Diagnostic delay and the potential of two fusion antigens for the diagnosis of Tuberculosis in Northeast Ethiopia

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Abstract

Background: Tuberculosis (TB) is a major public health problem in the Horn of Africa, Ethiopia having the highest burden which is related to continued transmission of the disease to uninfected individuals as a result of delayed diagnosis and treatment. Early detection and effective treatment are pre-requisites to bring the high TB load under control. In this regard, early health seeking action from patients' side and prompt diagnosis as well as initiation of treatment from health system's side are essential steps. Currently, efforts are underway to identify simple, rapid and accurate diagnostic tests for TB. The aim of this study was to evaluate the potential of rESAT-6-CFP-10 and rα-crystallin-MPT-83 in the diagnosis of pulmonary TB. Besides, we have assessed delay in the diagnosis and treatment of TB patients in Northeast Ethiopia.

Methods: To evaluate the two antigens, 328 pulmonary TB suspects who reported to selected health facilities were included consecutively. Sputum and serum samples were collected from all participants. Culture, which was used as reference standard, was done on all sputum samples. ELISA was run on 204 serum samples using the two antigens. To assess delay, 216 TB patients who visited DOTS clinics of two health facilities in Afar Region were included consecutively from September 2009 to February 2010. Time from onset of symptoms till first consultation of formal health providers (patients' delay) and time from first consultation till initiation of treatment (health system's delay) were analyzed.

Results: The sensitivity and specificity of rESA-6-CFP-10 antigen were 57.3% and 71.3%, respectively whereas the sensitivity and specificity of rα-crystallin-MPT-83 antigen were 20.2% and 92.2%, respectively. The median patients' and health system's delay were 20 and 33.5 days, respectively. The median total delay was 70.5 days with a median treatment delay of 1 day. Self-treatment and first visit to non-formal health providers were found to be independent predictors of patients' delay. On the other hand, having extra-pulmonary TB and a first visit to health posts/clinics, health centers and private clinics were found to be independent predictors of health system's delay.

Conclusion: The performance of the two antigens was low and therefore, they can't be used as a substitute or supplementary test in the study area. There is a long delay in the diagnosis and initiation of treatment and this was mainly attributable to the health system. Therefore, the quest for simple, accurate and rapid tests should be a priority in TB control programmes.

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Interpretation of locally used words

Health post/ clinic: The lowest health facility in the hierarchy of government health facilities staffed with junior nurses and health assistants.

Health center: The next higher level from health posts/ clinics and it is staffed with nurses of different category, health officers, doctors and laboratory technicians. However, the professional mix varies from area to area and currently, in remote areas like Afar Region, it is hardly possible to find a medical doctor working in a health center.

Zone The highest administrative unit next to regions

Woreda Each Zone is divided into smaller administrative units called Woreda

Dagu A mode of face-to-face communication among the Afar people. It is common among the Afars to stop a passerby for *Dagu* and ask each other information with regard to current happenings. It is a cultural responsibility to share information to others promptly.

Debora A traditional Afar house made of stick covered with mats. It is easily disassembled and transferred from place to place on camel. Two typical Afar houses are depicted on the cover page of this thesis.

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List of abbreviations

AIDS	Acquired Immunodeficiency Syndrome
AFB	Acid Fast Bacilli
ALIPB	Aklilu Lemma Institute of Pathobiology
AUC	Area Under the Curve
BCG	Bacillus Calmette Guerin
CDR	Case Detection Rate
CI	Confidence Interval
DOTS	Directly Observed Treatment Short Course
ELISA	Enzyme-linked Immunosorbent Assay
HIV	Human Immunodeficiency Virus
IFN-γ	Interferon gamma
IGRA	Interferon Gamma Release Assay
IQR	Inter Quartile Range
LR-	Negative Likelihood ratio
LR+	Positive likelihood ratio
OD	Optical Density
ODadj.	Adjusted Odds Ratio
ORc	Crude Odds Ratio
RD1	Region of Difference 1
ROC	Receiver Operating Characteristic
TB	Tuberculosis
TST	Tuberculin Skin Test
WHO	World Health Organization

