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Original Research

Preliminary Study on the Epidemiology of Tuberculosis in Nekemte and Its Surroundings -Western Ethiopia

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Abstract

Epidemiological study for Tuberculosis (TB) has been conducted in developed and developing countries to accesses the burden of the disease in their countries. However, this study was lacking in Nekemte and its surroundings, western Ethiopia. Therefore, present study was designed for cross-sectional study to generate preliminary epidemiological information on TB in Nekemte and its surroundings, Western Ethiopia. Clinical examination, chest X-ray, acid-fast staining, culture and polymerase chain reaction (PCR) were used in the study. In addition, five years Nekemte Hospital and Nekemte Health center data were analyzed to assess the five years situation of TB, TB and HIV co-infection, and treatment outcome. The results of the study reveals that out of 30,945 TB cases registered from 2003-2008, 19,494 (63%) had pulmonary TB (PTB) and 11,451 (37%) suffered from extra-pulmonary TB (EPTB) (χ^2=81.936; p<0.001). From 19,494 registered PTB cases, smear positive PTB accounted for 2,462 (12.6%) of cases. The incidences of all forms of TB were gradually declining in the last five years, while the trend of smear positive TB was almost constant, according to retrospective analysis. The disease was also showed to be more prevalent in 15- 64 age groups (χ^2 =288.035; p<0.001). In addition, around 11.5% of HIV positive patients were positive for TB. During two months study period, 520 new TB cases were registered. Out of these, 68 (13.1%) were smearing positive. The extrapolated annual incidence of clinically diagnosed and smear positive TB in the study area were 124.8/100,000 and 6.32/100,000 population, respectively. Out of 25 isolate analyses by PCR, 15 were *M. tuberculosis* (Mtb), 4 were mixed with Mtb and *M. bovis* (Mb), 2 were unknown band and five were negative. The retrospective and present study showed that the significance of TB problem in the study area was not at the level to be undermined

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INTRODUCTION

Tuberculosis (TB), one of the most wide spread infectious disease, is the leading cause of death in the world. It has been known in human history since ancient time (Zink *et al*., 2003) and still it remains an important public health problem (WHO, 2008). According to the World Health Organization (WHO), currently, nearly 2 billion peoples (approximately one-third of the world's population) have been exposed to TB pathogen (WHO, 2008). Annually, 9.2 million people become ill with TB, and 1.7 million people die from the disease worldwide (CDC, 2005; WHO, 2008). Developing countries bear the highest burden of the TB epidemic. An estimation of 95% of TB cases and 98% of TB deaths occurs in the developing parts of world, where peoples are especially vulnerable to TB because of poor living conditions and limited access to treatment (WHO, 2006; Raviglione and O'Brien, 2004). Estimation shows that in sub-Sahara Africa alone, there are 1.3 million new cases of TB and 50,000 deaths annually (Porter and Adam, 1992). The young and most

productive segments of the population is seriously affected by the disease with the infection rate goes up to 50% (Styblo *et al*., 1996).

In Ethiopia, TB is among the leading cause of mortality and morbidity (Azbite, 1991; FMOH, 2008). Over one third of the population has been exposed to TB (WHO, 2005), and it has becoming a disease of major public health important, due to low health services coverage and poorly developed health information system in the country (WHO, 2005). Sero-prevalence rates of HIV among adult TB patients are 11% and 31% according to WHO report and more recent nation data from 1999 E.C., respectively (FMOH, 2008).

Nekemte Hospital is a Referral hospital which is found in Nekemte Referral Town, East Wollega Zone, Western Ethiopia. It was estimated that over 2.5 million of people underwent treatment in this hospital. Currently, the Hospital gives diagnostic services for TB patients and it has not yet started the direct observed treatment short course (DOTS) program. Even though, there were no documents so far that shows specific figure of the TB problem in these part of Ethiopia, the hospital and other health center data show that the disease is a leading cause of mortality and morbidity. Similarly, the HIV/AIDS sero-prevalence for this area was found to be high according to surveillance conducted in the past which may be one of a contributing factor for this high public important of TB in the district. This study was, therefore, designed to generate preliminary epidemiological information on TB in Nekemte and its surroundings -Western Ethiopia.

