals in the morning sample than spot sample which was similar to Myneedu et al. (2011) and (Ryu 2015). Among the 280 smear from suspected patients, 10.7% showed positive results with male more affected than female while highest infected group was 56-65 (Dhakal et al. 2018). The prevalence of TB in suspected patients was highest in age above 60 while least in age below 15 with 32.54% and 5.79%. The male had smear positivity 1.67 times more than females (Sah et al. 2020). The prevalence of TB in Chronic Kidney Disease was found to be 13.7% (Pradhan & Sigdel 2020).

Knowledge, Attitude and Practice of TB

Most of the countries with TB burden lies in the Asian continent. One of such reason include the lack of knowledge about mode of transmission, causative agent, prevention of the disease. The perception towards the disease also affect the control programme in both public as well as health worker. Due to lack of social – economic factors and lack of knowledge, WHO (2018) estimated south- East Asian countries account for 44% of total global TB burden. Higher prevalence might be due to lack of awareness program and knowledge in these countries. Although the TB drugs are provided free of charge in Tajikistan, a study concluded that the average knowledge about the disease in workers was low and misconceptions was frequent (Gilpin et al. 2011). The lack of knowledge was far greater in the rural area than urban areas regarding all aspects of knowledge of TB in Pakistan (Mushtaq et al. 2011). Education and low economic status was associated with poor knowledge about TB with majority of them being female (Konda et al. 2016). Sound knowledge is prerequisite for treatment adherence, in terms of that, nearly half of the patients were aware of it (Kulkarni et al. 2016). Studies suggest that there is more need to educate the female and poor marginalized to obtain satisfactory awareness (Khalil et al. 2011). According to Kala et al. (2016) females, older generation and people without schooling are contributing factors of the TB. In a study 31.47 % correctly answered mode of spread while 62.07%, agreed that cough was the most common symptom (Das et al. 2012, Kala et al. 2016). In a study by Vidhani & Vadgama (2012) half of the patient knew about causative organism while 9% knew that BCG was and

effective preventive vaccination.

Regarding the knowledge on TB prevention using mask and causative agent, the study showed poor awareness which was less than national data (Amgain et al. 2014). Majority of the respondents believed that the cause if smoking and alcohol while few assumed it to be a sign of curse revealing that still there are misconception about the disease. In comparison to the etiology and symptoms which had satisfactory results, knowledge on proper disposal seemed to be far scarce which is also an important aspect to control the disease (Manandhar & Krishna 2012). Media and education play important role in spread of knowledge of the disease as in a study in Lalitpur higher secondary students, showed fluent knowledge on transmission, etiology and prevention of the disease(Sah et al. 2016). Karki et al. (2018) Showed that majority of the respondents have satisfactory knowledge on the disease and the main credit for this was assigned to the media including radio, television and newspaper. In a similar study in Bhaktapur, it was found that inadequate knowledge for prevention of TB in comparison to the other aspect of the disease (Karki et al. 2019).

3. MATERIALS AND METHOD

3.1 Clinical setting

The Study was conducted at National Tuberculosis Centre (NTC) Thimi, Bhaktapur from December 2019 to February 2020. Bhaktapur is a small city located east corner of Kathmandu valley. It is situated at the longitude of 85.3291289° E and latitude of 27.6647487° N with an elevation of 1316m. The National Tuberculosis Centre in Thimi, Bhaktapur was established in 1989 with the cooperation of Japan International Cooperation Agency. The NTC is responsible for making guidelines, policy control and supervision of National Tuberculosis Program (NTP). It also quantifies and supply anti tuberculosis drugs both being first and second line drugs from Global drug Facility. It is also the only National Tuberculosis Reference Laboratory in Nepal.

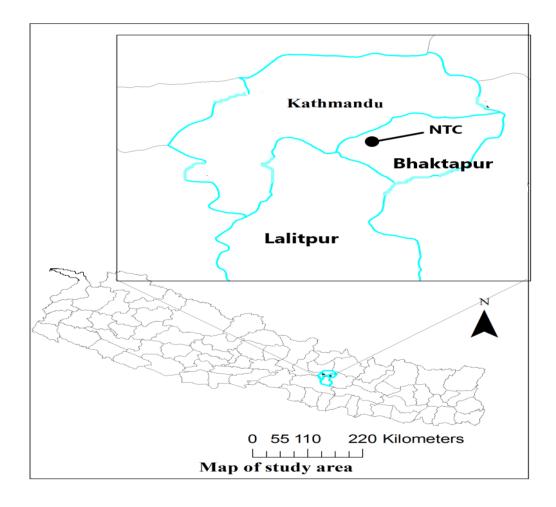
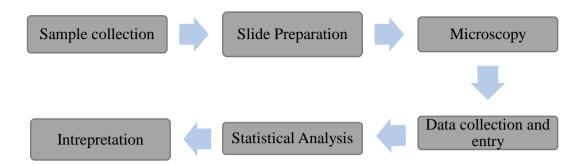


Fig 1. Site of laboratory setting

3.2 Study design



3.3 Materials

3.3.1 Apparatus Required

- Microscope
- Spirit lamp
- Glass slides
- Disposable containers
- Cotton
- Staining pan
- Forceps

3.3.2 Chemicals Required

- Sulphuric Acid 25%
- 0.1% Methylene Blue
- Auramine- Rhodamine solution
- Distilled water

3.4 Methods

3. 4.1 General screening of suspected patients

3.4.1.1 Sample collection

Routine fresh clinically suspected pulmonary TB patient's early morning sputum was collected in vial by giving them instruction. Only the thick purulent avoiding saliva was used for smear preparation.

3.4.1.2 Slide preparation

a. Acid Fast Bacillus (AFB) Stain

Sputum sample is smeared evenly on the slide using bamboo stick. The smear is air dried. It is then fixed with methanol. The slides are placed in serial order on the staining rack with smeared slides facing upward ensuring slide do not touch each other (Hooja et al. 2011).

b. Auramine Staining

The smear was placed of the staining rack over the sink, leaving some distance between the slides. Freshly prepared Auramine O solution was poured over the slides to completely cover it. The slides were left for 20 minutes and washed with tap water. The slides were covered with 25% sulphuric acid. After 2, 3 minutes of Decolorizing by sulphuric acid, it was cleaned with clean water. The slides were flooded with 0.1% Methylene Blue as counterstain for about 1 minute. After washing with tap water, the slides were dried at room temperature (Truant, 1962).

c. Fluorescence Microscopy

Compared to Conventional Microscopy, Fluorescence Microscopy has high sensitivity and similar specificity (Steingart et al. 2006). On observation, the *Mycobacterium* appears Bright yellow rods in black background. The *Mycobacterium* were observed in 40X magnification under light microscope.