Chapter 1: Introduction

1.1. Ethiopia: Country profile

1.1.1. Population and demography

Ethiopia is located in the Horn of Africa sharing borders with Sudan in the west and northwest, Kenya in the south, Somalia and Djibouti in the east, and Eritrea in the north and northeast. It is one of the ancient countries in the world with an estimated land area of 1.1 million square kilometres. Ethiopia, the second most populous country in Africa next to Nigeria, has an estimated population of 88 million [1] with annual growth rate of 2.6%. The majority (84%) of the population live in rural areas [2].

Administratively, Ethiopia is a Federal Democratic Republic since 1995 with 9 National Regional autonomous states. The federal government is responsible for national defence, foreign relations and general policy of common interest and benefits [3].

Ethiopian is one of the countries with the lowest on the Human Poverty Index ranking 92 out of 95 countries and about 45% of the population lives below the poverty line [4]. The adult literacy rate is estimated to be 36%. Ethiopia's economy is largely based on agriculture, which accounts for 54% of the Gross Domestic Product. Agriculture employs 80% of the population and contributes to 90% of the export [5].

1.1.2. National health profile

The country's health problems mainly emanate from potentially preventable infectious diseases and nutritional deficiency states with high mortality and morbidity. However, non-communicable diseases like diabetes are also emerging as public health problems. In 2006, the life expectancy at birth was 53.4 and 55.4 for males and females, respectively. Infant and under-five mortality rates were reported to be 77 and 123 per 1000 live births, respectively [6].

Access to basic sanitation and safe water is limited with only 22% and 13% of the population having a sustainable access to safe water and basic sanitation, respectively. Nutritional deficiency states are also major problems with prevalence of underweight and stunting among the population being 38% and 47%, respectively [4].

There is an ongoing effort to expand access to health services. However, the total health expenditure is 6 USD per capita per year [4] and the health worker to population ratio is very low. For example, the doctors and nurses to population ratios are 1 to 42,706 and 1 to 4,207, respectively [6].

Human Immunodeficiency Virus/ Acquired Immunodeficiency Syndrome (HIV/AIDS), malaria and tuberculosis (TB) are major health problems in the country. The national HIV prevalence is estimated to be 2.1% although the urban population is disproportionately affected [6]. Malaria has resulted in series of epidemics with high morbidity and mortality. The annual number of malaria cases reported in 2003 was 8 per 1000 population [4].

Ethiopia carries the highest burden of TB in the Horn of Africa [7] and it stands 7th among the 22 high-burden countries in the world. The national notification rate (new and relapse) for TB was 151 per 100,000 population [8]. In response to the problem, Directly Observed Treatment Short Course (DOTS), the WHO's center-piece for TB control has been introduced in the country in 1994 and has been progressively expanded with decentralization of treatment centres. However, with only 60% of the population accessing health services within 10 km walk [4], management of TB under the DOTS strategy is a serious challenge and hence patients are diagnosed late and Case Detection Rate (CDR) is far below the WHO target.

1.2. Literature Review

1.2.1. Global burden of TB

TB is among the top 10 causes of death [9] and it is only outranked by HIV/AIDS among infectious causes of death worldwide [10]. In low income countries, it is estimated that TB accounts for 26 % of avoidable deaths. The majority affected are those between 15 to 59 years of age, the productive work force [11].