MATERIALS AND METHODS

Study Area and Study Participants

This cross-sectional study was conducted at Nekemte Referral Hospital, Western Ethiopia. The study participants were patients exhibiting the typical clinical signs and symptoms of TB visiting the out patients department (OPD) of the Hospital that with those have no history of starting anti-TB, able to expectorate sputum, above the age of five years, and voluntary to participate in the study.

Sample Size

Sample size was determined based on the period of data collection (two months). During these periods, 520 clinical suspected TB cases were visited the hospital and investigated. The sputum samples were collected and cultured for 153 TB suspected patients because of limitation of resource to culture samples from all study patients.

Diagnosis and Laboratory Procedure

Clinical examination, chest radiography and microscopic examination of the study patient conducted at Nekemte Hospital as routine diagnostic procedure, and those patients positive for the disease was order to inter TB drug regime based on the discussion of physician.

For isolate and characterize species of mycobacterium from PTB at the study area, the collected sputum sample was stored at deep freezer (-20 ^{0}C) immediately until transported to Aklilu Lemma Institute of Pathobiology, Addis Ababa University for TB culture after acid fast examination done at the study site. The stored sputum samples were transported using cold chains every seven days to the TB laboratory for culture and PCR processing.

Lowenstein-Jensen (LJ) media was used for culturing the sputum samples. The sputum sample was decontaminated by adding equal volume of 4% NaOH solution, mixed very well and centrifuged at 3000 rpm for 10 min. The supernatant was decanted and the sediment was resuspended by mixing. Then a drop of phenol red was added to the suspension as indicator, and neutralized by 2 µl HCL through adding drop by drop until the color of the phenol red changed from purple to yellow. Then, one to two drops of the inoculums was added to the LJ medium. The inoculated tube was incubated in slant position with the tube screw caps closed for one week at 37 ^{0}C, and then put in up-right position until complete growth is seen in the some condition.

For isolate and characterize species of mycobacterium from PTB, RD4 deletion typing was used based on method described by Cleaveland *et al*. (2007). The primers used in this study was RD4 flank FCTCGT CGAAGGCCACTAAAG; RD4 flan RAAGGC GAACAGATTCAGCAT; and RD4 intF; ACA GGCTGGCGAAGTATAGC. If RD4 is present (i.e *M. tuberculosis and M. africanum*) a productive size of 335bp (RD4-intF + RD4-flankR) was obtained; if it is deleted (*M. bovis*); then a product of 446bp (RD4-flankF + RD4-flankR) was obtained.

Retrospective Data Record Analysis

Nekemte Hospital case book was used to assess the situation of TB in the last five years (2003-2008) in Nekemte and its surroundings. The forms of TB, sex and age pattern, as well as TB and HIV co-infection were assessed. Regarding treatment outcome of TB patients in Nekemte and its surrounding for the last five years (2003-2008), data were collected from Nekemte Health Center since the Nekemte Hospital was not yet started the DOTS programs and the data were not available.

Ethical Consideration

The study was ethically cleared by the Institutional Ethical Clearance Committee, Aklilu Lemma Institute of Pathobiology, Addis Ababa University. After the detailed purpose of the study was explained to each presumptive patient and those voluntary to participate in the study are confirmed their willingness by giving their oral or written consents.

Statistical Analysis

All data were analyzed by using SPSS for windows version 15.0 and Microsoft Excel. Descriptive statistics was used for analysis and expression of the result. *Chi*-square tests were used to compare difference between groups, and comparisons (difference) at $p<0.05$ were considered as statistically significant in this study. Strength of agreement between the TB diagnoses methods was assessed by using kappa.