3.4.2 Questionnaire Survey

To fulfill the quantitative research purpose, the close ended questionnaire was prepared. They were based on previous studies (Manandhar & Krishna 2012, Paudel 2017, Sanusi et al. 2017). About 100 participants were selected randomly for interview and answers were marked as multiple responses (Ato & Sis 2019).

3.4.3 Data collection and Analysis

The smear positive samples among the screened slides were used as raw data which was filled in datasheet. The data were analyzed using Chi- square test and one way Anova in the MS excel 2013.

4. RESULTS

4.1 Prevalence of TB among suspected patients visiting NTC

The sputum sample of 765 TB suspected patients were collected and screened showed 53 (7 %) to be AFB positive (Fig 2).

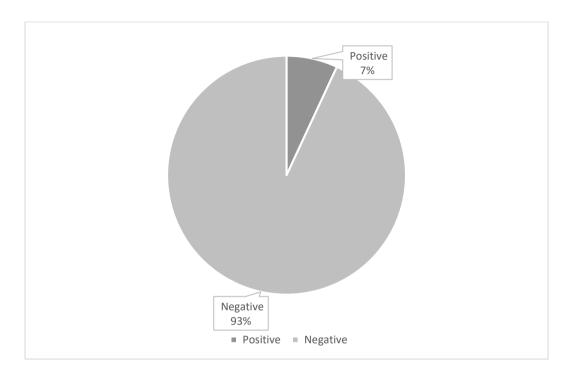


Fig 2. General prevalence of PTB among suspected patients

4.2 Age and sex wise prevalence of PTB

Sex wise prevalence of Pulmonary Tuberculosis (PTB) among suspected patients showed maximum (62%) in males as compared to females (38%) which showed insignificant association (χ^2 =0.903, P > 0.05) (Fig 3). Age specific prevalence was highest among the suspected patients of age groups 51-60 year accounting for 22.64% followed by 41-50, 21-30, 0-20, 61-70, 31-40 and least in 70+(Fig 4). This was found to be statistically significant (F=39.6, P<0.05) between age groups.

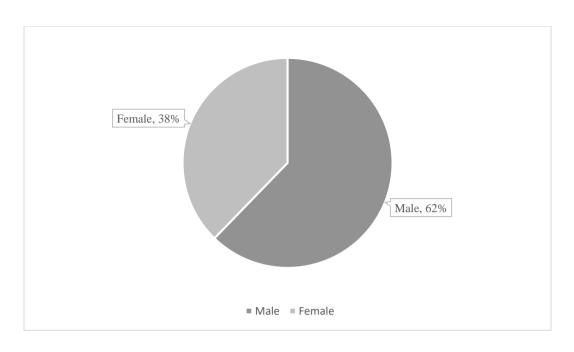


Fig 3. Gender wise distribution of positive cases

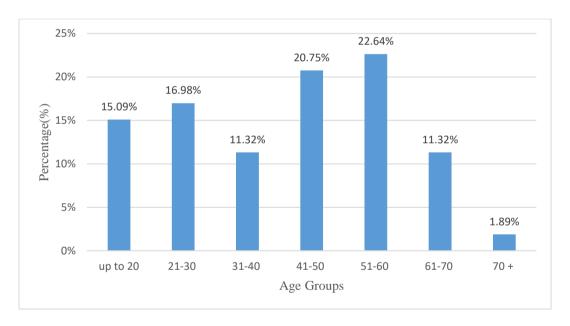


Fig 4. Distribution of positive cases on the basis of age

4.3 Knowledge regarding TB

Table 1. Knowledge about tuberculosis (Multiple response).

Variables	Male (%) N= 61	Female (%) N=39	Total (%) N=100	p-value
Risk Factors				
Alcohol	22(36.06)	4(10.25)	26(26)	
M. Tuberculosis	9(14.75)	10(25.64)	19(19)	
Smoking	29(47.52)	6(15.38)	35(35)	0.049375
polluted				
environment	15(25.59)	10(25.64)	25(25)	
Transmission				
Contagious	43(70.49)	15(38.46)	58(58)	0.461862
Non contagious	9(14.7)	5(12.82)	14(14)	
Sign and				
Symptoms				
Cough	18(29.5)	12(30.76)	30(30)	
Blood in				
sputum	33(54.09)	11(28.20)	44(44)	0.017064
Loss of appetite	23(37.7)	11(28.20)	34(34)	
loss of weight	17(27.86)	7(17.94)	24(24)	
Affected				
Organ				
Lungs	43(70.49)	19(48.71)	62(62)	
Bone	5(8.19)	4(10.25)	9(9)	0.577737
skin	2(3.27)	1(2.56)	3(3)	
mode of				
transmission				
cough/ sneeze	29(47.52)	16(41.02)	45(45)	
Touch	5(8.19)	0(0)	5(5)	0.467561
Blood	1(1.63)	1(2.56)	2(2)	
Don't know	29(47.52)	19(48.71)	48(48)	
sputum				
management				
Bury	23(37.7)	8(20.51)	31(31)	
Chemo	2(3.26)	7(17.94)	9(9)	
Burn	5(8.19)	0(0)	5(5)	0.489167
Don't know	35(57.37)	23(58.97)	58(58)	
Prevention				
Mask	24(39.34)	18(46.15)	42(42)	
Avoid alcohol	29(47.52)	6(15.38)	35(35)	
Avoid smoking	31(50.81)	11(28.20)	42(42)	0.018731
Distant from			·	
patient	18(29.5)	9(23.07)	27(27)	
Don't know	16(26.22)	18(46.15)	34(34)	

Treatment Duration				
12 months	4(6.55)	3(7.69)	7(7)	
8 months	8(13.11)	3(7.69)	11(11)	
6 months	24(39.34)	14(35.89)	38(38)	0.370521
4 months	9(14.75)	0(0)	9(9)	
Don't know	21(34.42)	20(51.28)	41(41)	

About 72% of the respondents had heard about the TB formally or informally. Statistically, there was significant difference between awareness between male and female (F= 12.594, P < 0.05). The results showed there is significant differences in the opinion of male and female regarding actiology, signs and symptoms, and prevention (P<0.05) while in others cases transmission, affected organ, mode of transmission, sputum management and treatment duration, the differences was not significant (P>0.05). Overall, only 38.9% had good knowledge while 61.1% had poor knowledge regarding the disease. Most respondents viewed smoking as main cause (35%) of TB rather than Mycobacterium (19%), among them 58% of respondent viewed it as contagious. There was higher response for blood in sputum (44%) compared to cough, weight loss and loss of appetite. In the present study 62% of respondents knew that the affected organ is lungs while 9% knew that it can also affect bone and 4% believed it can affect skin as well. Majority of the respondents (48%) didn't knew the method of transmission while 45% agreed that it could be transferred through coughing and sneezing. 5% and 2% claimed that it could be transferred through touch and blood.