In 2006, the global estimated total number of TB cases was 14.4 million, 9.2 million of these being new cases. Of the new cases, 4.1 (44%) million were reported to be smear positive whereas 8% were HIV co-infected. The mortality related to TB for the same year was estimated to be 1.7 million deaths of which 0.2 million were HIV co-infected [8]. HIV is

responsible for recent increase in the global burden of TB and it was estimated that in 2000, 11% of all TB cases were HIV infected but the range across WHO regions was wide with as high as 38% in Africa and as low as 1% in Western Pacific Region [10]. In 2003, the African Region which is home only to 11% of the world's population took the lion's share of TB-related burden with 27% of the cases and 31% of the deaths being from this region [12]. In 2005, TB became declared as an emergency health problem in the WHO African Region [13].

Worldwide, 22 countries account for 80% of new TB cases and are labelled as high-burden countries [8,10,14]. However, the African Region has the highest estimated incidence rate (363 per 100, 000 population compared to 139 per 100,000 population for the world) in the world and 12 of the 15 high TB incidence rate countries in the world are from Africa [8].

The global prevalence of TB started to fall in 1990 and continued till 2006 despite the HIV epidemics because HIV in Africa caused a smaller increase in prevalence than incidence or death. However, halving prevalence and death rate by 2015 is unlikely because of poor progress in African and European Regions where both prevalence and death rates increased from 1990 to 2006. The death rate estimate for Africa in 2006 was 83 per 100,000 population which is much higher compared to the Region's target, 21 per 100,000 [8].

1.2.2. Epidemiology of TB

TB is an infectious disease caused by *M. tuberculosis* complex. It is primarily transmitted from man to man through inhalation of suspended infectious droplets in the air containing live bacilli. However, transmission can occur from animals to humans or vice versa [15].

It is estimated that a third of the world population is infected with *Mycobacterium tuberculosis*. In high income countries, more than 80% of infected individuals are above 50 years of age as opposed to the situation in low income countries where 75% of infected individuals are below 50 years of age and this is a reflection of differences in population structure and transmission levels [11].

Smear positive pulmonary TB patients are the most important source of infection compared to smear negative patients [16]. However, smear negative patients which account for half of

pulmonary TB cases do as well have significant contribution to transmission with a relative transmission of 22% compared to smear positives [17].

Although the prevalence of infection is high, only a small fraction of those infected develop the disease. It is not clear why some do and some do not progress to disease after infection. Among the different factors identified as important risks for the development of TB include a recent infection (< 1 year) with the bacilli, HIV/AIDS, immunosuppressive drugs, underweight, diabetes, smoking, malnutrition, alcoholism, crowded living conditions, indoor air pollution and genetic factors with inherited susceptibility and being male [16,18].

Epidemiological data has consistently shown that TB is primarily a disease of men. Although differences in accessing health services may contribute to the differences among men and women, it seems that there is a real epidemiologic difference in terms of risk of exposure and susceptibility for developing disease [14]. In terms of Disability Adjusted Life Years lost, the overall burden of TB is 23% higher in males than females except among HIV co-infected patients where the reverse is true. Moreover, the prevalence of TB is estimated to be 2.6 times higher among the poor than among the non-poor [9].

Depending on the organs involved, patients are grouped as having pulmonary TB or extra-pulmonary TB. Pulmonary TB primarily affecting the lungs accounts for 80-85% in HIV negative TB patients whereas extra-pulmonary TB which involves organs other than the lungs accounts for 15-20% of TB cases. However, in HIV infected patients, the proportion of extra-pulmonary TB may reach as high as 50% [19].

Although any organ can be involved, lymph nodes are the primary site of extra-pulmonary TB accounting for 35-50% of the cases. It is relatively common in children, young adults and females. Single lymph node involvement occurs in 70% of the cases in HIV negative patients; on the other hand, multiple lymph node involvement in HIV positive patients reaches up to 90% [19].

The main isolate from TB patients is *M. tuberculosis*. Another important species that contributes to TB prevalence is *M.bovis.* The prevalence of *M.bovis* among TB patients is not well established in Africa; however, there is evidence that it significantly contributes to the overall burden of TB especially in pastoral areas of Africa as supported by a review

article [20]. In a study carried out in pastoral areas of Tanzania, 28.6% of the isolates from TB lymphadenitis patients were *M.bovis* [21].