RESULTS

Result of Retrospective Data Analysis

Demographic Data

Demographic data of TB patients were presented in Figure 1. Out of 30,945 TB cases registered in the last five years, 16,306 (52.7%) were males (χ^2 =30.205; $p<0.001$), while out of all PTB and EPTB registered, 55.6% were females and 52% were males, respectively. The annual trends of TB for both sexes were gradually declined in the last five years.

The mean age of all registered TB case was 35.35. In both form of TB (ETB and EPTB) the age group that most common affected by this disease was 15-44 (47.10%) followed with 45-64 (35.50%), and 5-15 (14.39%). But the disease was less commonly in the age groups less than five years (0.74%) and greater than sixty four years age (2.72%) groups (χ^2 = 288.035; $p<0.001$) (Figure 2).

In both sexes the disease was showed to be higher in 15-44 and followed with 45-64 ages group. However, in below 15 age groups, around 54.4% active TB recorded in female while in above 15 years old TB was prevalent in males (χ^2=30.205; $p<0.001$).

Pattern of Tuberculosis in Nekemte and its Surroundings

Out of 30,945 TB cases that registered from 2003-2008, 19,494 (63%) had PTB and 11,451 (37%) suffered from EPTB (χ^2=81.936; $p<0.001$). From 19,494 PTB cases in the last five years, smear positive PTB accounted for 12.6%. The trend of TB incidence in the last five years showed gradual decline per year with slight variation between years (Figure 3). As indicated by Figure 3, the incidence of PTB was higher (63%) than that of EPTB (χ^2 =81.936; $p<0.001$). The trend of smear positive PTB was found to be almost constant in the last five years while smear negative PTB showed gradually declining (χ^2=81.936; $p<0.001$).

Trend of TB Treatment Outcomes in Nekemte and its Surrounding

The trends of relapse, death and defaulter of TB chemotherapy in Nekemte and its surroundings were shown by the Figure 4. The numbers of relapse and death cases were generally showed increment in the last five years. Unlikely to the number of relapse and death cases, the defaulter cases showed gradual declining in the last five years (χ^2=108.76, $p<0.001$). Regarding to failure cases in the study area, there was no any cases that recorded as treatment failure.

Even though, the overall sex difference in treatment outcome was not showed any statistical significant difference between both sexes (χ^2= 2.86; $p>0.05$), the relapse cases were slightly higher in females 16 (53.3%) than in males, while defaulter 46 (52.3%) and death 44 (58.7%) cases were occurred highly in males.

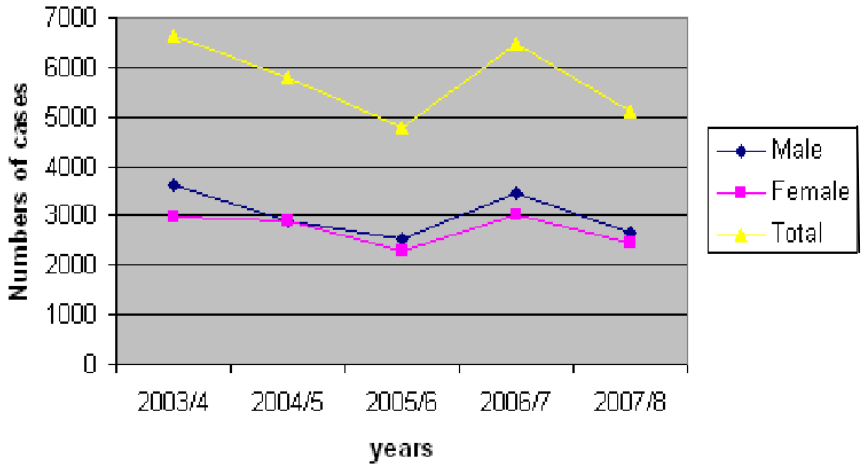


Figure 1. Sex distribution of TB during 2003-08 in Nekemte and its surroundings.