As for the sputum management 58% didn't knew about the disposal of sputum. On the other hand 31 % correctly answered about burying the sputum, chemotherapy 9% and burning 5%. For the prevention, most of the respondents (42%) viewed using mask and avoiding smoking can help to prevent the infection while 35% believed the TB can be prevented by avoiding alcohol, 15% believed to make distance from the patient and 34% didn't knew about the ways of prevention.

5. DISCUSSION

Tuberculosis remains a major threat to in our country especially in the poor and marginalized population. About 765 total sputum samples were collected and examined in the laboratory using auramine staining and fluorescence microscopy. Fifty three (7%) were found to be AFB positive which was similar to Amgain et al. (2013), Shah et al. (2014) and Adane et al. (2020). This suggest that in the present study, result might have been different if culture method was employed instead of microscopy (Chisti et al. 2014). However other studies (Acquah et al. 2012, Paudel 2017, Dhakal et al. 2018) showed higher positivity than present study with 13%, 12% and 10.7% respectively. The higher prevalence might be due to lack of awareness, poor living standard and malnutrition (Jasmine et al. 2018). The prevalence was very high than present study 27%, 8.1% and 17% (Dey et al. 2003, Kebede & Wabe 2012, Giri et al. 2013). This might be because of HIV and TB coinfection suggesting HIV being risk factor of TB (Giri et al. 2013). In contrast, many other studies (Sharma 2008, Amare et al. 2013, Dhanaraj et al. 2015, Eliso et al. 2015) showed quite less prevalence than present study. The possible explanation for this could be that there has been effective TB control strategy as DOTS and early detection and treatment (Eliso et al. 2015, Sharma et al. 2015). The variation for all these dissimilarity may be caused due to different in population and selection bias, overcrowding and respiratory disease due to pollution in urban areas (Kompala et al. 2013).

There are many factors that contribute to difference in disease in male and female. One of such factor is being Immunoglobulin M and G being more in female than male specially in younger age (Oyeyinka et al. 1984). Hence, it is essential to study the patterns of diseases in relation to sex. Many studies seem to be coherent to the given fact as well. Previous studies (Bhat et al. 2009, Manandhar & Krishna 2012, Raj et al. 2012, Amare et al. 2013, Nugussie et al. 2017, Sah et al. 2020) showed that the prevalence of PTB was more in males than in females. The same pattern was observed in this study as well. It may be because males are more exposed to the environment in terms of social gathering and longer duration of employment which make them more susceptible to TB. The current study showed males were 62% infected which was consistent to the Bam (2003) and Bhatt et al. (2009) which was 65% and 64%. It might be due to sampling bias as more males was included

than females. Hence gender disparity in our society play important role in communicable disease thus hiding true nature of the disease. Contrasting to the study and above, showed male (50.90%) and female (49.10%) prevalence being very similar (Jasmine et al. 2018), the male to female ratio was 1.03:1 which was different from ours 1.69:1. This suggest that sampling size of male and female affects the prevalence considerably. This explanation is also coherent with other studies (Rao 2009, Shrestha et al. 2013) where higher sample bias regarding male's population in sample size resulted more prevalence in male.

The immune system has significance role in determining susceptibility of the disease. The immature immune system in the young age and waning of immune system in the old age (Schaaf et al. 2010, Kogut et al. 2012) becomes significant reason to study the disease in the perspective of the age. In the present study, age specific prevalence was highest in age group 51 – 60 years (22.64%). This finding was similar to the results (Datta et al. 2001, Shrestha et al. 2013, Paudel 2017, Dhakal et al. 2018, Sah et al. 2020) which showed highest prevalence in 41+ age groups. This can be explained on the fact that 41+ age group are more likely to get TB due impaired immune function.

Social attitude play major role in the disease spread and management. In spite of growth in social communication technology, the lack of proper knowledge in the general public cannot be ruled out. Accordingly, low knowledge of TB is one of the leading cause of increasing TB patients (Khan et al. 2011). Further, proper knowledge about the disease directly correlates with the early detection of the disease (Amgain et al. 2014), thus supporting the cause for this kind of research. In the current study, there were 61 % male and 39% female. It was consistent with other studies (Singh et al. 2006, Manandhar & Krishna 2012, Khan et al. 2020). This might have been due to the fact that health seeking attitude is less in female as compared to male as due to stigmatized society and fear that it would cause problem in marriage (Abebe et al. 2010, Khan et al. 2020). The current study, 72% were found to be aware about the TB. This was coherent with similar study conducted by (Singh et al. 2006, Rami et al. 2015, Sivakumar et al. 2018) but other studies showed (Das et al. 2012, Suleiman et al. 2014) almost all had heard about the disease being main contributing factor was television and other mass media program conducted by Government of India. This emphasize that even in the cities,

still there is lack of adequate information about the disease to general population and effective mass media program is still lacking.

Only few of the respondents (19%) believed that the main cause of the disease is bacterium suggesting that most of the general population are ignorant about the disease which was also similar to study by (Das et al. 2012, Vidhani & Vadgama 2012, Hassan et al. 2017). The study as well as pre mentioned studies, cause of TB was high in wrong beliefs such smoking and alcohol. This misconception might have arisen due to social perception regarding smoking and alcohol. About 58% respondents believed that the disease is contagious. Previous study (Manandhar & Krishna 2012, Agho et al. 2014, Paudel 2017, Sivakumar et al. 2018) showed contradicting results where more respondents agreed that TB is contagious. This finding also support the fact that proper and effective campaign is still unreached to majority of the people. As for the symptoms, majority (44%) of the interviewers believed blood in sputum to be major symptom followed by loss of appetite and cough. Previous study by (Khatri & Thapa 2018) showed an outstanding 100% result. The explanation for this could be that it was community based survey and possibly there might have been campaign regarding TB in the past.

Similarly many other study showed (Priyanka & Dahal 2016, Sanusi et al. 2017) higher results in terms of symptoms. This might have because majority of the people do not consider coughing could be related to some disease unless blood is seen in sputum. Gilani & Khurram (2012) Stated that, good information on symptoms is a must for effective prevention procedure, hence more community based or nationwide campaign could be implemented to bring more people aware about symptoms and other contributing factor of the disease. Similarly, the study revealed matching results about correct way of transmission of disease being cough and respiratory tract (45%) by Rami et al. (2015) but less than past studies ranging from 60 to 80% (Mokhtar et al. 2012, Agho et al. 2014, Paudel 2017, Dakhode et al. 2019). This varying knowledge could be due to non-uniformity arisen due to geographical variances and literacy status.