In Ethiopia, the prevalence of bovine TB among humans is not well established. A review on bovine TB in animals and humans indicated a prevalence of 16.3% and 29.2% among human patients based on two reports [22]. Raw milk consumption is a common practice in the country. In a study addressing epidemiology of bovine TB in cattle and farmers' awareness, it was found out that nearly 50% of adults drank raw milk regularly and more than 60% of respondents did not regularly boil milk for their babies. Moreover, their knowledge on bovine TB was found to be poor [23]. With the background of large livestock, high HIV prevalence and a common practice of drinking raw milk in rural areas, the risk of spillover of zoonotic bovine TB to rural communities is expected to be high in the country [22].

1.2.3. Immunopathogenesis of TB

Efforts to understand the pathogenesis of TB started long before its aetiology was described and late in the 19th century, Robert Koch made a tremendous contribution in this regard. He identified *M.tuberculosis*, developed staining and culture techniques, described mode of transmission, prepared tuberculin [24] and attempted to develop a therapy [25]. Subsequently, advances have been made in the immunology, pathogenesis and molecular biology of *M.tuberculosis.*

The tubercle bacilli primarily enter via the respiratory route as respiratory droplet nuclei. Subsequently, droplets get deposited on the alveolar space and bacilli are taken up by macrophages. Infected macrophages either remain in the lungs or get disseminated to other organs of the body with subsequent risk of extra-pulmonary TB. Moreover, *M.bovis* is primarily a zoonotic infection transmitted from animals to humans and manifest as extra-pulmonary TB.

Cell mediated immunity plays a major role in controlling infection. Once the bacilli are engulfed, activated alveolar macrophages are able to transfer the phagosome-endosome cargo containing the bacilli into lysosome where the pathogen is effectively destroyed. However, some bacilli are able to escape lysosomal delivery and hence survive inside macrophages [26].

Phagocytosis of the pathogen is accompanied by an inflammatory immune response with accumulation of different immune cells and subsequent formation of granuloma. The granuloma characteristically contains a small number of infected phagocytes surrounded by activated monocytes /macrophages which in turn is surrounded by activated lymphocytes [25].

If the immune response is able to contain the infection, the granuloma shrinks. However, failure to contain the infection results an increase in size and cellularity of the granuloma with necrosis at its centre. A granuloma close to the surface of a bronchus may rupture as a result of tissue destruction and a breach on mucosal surface. This results in the classic symptoms of TB, a persistent cough associated with bloody sputum and it is commonly referred to as cavitory TB. Patients with such type of lesion are highly infectious [25].

Tissue destruction in TB is immunopathological in nature resulting from the effects of both the pathogen and the host immune response. The host immune response involves an array of immune cells and inflammatory cytokines interacting with different mycobacterial antigens and the balance between these is believed to be reflected by the granuloma formation [25].

Generally, infection is asymptomatic (latent TB) and the lifetime risk of TB after infection is about 10% [16]. The presence of asymptomatic TB infection poses a challenge to differentiate between active and latent TB using immune-based tests.

1.2.4. Management of TB

Management of TB patients involves early (& accurate) diagnosis, and appropriate treatment to reduce transmission, morbidity, mortality and development of drug resistance. The entry point to this effort is suspecting TB in patients with suggestive symptoms. Clinically, patients with pulmonary TB mainly present with productive cough, haemoptysis, shortness of breathing, chest pain and other constitutional symptoms. On the other hand, clinical presentation of patients with extra-pulmonary TB depends on the organs involved [27].

Currently, detection of patients with TB requires that patients are aware of their symptoms and have access to health facilities. Once they come in contact with a health facility, the diagnosis of TB depends on clinical suspicion and subsequent laboratory confirmation which

in turn depends on the type of test available and the skills of the laboratory personnel. In this complex continuum, anything could go wrong and patients may remain undetected leading to high morbidity and mortality as well as continued transmission.