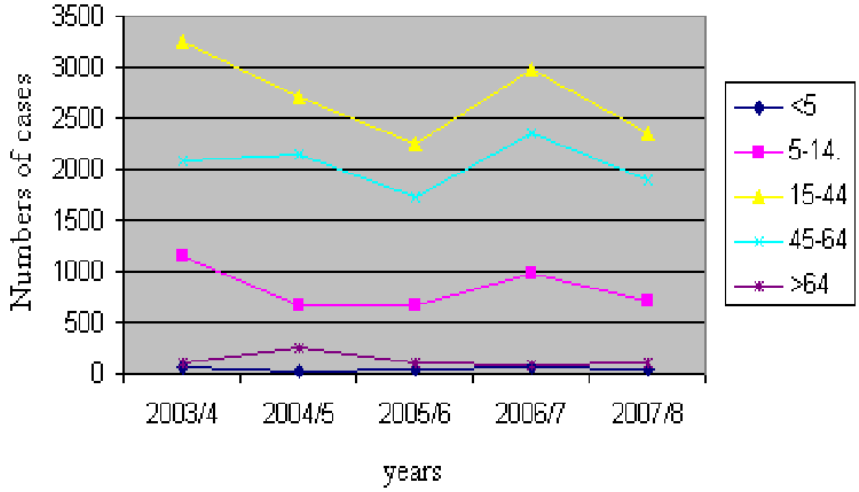


Figure 2. Age distribution of TB cases that registered during 2003-08 in Nekemte & its surroundings.

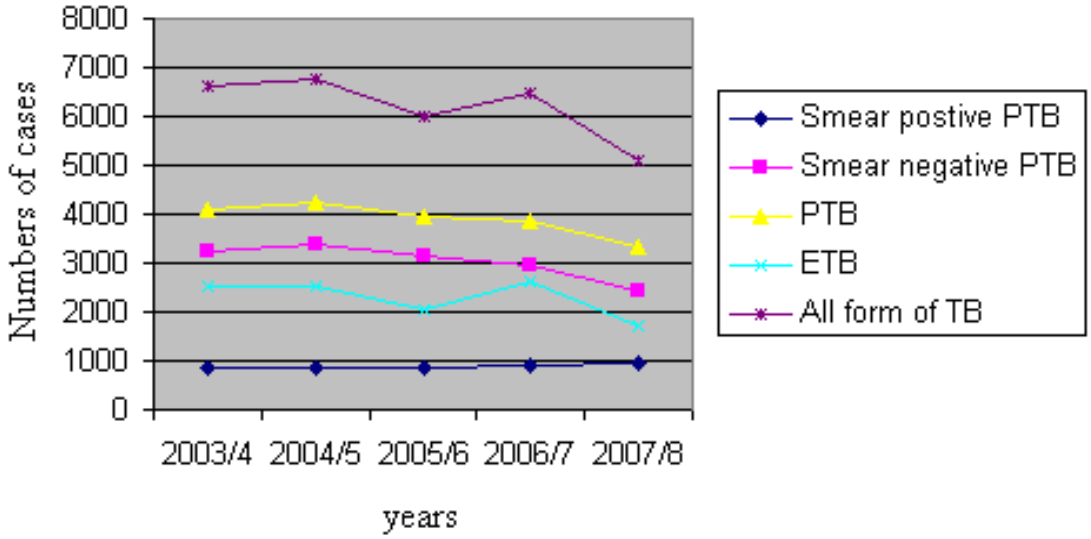


Figure 3. Trends of all form of TB, PTB, EPTB, smear positive and smear negative PTB that registered in the last five year in Nekemte and its surroundings.