Most of the respondents stated that lungs are the most affected organ 83.78% which showed good knowledge greater than similar survey by (Paudel 2017) where only 47% were aware about the main affected organ. This implies that present study

showed good knowledge regarding the site of TB infection. As for the prevention, only 23.33 % correctly said covering mouth with mask. This was found to be more or less consistent with the similar structured study (Karim 2018, Khan et al. 2020) giving results 19% in both. This was less in comparison to preceding studies (Manandhar & Krishna 2012, Paudel 2017, Karki et al. 2019) where more than half knew about the prevention. This signifies education play important role for increasing knowledge (Karki et al. 2019). It showed that knowledge regarding the prevention of the TB was below satisfactory. In the suspected patients visiting NTC, only 30.10% of the patients practiced safe sputum disposal which was less than (Dzeyie et al. 2019). Only 45% knew about duration of time being 6 months. Although this finding was high compared to similar study in Malaysia by Sanusi et al. (2017) where only 11.1% showed true treatment duration. Other studies showed (Khalil et al. 2011, Anaam et al. 2018, Singh et al. 2019) very high results which were 95.5%, 88.9% and 81.8% respectively. The might have due to lack of proper counseling about the curability of the disease seen in general population.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

It is well established fact that TB is one of the contagious disease which our country has been combating with the objective to completely eradicate the disease. The result showed that 7 % prevalence in the suspected sputum samples which is higher than national level. The prevalence in gender wise showed males being slightly more infected than females due to sampling bias. Highest age group with prevalence was 51- 60 showing productive age being more susceptible to disease signifying more health seeking behavior in this age group. The results shows still there is less than satisfactory knowledge on tuberculosis disease.

6.2 Recommendations

- ➤ Since number of males seeking health were more than female, effective measure like telemedicine to ensure equality for health seeking behavior should be implemented.
- ➤ Mass communication program from government level should focus keeping in mind about cultural, social and economic factors.
- ➤ Information regarding cause, treatment and treatment measures must be enhanced using social media, mass communication and other methods,

REFERENCES

- Abebe, G., Deribew, A., Apers, L., Woldemichael, K., Shiffa, J., Tesfaye, M., et al. 2010. Knowledge, health seeking behavior and perceived stigma towards tuberculosis among tuberculosis suspects in a rural community in southwest Ethiopia. PLoS One 5(10):e13339.
- Acquah, S., Quaye, L., Ziem, J., Kuugbee, E., Iddrisu, A. and Sagoe, K. 2012. Prevalence of smear positive tuberculosis among outpatient attendees, the case of the Tamale Teaching Hospital. Journal of Medical and Biomedical Sciences **1**(4):34-41.
- Adane, A., Damena, M., Weldegebreal, F. and Mohammed, H. 2020. Prevalence and associated factors of tuberculosis among adult household contacts of smear positive pulmonary tuberculosis patients treated in public health facilities of Haramaya district, Oromia region, eastern Ethiopia. Tuberculosis Research and Treatment **2020**.
- Adhikari, N., Joshi, L., Subedi, B., Acharya, D., Adhikari, M., Thapa, P., et al. 2019. Tuberculosis in Nepal: Situation, Challenges and Ways Forward. SAARC Journal of Tuberculosis, Lung Diseases and HIV/AIDS **17**(1):34-40.
- Afroz, H., Ali, M. A., Akter, S., Khan, Z. U. and Datta, S. Occurrence and prevalence of pulmonary tuberculosis in rural communities in Narsingdi, Bangladesh.
- Agho, K. E., Hall, J. and Ewald, B. 2014. Determinants of the knowledge of and attitude towards tuberculosis in Nigeria. Journal of health, population, and nutrition **32**(3):520.
- Ahmed, M., Omer, I., Osman, S. M. and Ahmed-Abakur, E. H. 2017. Association between pulmonary tuberculosis and Type 2 diabetes in Sudanese patients. International journal of mycobacteriology **6**(1):97.
- Ai, J.-W., Ruan, Q.-L., Liu, Q.-H. and Zhang, W.-H. 2016. Updates on the risk factors for latent tuberculosis reactivation and their managements. Emerging microbes & infections 5(1):1-8.
- Al-Rahamneh, M. J., Al-Rahamneh, A., Guillén-Grima, F., Arnedo-Pena, A. and Aguinaga-Ontoso, I. 2018. Mortality trends for tuberculosis in European Union countries, 2000–2010. Enfermedades infecciosas y microbiologia clinica **36**(6):342-351.
- Alam, S. and Roy, C. 2019. Assessment, monitoring, and awareness of garment workers

- regarding the prevalence of tuberculosis in Savar, Dhaka.
- Ali, S., Haileamlak, A., Wieser, A., Pritsch, M., Heinrich, N., Loscher, T., et al. 2015. Prevalence of pulmonary tuberculosis among prison inmates in Ethiopia, a cross-sectional study. PLoS One **10**(12):e0144040.
- Amare, H., Gelaw, A., Anagaw, B. and Gelaw, B. 2013. Smear positive pulmonary tuberculosis among diabetic patients at the Dessie referral hospital, Northeast Ethiopia. Infectious Diseases of poverty 2(1):6.
- Amgain, K. and Maharjan, M. 2013. Prevalence of Pulmonary Tuberculosis in Jutpani VDC, Chitwan, Nepal. M.sc. Thesis. Central Department of Zoology, Tribhuvan University
- Amgain, K., Maharjan, M., Paudel, D. P., Dhital, M., Amgain, G. and Paneru, D. P. 2014.

 Awareness and attitude of pulmonary tuberculosis patients toward tuberculosis: A cross-sectional study from Chitwan district of Nepal. International Journal of Health & Allied Sciences 3(4):216.
- Anaam, M. S., Alsahali, S., Alfadly, S. O., Al-Musali, M., Siddeeg, K. and Aldhubhani, A.
 H. 2018. Knowledge of Tuberculosis and Influencing Factors among New Pulmonary Tuberculosis Patients in Yemen. Journal of Pharmacy Practice and Community Medicine 4(4).
- Ardi, M., Tahmir, S. and Pertiwi, N. 2018. Knowledge and attitudes with family role in prevention of pulmonary tuberculosis in Maros, Indonesia. Journal of Physics Conference Series.
- Ato, M. L. and Sis, M. T. 2019. Assessment of knowledge, attitude and practice of tuberculosis transmission and prevention among tuberculosis patients at Kuyyu Hospital, NorthShoa Zone, Oromia Regional State, Ethiopia. Anatol J Family Med 2:91-97.
- Bam, T. S. 2003. Factors Affecting Patient Compliance with Directly Observed Treatment Short Course in Kathmandu Urban Areas Nepal. Mahidol University. p.
- Belizario, V., Totanes, F. I., Asuncion, C. A., De Leon, W., Jorge, M., Ang, C., et al. 2014. Integrated surveillance of pulmonary tuberculosis and paragonimiasis in Zamboanga del Norte, the Philippines. Pathogens and global health **108**(2):95-102.
- Bhat, J., Rao, V. G., Gopi, P. G., Yadav, R., Selvakumar, N., Tiwari, B., et al. 2009. Prevalence of pulmonary tuberculosis amongst the tribal population of Madhya Pradesh, central India. International journal of epidemiology **38**(4):1026-1032.
- Bhatt, C., Bhatt, A. and Shrestha, B. 2009. Nepalese People's knowledge about