To improve case detection as well as treatment success, WHO launched DOTS in the early 1990s. One of the five key elements of DOTS is diagnosis of TB using smear microscopy which suffers from low sensitivity. Under the DOTS programme, WHO aimed to detect 70% of the smear positive cases and treat 85% of them successfully by 2005. However, CDR remained low standing at 61% in 2006. In fact, the case detection rate has begun falling in China and India whereas in Africa, it remained far below the target currently estimated to be only 46%. Even worse, the CDR in Ethiopia is just 27%, the lowest next to Nigeria among high-burden countries [8]. However, the concept of CDR is debated and it seems difficult to assess the performance of countries towards detecting smear positive pulmonary TB patients.

CDR is the proportion of incident smear-positive TB cases detected through a TB program. The denominator of CDR, incidence rate, has been estimated based on annual risk for TB infection. It has been estimated that a 1% annual risk of infection without control measures would correspond with an incidence for new cases of smear-positive TB of approximately 50 per 100,000. The annual risk of infection, on the other hand, is measured through tuberculin surveys. Tuberculin survey has a major draw-back: cross-reaction with BCG and environmental mycobacteria. Moreover, the relationship between risk of infection and incidence rate is affected by HIV infection and the quality of control measures. Because of the above problems, the validity of CDR as an indicator is being debated and alternative indicators are suggested. One such indicator suggested is patient diagnostic rate which is defined as the number of newly reported cases of smear-positive TB per 100,000 population per year divided by the prevalence of new cases of smear-positive TB per 100,000 population [28]. However, this requires countries to undertake prevalence surveys every 5 to 10 years.

Another study proposes the use of treatment delay (time interval between onset of symptoms and initiation of treatment) to monitor infectious pool. According to this study, a systematic recording of treatment delay in TB treatment clinics could help in estimating the infectious pool as well as monitoring programme performance with regard to TB control [29].

The low CDR is in sharp contrast to treatment success which is very close to the set target at global as well as regional level. Although successful treatment of cases is important to prevent drug resistance, the ultimate control of TB depends on a balanced effort in detecting as well as treating TB patients or else diagnostic delay with late detection will lead to perpetuation of the epidemic especially in high burden countries where the laboratory diagnosis is poor [30].

Diagnostic delay is defined as patients' delay in seeking health care, health providers' delay in making prompt and accurate diagnosis with subsequent initiation of treatment or both. In some studies, the acceptable period from onset of symptoms till reporting to a health facility was taken as 30 days [31-33]. Another study in Australia used 30 days as a cut-off for delay from onset of symptoms till initiation of treatment based on a suggestion from a panel of experts. Similarly, the acceptable period from determination of sputum positivity to initiation of treatment was taken as 3 days [34]. On the other hand, others take the median value of observed data as a cut-off point [35-37].

According to a review on diagnostic delay, the majority of the studies reported a total delay ranging from 60 to 90 days. The contribution of patients and health systems to the total delay is not consistent across many studies; in some studies, the major portion of total delay was attributable to patients whereas in others health systems take the major share. Although a number of risk factors for delay were reported, there was no consistency among the different studies. Some of the risk factors reported include initial visit to traditional or unqualified practitioners, initial visit to private practitioners, old age, female sex, poverty, and low educational level [38].

A vicious circle of repeated visits at the same level has been especially found to be an important reason for longer delay and three groups of health care providers were particularly found to be linked to the vicious cycle: low level government health facilities, private practitioners and unqualified practitioners [38]. Apart from low awareness, lack of simple and accurate diagnostic tests could play a central role for such a vicious cycle.