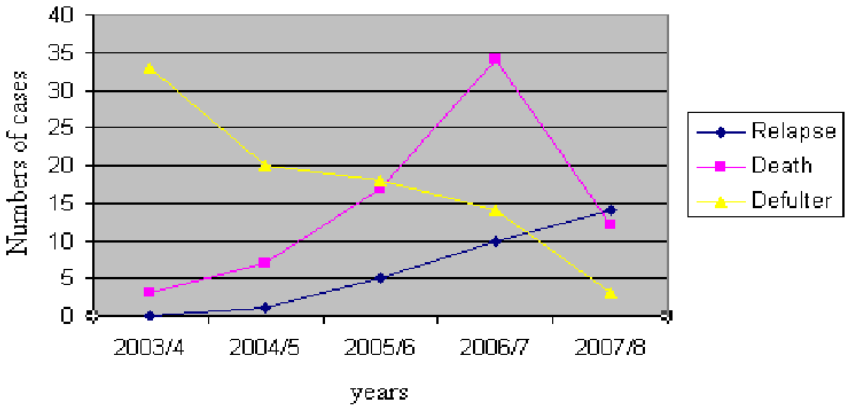
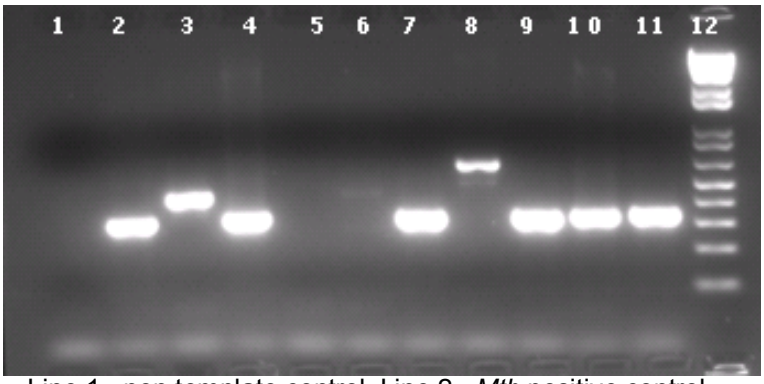


Figure 4. Trends of relapse, death and defaulter cases for TB treatment in Nekemte and its surroundings during 2003-08.



Line 1= non template control, Line 2= *Mtb* positive control,
Line 3 = *Mb* positvecontrol, Line 4-11 =sample, Line12 =1kb Ladder

Figure 5. RD4 gel results of some representative samples.

Prevalence of TB-HIV Co-infection in Nekemte and its Surroundings

The data for TB and HIV co-infection were only available from September 2007 to October 2008 at Nekemte Hospital. During this period, 1302 HIV positive patients were tested for TB and out of these 146 (11.21%) were found to be positive for TB as indicated in Table 2, Therefore, the prevalence of TB and HIV co-infection during this period of time in Nekemte and its surroundings was found to be 11.21% (146/1302).

Incidence of Tuberculosis in Nekemte and its Surroundings

On the base of clinical examination, 520 new TB cases were recorded during the two months of data collection period. Out of these, 68 (13.1%) were positive for acid fast staining, and 53% were males. The mean age of the study subjects were 32.5 and the disease was mostly recorded in 15-44 age groups.

Extrapolation from two month record of TB patients, on average 3,120 new clinically diagnosed and 408 smear positive patients would visit the Nekemte Hospital annually. Thus, the annual incidence of clinically diagnosed and smear positive TB in Nekemte and its surrounding was 124.8/ 100,000 and 16.32/ 100,000 population, respectively.

Polymerase Chain Reaction (PCR)

Twenty five (25) isolate were processed for identification by PCR (Figure 5), Out of these, 14 (56%), 4 (16%) and 2 (8%) were identified to be Mtb, mixed infection of two species (Mtb and Mb) and unknown band, respectively. The remaining five samples (20%) were negative by PCR.

DISCUSSION

The retrospective data analysis showed that out of all forms of TB registered in the last five years, 53% were males which was consistent with the report of other studies conducted in different part of the country (Kassu *et al*., 2007; Shargie and Lindtjorn, 2005) and the recent report of WHO (2008). This result reflects sex difference in the disease risk or access to treatment. The sex distribution of the forms of TB recorded in the present study is similar with that reported in Nepal (Chandrasekhar *et al*., 2008).