- Tuberculosis. SAARC Journal of Tuberculosis, Lung Diseases and HIV/AIDS **6**(2):31-37.
- Boskovska, K., Naceva-Fustic, S., Simonovska, L., Dilberovska, M., Dacevski, D., Popova, G., et al. 2018. Comparison of IFN-γ Levels in Children with Tuberculosis Disease (TB) and Latent Tuberculosis Infection (LTBI). Open Access Macedonian Journal of Medical Sciences **6**(11):2091.
- Cole, E. and Cook, C. 1998. Characterization of infectious aerosols in health care facilities: an aid to effective engineering controls and preventive strategies.

 American Journal of Infectious Control 26(4): 453-464.
- Chadha, V. K., Kumar, P., Anjinappa, S. M., Singh, S., Narasimhaiah, S., Joshi, M. V., et al. 2012. Prevalence of pulmonary tuberculosis among adults in a rural sub-district of South India. PLoS One **7**(8):e42625.
- Chisti, M. J., Graham, S. M., Duke, T., Ahmed, T., Ashraf, H., Faruque, A. S. G., et al. 2014. A prospective study of the prevalence of tuberculosis and bacteraemia in Bangladeshi children with severe malnutrition and pneumonia including an evaluation of Xpert MTB/RIF assay. PLoS One **9**(4):e93776.
- Dakhode, S., Muntode, P. and Gaidhane, A. 2019. A Cross Sectional Assessment of Tuberculosis (TB) Related Knowledge and Awareness among Urban Slum Dwellers in Wardha District. Indian Journal of Public Health Research & Development **10**(12).
- Das, P., Basu, M., Dutta, S. and Das, D. 2012. Perception of tuberculosis among general patients of tertiary care hospitals of Bengal. Lung India: Official Organ of Indian Chest Society **29**(4):319.
- Datta, M., Radhamani, M., Sadacharam, K., Selvaraj, R., Rao, D., Rao, R., et al. 2001. Survey for tuberculosis in a tribal population in North Arcot District. The International Journal of Tuberculosis and Lung Disease 5(3):240-249.
- Derseh, D., Moges, F. and Tessema, B. 2017. Smear positive pulmonary tuberculosis and associated risk factors among tuberculosis suspects attending spiritual holy water sites in Northwest Ethiopia. BMC infectious diseases **17**(1):100.
- Dey, S., Pal, N. and Chakrabarty, M. 2003. Cases of human immunodeficiency virus infection and tuberculosis--early experiences of different aspects. Journal of the Indian Medical Association **101**(5):291-292, 294, 296 passim.
- Dhakal, A., Nepal, S., Atreya, A. and Rijal, B. 2018. Baseline Study of Sputum Microscopy for Diagnosis of Tuberculosis in Western Region of Nepal. Medical Journal of

- Shree Birendra Hospital **17**(2):19-24.
- Dhanaraj, B., Papanna, M. K., Adinarayanan, S., Vedachalam, C., Sundaram, V., Shanmugam, S., et al. 2015. Prevalence and risk factors for adult pulmonary tuberculosis in a metropolitan city of South India. PLoS One **10**(4):e0124260.
- Diel, R., Loddenkemper, R. and Nienhaus, A. 2016. Consequences of tuberculosis among asylum seekers for health care workers in Germany. Journal of Occupational Medicine and Toxicology **11**(1):4.
- Dim, C. C. and Dim, N. R. 2013. Trends of tuberculosis prevalence and treatment outcome in an under-resourced setting: the case of Enugu state, South East Nigeria. Nigerian medical journal: journal of the Nigeria Medical Association **54**(6):392.
- DoHS. 2020. Annual Report of FY 2075/076, Department of Health Services, Kathmandu, Nepal.
- Dzeyie, K. A., Basu, S. and Dikid, T. 2019. The knowledge, attitude, and practices relating to tuberculosis among drug-resistant tuberculosis patients. Indian Journal of Medical Specialities **10**(2):76.
- Ekrakene, T. and Igeleke, C. 2010. Prevalence of pulmonary tuberculosis among active and passive smokers of cigarette in Benin City, Nigeria. International Journal of Biomedical and Health Sciences **6**(1).
- Eliso, E., Medhin, G. and Belay, M. 2015. Prevalence of smear positive pulmonary tuberculosis among outpatients presenting with cough of any duration in Shashogo Woreda, Southern Ethiopia. BMC public health **15**(1):1-6.
- Erawati, M. and Andriany, M. 2020. The Prevalence and Demographic Risk Factors for Latent Tuberculosis Infection (LTBI) Among Healthcare Workers in Semarang, Indonesia. Journal of Multidisciplinary Healthcare **13**:197.
- Estevan, A. O., Oliveira, S. M. d. V. L. d. and Croda, J. 2013. Active and latent tuberculosis in prisoners in the Central-West Region of Brazil. Revista da Sociedade Brasileira de Medicina Tropical **46**(4):515-518.
- Gilani, S. I. and Khurram, M. 2012. Perception of tuberculosis in Pakistan: findings of a nation-wide survey. Age (Years) **30**(965):35.
- Gilpin, C., de Colombani, P., Hasanova, S. and Sirodjiddinova, U. 2011. Exploring TB-related knowledge, attitude, behaviour, and practice among migrant workers in Tajikistan. Tuberculosis Research and Treatment 2011.
- Giri, P. A., Deshpande, J. D. and Phalke, D. B. 2013. Prevalence of pulmonary tuberculosis among HIV positive patients attending antiretroviral therapy clinic. North