1.2.5. Diagnostics for TB

Currently, the diagnosis of TB is largely based on a combination of clinical criteria and conventional laboratory diagnostics (smear microscopy, culture, tuberculin skin test and radiology). Of these, sputum microscopy is the routinely available and most important test in low and middle income countries. However, all the existing conventional diagnostic tests have serious limitations and diagnostic delay is a major problem. In a review on diagnostic delay, it was found that smear negative pulmonary TB, extra-pulmonary TB and HIV co-infected TB patients suffer from significant diagnostic delay [38].

In low income countries, diagnosis of smear negative pulmonary TB and extra-pulmonary TB rests on either clinical criteria alone or in combination with radiological findings because of the absence of other alternative diagnostic tools. However, the clinical presentation of TB is non-specific mimicking many diseases and hence it is not reliable for diagnosis. In addition to the conventional diagnostic tests, molecular and immune-based tests have been used in the diagnosis of TB. A brief description of the major diagnostic tests for pulmonary TB follows.

1.2.5.1. Radiology

The radiological findings in pulmonary TB are well described and it has a high sensitivity. However, in HIV infected TB patients, the radiological findings are variable with atypical findings and hence demanding experienced radiologists. Even then low specificity of this method remains a concern. In a study done in Kenya, the investigators found that radiology has a sensitivity of 92 % and a specificity of 63% [39]. Moreover, for low income countries, availability of equipment and running cost issues remain central problems.

1.2.5.2. Bacteriologic methods

Smear microscopy and culture are used as techniques of identifying bacilli from specimens. Smear microscopy on unprocessed sputum remains the standard diagnostic method for pulmonary TB in low and middle income countries where 90% of TB is found [40]. It is positive for those patients having 5,000 to 10,000 bacilli per millilitre of specimen [41]. Although its specificity is about 98%, the sensitivity is variable ranging from 20-80 % in pulmonary TB [17,41,42]; high in patients with advanced and cavitory TB whereas low in less advanced ones. The sensitivity further drops in HIV infected TB patients [30]. Moreover,

it needs two visits and technically burdensome requiring a good microscope as well as a trained expert [30] with external quality control in place.

Culture overcomes the low sensitivity of sputum microscopy. *M. tuberculosis* can be detected using culture at a concentration of 10 to 100 organisms per ml of specimen and hence the sensitivity ranges from 80-93% with a specificity of 98%. The major limitation of culture is the time it takes to yield results. Although liquid media seem to shorten the duration to yield results in 2 weeks time [41], it is still unacceptably long and hence is not important for quick clinical decision. Moreover, it requires a quality and dedicated laboratory with trained expertise making it expensive to run it [40] and hence few primary diagnostic laboratories in low income countries have culture facilities [17].

1.2.5.3. Molecular methods

Better diagnostic tests are being developed as a result of advances in molecular techniques. Polymerase chain reaction-based molecular tests are already in the market; Amplicor MTB test (Roche diagnostics System) and Amplifier Mycobacterial Tuberculosis Direct test are approved by Food and Drug Administration. The sensitivity of these tests is dependent on smear status of samples: in a review paper including those studies which used culture as a standard, among smear positives pooled sensitivity and specificity were 96% and 85%, respectively whereas in smear negative patients, pooled sensitivity and specificity were found to be 66% and 98%, respectively [43] making it less beneficial for smear negative patients. Moreover, they have low specificity under field conditions [44].

Besides, these tests are expensive and require sophisticated laboratories with trained expertise making them inaccessible for low and middle income countries. In a case study to assess the cost effectiveness of molecular tests, it was reported that they are not cost effective at the moment [45]. Moreover, the fact that these tests require specimens from affected areas makes them less appealing for patients with extra-pulmonary TB.

1.2.5.4. Immune-based tests

Immunological tests are meant to detect immune response (cellular or humoral) or antigens in body fluids. These tests are ideal since they do not depend on samples taken from affected

areas. Currently, cellular immune response is measured using tuberculin skin test (TST) and interferon gamma release assay (IGRA).