Even though, the mean age of all registered TB cases was 35.35, in both forms of TB, the age group that most commonly affected by this disease was 15-44 followed with 45-64. But the disease was less commonly registered in the others age groups. This result is consistent with the report of the other studies conducted in southern Ethiopia (Shargie and Lindtjorn, 2005), in Nepal (Chandrashekhar *et al*., 2008) and from the recent national report (FMOH, 2008). TB is prevalent in the age group 15-44 as this age group is sexually active may be co-infected with HIV, which is a risk factor for TB. Besides, TB is prevalent in male in all age group except in those years age below 15, which is consistent with the globally age pattern of TB where the number of male TB cases exceed that of female in all age groups except in children (WHO, 2008).

The finding from retrospective study indicated that the overall annual trends of all forms TB in the last five years (2003-2008) were observed to fall gradually. This is similar to the national and global TB incidence according to the recent report of WHO (2008). This could be due to the effect of DOTS program. This study also showed the higher incidence of PTB than the incidence of EPTB. This result supports the study that was conducted in south region Ethiopia where PTB accounted 67% of all from TB in the area (Shargie and Lindtjorn, 2005), and in northwestern Ethiopia where PTB accounted 64.2% from all form of TB, regardless of HIV status (Kassu *et al*., 2007). This result further confirms the presence of similarity in the forms of TB in different part of the country. Contrary to the trend of smear negative PTB, the trend of smear positive PTB was almost constant in the last five year. These could be due to the increasing quality of laboratory services and dissemination of HIV/AIDS in the study area.

Presently, DOTS program was implemented in the study area but the relapse and death cases were generally increasing while the defaulters were gradually declining per years in responding to DOTS program, which was similar to other studies done in south Ethiopia (Shargie and Lindtjorn, 2005), in northwestern shewa (Seyoum, 2007) and in Jijiga district (Mohammed, 2007). Thus, this increasing in the incidence of relapse from these studies in different part of the country may be an indication for dissemination of the drug resistance strain of TB in the country as the national incidence MDR-TB in relapse cases was 12% (WHO, 2008). The other possible reason could be a rapid dissemination of HIV/AIDS in the area would increase the incidence of relapse and death cases while the implementation of DOTS, increasing of the community knowledge about TB and health facilities in area might have contributed for this change in the defaulter rate.

Even though, overall sex difference in treatment outcome was not statically significant, there were low defaulter cases in females supporting the result of other studies (Shargie and Lindtjorn, 2005; Wally *et al*., 2001). The presence of slightly higher relapse rate in females and higher death rate in males were also found in this study of TB (Holmes *et al.*, 1998; Hudelson, 1996).

The incidence of TB in HIV positive individuals was found to be 11.21% in this study, which is higher than the national incidence (6.3%), according to the recent WHO report (WHO, 2008). This high incidence of TB and HIV co-infection in Nekemte and its surroundings may be due to the high HIV/AIDS dissemination in the study area that would have the effect on TB progression.

The extrapolated annual incidence of all from and smear positive TB in this study are lower than the national incidence of all form of TB per year (379/100,000 population) and smear positive TB (168/100,000 population) as the recent report of WHO (WHO, 2008). This may be due to the extrapolation may not indicate the real picture of TB in the study area as the incidence of TB is not evenly distributed with in 12 month of a year. The other possible reason is the incidence of TB in the study area may be lower than the national TB incidence.

PCR result showed that 56% infections were caused by Mtb while 16% were caused by mixed infection with Mtb and Mb. This finding showed lower proportion of Mtb infection as compared to other studies conducted in Addis Ababa with 98.37% (Bruchfeld *et al*., 2002) and in Nigeria around 85% (Cadmus *et al*., 2006) TB infection was caused by Mtb.

CONCLUSION

Result of the retrospective study showed the significance of the disease in the study area. The trends of all forms of TB, and defaulters were gradually declining in the last five years, which may be a promising achievement in the control of the disease in this area. However, the disease was more commonly in the productive age (15-64) groups of the population thereby affecting the economic development. In addition, the detection rate (incidence of smear positive TB cases) was almost constant while relapse and death rate were rising in associated to dissemination of HIV in the area. Thus, farther detail study has to be done in future to identify the contributing factors and the strain of the mycobacterium that circulate in the study area.

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