- American journal of medical sciences **5**(6):367.
- Hassan, A., Olukolade, R., Ogbuji, Q., Afolabi, S., Okwuonye, L., Kusimo, O., et al. 2017. Knowledge about tuberculosis: a precursor to effective TB control—findings from a follow-up national KAP study on tuberculosis among Nigerians. Tuberculosis Research and Treatment **2017**.
- Herzmann, C., Sotgiu, G., Bellinger, O., Diel, R., Gerdes, S., Goetsch, U., et al. 2017. Risk for latent and active tuberculosis in Germany. Infection **45**(3):283-290.
- Hooja, S., Pal, N., Malhotra, B., Goyal, S., Kumar, V. and Vyas, L. 2011. Comparison of Ziehl Neelsen & Auramine O staining methods on direct and concentrated smears in clinical specimens. The Indian journal of tuberculosis **58**(2):72-76.
- Houben, E. N., Nguyen, L. and Pieters, J. 2006. Interaction of pathogenic mycobacteria with the host immune system. Current opinion in microbiology **9**(1):76-85.
- Jasmine, R., Khan, A. A., Kamal, A. K. M., Karim, A. R. and Haque, M. M. 2018. Prevalence of Pulmonary Tuberculosis among Rohingya Refugees in Kutupalong Camp, Bangladesh. Journal of Armed Forces Medical College, Bangladesh 14(2):197-199.
- Jensen, M., Lau, A., Langlois-Klassen, D., Boffa, J., Manfreda, J. and Long, R. 2012. A population-based study of tuberculosis epidemiology and innovative service delivery in Canada. The International Journal of Tuberculosis and Lung Disease **16**(1):43-49.
- Jia, Z., Cheng, S., Ma, Y., Zhang, T., Bai, L., Xu, W., et al. 2014. Tuberculosis burden in China: a high prevalence of pulmonary tuberculosis in household contacts with and without symptoms. BMC infectious diseases 14(1):64.
- Kala, M., John, K. and Logaraj, M. 2016. A study on awareness on pulmonary tuberculosis among population covered under rural health training centre at Mamandur in Tamilnadu. Biomedical & Pharmacology Journal 9(2):651.
- Karim, F. 2018. Tuberculosis related knowledge among the high school students in a selected area of Bangladesh. Virus **13**:6.5.
- Karki, D. K., Singh, B. K. and Khanal, P. K. 2018. Contribution of media on knowledge generation about tuberculosis in Palpa district, Nepal. International Journal of Community Medicine and Public Health 5(11):4690.
- Karki, K., Sushmita, K., Shah, S. K., Singh, D. R. and Gosai, S. 2019. High School Students' Knowledge on Tuberculosis Prevention: A Quantitative Survey in Bhaktapur of Nepal. Majmaah Journal of Health Sciences **7**(3):1-9.

- Kebede, A. and Wabe, N. T. 2012. Medication adherence and its determinants among patients on concomitant tuberculosis and antiretroviral therapy in South West Ethiopia. North American journal of medical sciences **4**(2):67.
- Khalil, S., Ahmad, E., Khan, Z. and Perwin, N. 2011. A study of knowledge and awareness regarding pulmonary tuberculosis in patients under treatment for tuberculosis in a rural area of Aligarh–UP. Indian Journal of Community Health **23**(2):93-95.
- Khan, A., Shaikh, B. T. and Baig, M. A. 2020. Knowledge, awareness, and health-seeking behaviour regarding tuberculosis in a rural district of Khyber Pakhtunkhwa, Pakistan. BioMed research international **2020**.
- Khan, N., Abid, M., Singh, V., Chattopadhyay, P., Ghosh, A. and Kamal, K. 2011.

 Assessment of college students awareness about tuberculosis in Moradabad. Indian

 Journal of Pharmacy Practice 4(2).
- Khatri, N. and Thapa, B. 2018. Awareness regarding pulmonary tuberculosis among the middle aged adults of the selected community. Journal of Chitwan Medical College **8**(4):27-33.
- Kogut, I., Scholz, J. L., Cancro, M. P. and Cambier, J. C. 2012. B cell maintenance and function in aging. Seminars in immunology, Elsevier.
- Kompala, T., Shenoi, S. V. and Friedland, G. 2013. Transmission of tuberculosis in resource-limited settings. Current Hiv/aids Reports **10**(3):264-272.
- Konda, S. G., Melo, C. A. and Giri, P. A. 2016. Knowledge, attitude and practices regarding tuberculosis among new pulmonary tuberculosis patients in a new urban township in India. International Journal of Medical Science and Public Health **5**(3):563-570.
- Kulkarni, P. Y., Kulkarni, A. D., Akarte, S. V. and Rajhans, P. A. 2016. Positive impact of knowledge about tuberculosis and its treatment on treatment adherence among new smear-positive tuberculosis patients in ward E of Mumbai, Maharashtra, India. International Journal of Educational and Psychological Researches 2(1):26.
- Kumar, V., Abbas, A.K., Fausto, N. and Mitchell, R.N. 2007. Robbins Basic Pathology 8th ed. Saunders Elsevier, 516-522p
- Law, S., Piatek, A. S., Vincent, C., Oxlade, O. and Menzies, D. 2017. Emergence of drug resistance in patients with tuberculosis cared for by the Indian health-care system: a dynamic modelling study. The Lancet Public Health **2**(1):e47-e55.
- Lee, S. H. 2016. Tuberculosis infection and latent tuberculosis. Tuberculosis and respiratory diseases **79**(4):201-206.
- Linguissi, L. S. G., Mayengue, P. I., Sidibé, A., Vouvoungui, J. C., Missontsa, M., Madzou-

- Laboum, I. K., et al. 2014. Prevalence of national treatment algorithm defined smear positive pulmonary tuberculosis in HIV positive patients in Brazzaville, Republic of Congo. BMC research notes **7**(1):1-8.
- Loudon, R. G., Bumgarner, L. R., Lacy, J. and Coffman, G. K. 1969. Aerial transmission of mycobacteria. American Review of Respiratory Disease **100**(2):165-171.
- Mahato, R. K., LaohaSiRiWong, W., VaeteeWootaChaRn, K., Koju, R. and BhattaRai, R. 2015. Major delays in the diagnosis and Management of Tuberculosis Patients in Nepal. Journal of clinical and diagnostic research: JCDR 9(10):LC05.
- Manandhar, N. and Krishna, G. 2012. Knowledge about Tuberculosis in outpatients who visited College of Medical Sciences Teaching Hospital, Bharatpur, Nepal. Journal of Institute of Medicine Nepal **34**(1):33-36.
- Manandhar, N. and Krishna, G. 2012. Knowledge about Tuberculosis in outpatients who visited College of Medical Sciences Teaching Hospital, Bharatpur, Nepal. Journal of Institute of Medicine Nepal (JIOM Nepal) **34**(1):33-36.
- Marahatta, S. and Karki, B. 2016. Tuberculosis in Nepal: Diagnosis and Treatment Barriers. Journal of Manmohan Memorial Institute of Health Sciences 2:1-2.
- Mijiti, P., Yuehua, L., Feng, X., Milligan, P. J., Merle, C., Gang, W., et al. 2016. Prevalence of pulmonary tuberculosis in western China in 2010–11: a population-based, cross-sectional survey. The Lancet Global Health **4**(7):e485-e494.
- Mokhtar, K. S., Rahman, N., Shariff, N. and Nor, W. 2012. Tuberculosis in Malaysia: A study on the level of societal awareness and stigma. IOSR Journal of Humanities and Social Science **1**(4):59-64.
- Mushtaq, M. U., Shahid, U., Abdullah, H. M., Saeed, A., Omer, F., Shad, M. A., et al. 2011. Urban-rural inequities in knowledge, attitudes and practices regarding tuberculosis in two districts of Pakistan's Punjab province. International journal for Equity in Health **10**(1):8.
- Muvunyi, C. M., Masaisa, F., Bayingana, C., Mutesa, L. and Hern, T. C. 2010. Prevalence and diagnostic aspects of sputum smear positive tuberculosis cases at a tertiary care institution in Rwanda. African Journal of Microbiology Research 4(2):088-091.
- Myneedu, V., Verma, A., Sharma, P. and Behera, D. 2011. A pilot study of same day sputum smear examination, its feasibility and usefulness in diagnosis of pulmonary TB. The Indian journal of tuberculosis **58**(4):160-167.
- Nugussie, D. A., Mohammed, G. A. and Tefera, A. T. 2017. Prevalence of smear-positive tuberculosis among patients who visited Saint Paul's specialized Hospital in Addis

- Ababa, Ethiopia. BioMed research international **2017**.
- NTP. 2013. Annual report of tuberculosis 2012/2013, National Tuberculosis Control Programme, Kathmandu, Nepal.
- O'Garra, A., Redford, P. S., McNab, F. W., Bloom, C. I., Wilkinson, R. J. and Berry, M. P. 2013. The immune response in tuberculosis. Annual review of immunology **31**:475-527.
- Oyeyinka, G., Salimonu, L., Williams, A., Johnson, A., Ladipo, O. and Osunkoya, B. 1984.

 Range of normal serum immunoglobulin (IgG, IgA and IgM) values in Nigerians.

 African journal of medicine and medical sciences 13(3-4):169-176.
- Park, K. 2005. Park's textbook of preventive and social medicine. Preventive Medicine in Obstet, Paediatrics and Geriatrics.
- Paudel, S. 2017. Risk Factors of Multidrug-Resistant Tuberculosis. International Journal of Applied Sciences and Biotechnology **5**(4):548-554.
- Pradhan, R. R. and Sigdel, M. R. 2020. Prevalence, Clinical Presentation, and Outcome of Tuberculosis in Patients with Chronic Kidney Disease at a Tertiary Care Hospital in Nepal. International journal of nephrology **2020**.
- Priyanka, G. and Dahal, P. 2016. Stigma Related to Tuberculosis in Patients Taking DOTS Treatment from DOTS Center of Palpa District Hospital, Tansen, Palpa, Nepal. J Microbiol Modern Tech 1(1):104.
- Rai, D., Kshetry, N., Bhargava, D. and Pokhrel, B. 2007. 15. Comparison of Ziehl-Neelsen staining microscopy and immunochromatographic tuberculosis test for diagnosis of pulmonary tuberculosis. Journal of Institute of Medicine **28**(2).
- Raj, P., Prakash, R., Mishra, G., Singh, T., Poojary, S., Mehra, N., et al. 2012. Prevalence of smear-positive pulmonary tuberculosis in different ethnic groups in India: evaluation of public health. Public Health **126**(4):295-299.
- Rami, K., Thakor, N. and Patel, A. 2015. Awareness and knowledge about tuberculosis in patient of tuberculosis at GMERS Medical College and Hospital Dharpur, Patan, Gujarat. Int J Med Sci Public Health **4**(7):906-909.
- Rao, S. 2009. Tuberculosis and patient gender: An analysis and its implications in tuberculosis control. Lung India: Official Organ of Indian Chest Society **26**(2):46.
- Roy, R. C., Sahabuddin, M., Debnath, S. C., Hosaain, M. J., Azmol, M., Hossain, S. C. B., et al. 2019. Nutritional Status of the Adolescent Boys of a Recognized Charitable Orphanage in Dhaka City, Bangladesh. European J. of Medical and Health Sciences 1(3):10-18.

- Ryu, Y. J. 2015. Diagnosis of pulmonary tuberculosis: recent advances and diagnostic algorithms. Tuberculosis and respiratory diseases **78**(2):64-71.
- SAARC. 2019. Tuberculosis in the SAARC Region- an update, SAARC Tuberculosis Center, Kathmandu, Nepal.
- Sagir, G., Islam, R., Rashid, M. M., Hossain, M. A. and Haque, M. A. 2018. Knowledge of pulmonary tuberculosis among the patients under anti-tubercular therapy. Bangladesh Journal of Infectious Diseases 5(1):27-31.
- Sah, B., Sah, J., Shah, S. and KC, A. 2016. Knowledge on tuberculosis among students of higher secondary school, lalitpur, Nepal. MOJ Public Health **4**(4):00091.
- Sah, M., Maharjan, K., Aryal, P., Jha, A., Jha, G. and Shrestha, S. 2020. Pulmonary tuberculosis at Patan Hospital, Nepal: One year audit. Journal of Clinical Tuberculosis and Other Mycobacterial Diseases 22:100207.
- Salleh, S., Rahman, N., Rahman, N. and Haque, M. 2018. Knowledge, attitude and practice towards tuberculosis among community of Kulim Municipal Council, Kedah, Malaysia. Int Med J 25:299-303.
- Sanusi, S. B., Talip, B. A. and Mohamed, M. 2017. The Descriptive Study of Knowledge and Awareness of Tuberculosis Among Students in Universiti Tun Hussein Onn Malaysia. Journal of Science and Technology **9**(1).
- Schaaf, H. S., Collins, A., Bekker, A. and Davies, P. D. 2010. Tuberculosis at extremes of age. Respirology **15**(5):747-763.
- Shah, N. S., Yuen, C. M., Heo, M., Tolman, A. W. and Becerra, M. C. 2014. Yield of contact investigations in households of patients with drug-resistant tuberculosis: systematic review and meta-analysis. Clinical infectious diseases **58**(3):381-391.
- Sharma, S. 2008. A study on prevalence of Pulmonary Tuberculosis among the suspected cases visiting in Gorkha District Hospital, Nepal and TB awareness among them (M. Sc. Thesis in parasitologiy). Central Department of Zoology, Tribhuvan University.
- Sharma, S., Goel, A., Gupta, S., Mohan, K., Sreenivas, V., Rai, S., et al. 2015. Prevalence of tuberculosis in Faridabad district, Haryana state, India. The Indian journal of medical research **141**(2):228.
- Shimazaki, T., Taniguchi, T., Saludar, N., Gustilo, L., Kato, T., Furumoto, A., et al. 2018. Bacterial co-infection and early mortality among pulmonary tuberculosis patients in Manila, The Philippines. The International Journal of Tuberculosis and Lung Disease 22(1):65-72.

- Shrestha, L., Jha, K. and Malla, P. 2010. Changing tuberculosis trends in Nepal in the period 2001-2008. Nepal Med Coll J **12**(3):165-170.
- Shrestha, S., Shakya, B. and Shrestha, P. 2013. Prevalence of Pulmonary Tuberculosis among the suspected patients visiting tertiary care hospital in Birgunj, Nepal. Janaki Medical College Journal of Medical Science 1(1):48-52.
- Singh, H., Bhardwaj, A., Khan, Z. A., Mittal, A., Nazir, M. and Jain, P. 2019. A Study of Knowledge and Awareness Regarding Pulmonary Tuberculosis in Patients Under Treatment for Tuberculosis in Tuberculosis Unit Ambala City (Haryana). Indian Journal of Public Health Research & Development **10**(10).
- Singh, U., Bala, A. and Goel, R. 2006. Knowledge about tuberculosis in senior school students of Punjab. Indian J Community Med **31**:93-95.
- Sivakumar, P., Ahuja, A. and Venkatachalam, T. 2018. A Study of Sociodemographic Profile, Awareness and Knowledge about Tuberculosis in Patients of Tuberculosis at Dots Centre. Current Research in Pharmaceutical Sciences:258-262.
- Steingart, K. R., Henry, M., Ng, V., Hopewell, P. C., Ramsay, A., Cunningham, J., et al. 2006. Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review. The Lancet infectious diseases **6**(9):570-581.
- Suleiman, M., Sahal, N., Sodemann, M., Elsony, A. and Aro, A. 2014. Tuberculosis awareness in Gezira, Sudan: knowledge, attitude and practice case-control survey. EMHJ-Eastern Mediterranean Health Journal. 2014; 20 (2): 120-129.
- Takayama, K., Wang, C. and Besra, G. S. 2005. Pathway to synthesis and processing of mycolic acids in Mycobacterium tuberculosis. Clinical microbiology reviews **18**(1):81-101.
- Tavares, A. M., Fronteira, I., Couto, I., Machado, D., Viveiros, M., Abecasis, A. B., et al. 2017. HIV and tuberculosis co-infection among migrants in Europe: A systematic review on the prevalence, incidence and mortality. PLoS One **12**(9):e0185526.
- Vidhani, M. and Vadgama, P. 2012. Awareness regarding pulmonary tuberculosis-a study among patient taking treatment of tuberculosis in rural Surat, Gujarat. Natl J Med Res 2(4):452-455.
- Walls, T. and Shingadia, D. 2007. The epidemiology of tuberculosis in Europe. Archives of disease in childhood **92**(8):726-729.
- Wijesinghe, P. R., Palihawadana, P., De Alwis, S. and Samaraweera, S. 2013. Annual risk of tuberculosis infection in Sri Lanka: a low prevalent country with a high BCG vaccination coverage in the South-East Asia Region. WHO South-East Asia journal

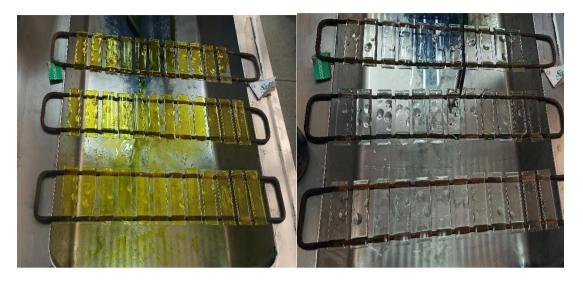
- of public health **2**(1):34-40.
- WHO. 2017/18. Global Tuberculosis report, World Health Organization, Geneva.
- WHO. 2020. Global Tuberculosis report, World Health Organization, Geneva.
- Zaman, K., Yunus, M., Arifeen, S., Baqui, A., Sack, D., Hossain, S., et al. 2006. Prevalence of sputum smear-positive tuberculosis in a rural area in Bangladesh. Epidemiology & Infection **134**(5):1052-1059.
- Zangpo, T., Tshering, C., Tenzin, P., Rinzin, C., Dorji, C., Nima, G., et al. 2019. Comparative Study of Pulmonary and Extra-Pulmonary Tuberculosis in Bhutan (2015-2017). SAARC Journal of Tuberculosis, Lung Diseases and HIV/AIDS 17(1):8-16.

APPENDIX 1

Questionnaire	e related to Knowled	ge of Tubercu	losis:	
Name:			Age/Sex:	
Occupation:				
Educational St	atus:			
1. Do you kno	ow about TB?			
a. Yes		b. No		
2. If yes, what	are the clinical featu	ires?		
a. Cough	b. Blood in Sputum	c. Headache	d. Vomiting	
3. What type	of disease is TB?			
a. Communica	ble b. Non-Communic	cable		
4.How does T	B spread?			
a. Coughing b.	Blood c. Touch			
5.TB occurs in	n			
a. Lungs	b. Skin	c. live	er d. Bor	ne
6. How do you	ı dispose the sputum	?		
A. Bury	b. incernation c. che	motherapy d	disinfectant e	e. don't know
8. What is/ ar	re the cause of TB?			
a. Smoking b.	Alcohol c. Polluted er	vironment d. I	nfection of Myo	cobacterium
tuberculosis				
9. What are t	he preventive measu	res of TB?		
a. Avoiding sn	noking b. Avoiding alo	cohol c. Person	al protection li	ke mask
d. Avoiding pe	ersonal contact to the	ΓB patients		
10. What is th	e duration of treatm	ent of TB?		
a. 12 months	b. 8 months	c. 6 months	d. 4 months	e. don't know

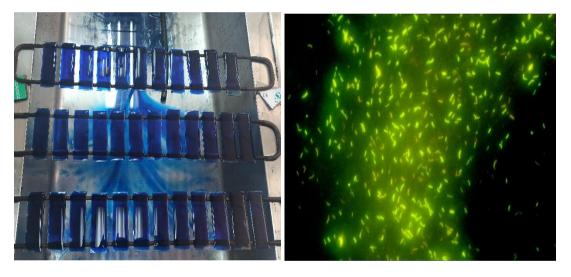
APPENDIX 2

PHOTOGRAPHIC PLATE



Staining with Auramine

Destaining With Sulphuric Acid



Counter staining with Methylene Blue Mycobacterium in Fluoroscence Microscopy