TST involves intradermal injection of TB antigens and subsequent measurement of the size of induration. It has been used for epidemiologic surveys and in some cases for diagnostic purpose; in high income countries, it is used to diagnose latent TB. It has low specificity and cannot differentiate active from latent TB, Bacillus Calmette Guerin (BCG) vaccination as well as infection with environmental mycobacteria. Therefore, in areas where TB is endemic, BCG vaccination is a routine practice or infection with environmental mycobacteria is common, its role in the diagnosis of TB is limited [17]. Moreover, TST tends to be negative in patients with clinical TB [46] and HIV infected patients [47].

IGRA assesses the cellular immune response to specific TB antigens through measurement of interferon gamma (IFN-γ) produced by T-cells. Currently, there are two commercially available tests: QuantiFERON-TB Gold and T-SPOT.TB tests. These tests have a sensitivity ranging from 80 to 95% and are highly specific (90-100%) for TB infection. Moreover, they are unaffected by BCG vaccination status. Therefore, they are promising tools for screening TB infection with several advantages over TST like one patient visit, higher specificity and are ex vivo tests. However, they have low specificity for active TB [41]. In endemic countries where latent TB infection is prevalent, a good immune-based test should differentiate active TB from latent infection.

As a result of the limitations of the existing tests, diagnosis of TB poses a formidable challenge with a consequence of health system's delay. Therefore, simple, accurate, affordable and point of care diagnostic tools are in urgent need either to replace or supplement the existing tools especially in resource constrained areas. Immune-based tests based on antibody detection have the potential to fulfil these demands.

Although the role of humoral immunity in TB infection is not well established, exposure to *M.tuberculosis* antigens results in the production of antibodies which could be used for diagnosis. In this regard, the first attempt was made in 1898 by Arloing as a technique of haemagglutination. However, the sensitivity and specificity remained unacceptable and the progress was slow. In 1972, Enzyme Linked Immunosorbent Assay (ELISA) was used for the

first time and since then evaluation of different potential antigens was carried out extensively [48]. Evaluation of serological tests before 1990 was done on crude antigens and hence specificity was low because of cross-reaction with other antigens. After 1990, purified and recombinant antigens were introduced with subsequent improvements in the specificity of the tests [48,49].

To this end, a number of potential antigens have been identified and evaluated for their diagnostic potential for TB using serum samples. A review of studies on serological tests for TB described the different potential antigens identified and evaluated. Among these antigens, the 38kDa and A60 have been extensively evaluated either in combination with other antigens or alone [48]; the tests are either in ELISA or rapid formats such as immunochromatographic tests and primarily detect IgG, IgA and IgM. Currently dozens of such tests are commercialized in rapid formats and used in low and middle income countries with estimated annual sales volume of 3,000 to 300,000 [44]. Moreover, it is estimated that more than 40 types of rapid serologic tests have already entered in the market [40] and mostly also left.

A review of studies done on commercial tests for the diagnosis of pulmonary TB [49] and extra-pulmonary TB [50] showed variation in accuracy of tests across studies with sensitivities ranging from 0% to 100% and specificities ranging from 47% to 100%. This variation was attributed to differences in the antigen used, antibody detected, setting, stage of disease, study design, and status of specimen (fresh or archived). Moreover, the type of TB has an influence on the performance of these tests. Sensitivity is low in smear negative pulmonary TB [51, 52], extra-pulmonary TB [51-54], and HIV co-infected patients compared to HIV negative smear positive pulmonary TB [54]. Moreover, a recent head to head evaluation of 19 commercial rapid tests using culture as a reference in pulmonary TB patients revealed a low sensitivity (0.97% to 59.7%) and a variable specificity (53% to 98.7%). Generally, those tests with high sensitivity have low specificity and vice versa [40].

There are a number of antigens evaluated in-house and not yet commercialized; some of these antigens are reported to be promising. Moreover, protein antigens achieved high specificity; combined antigens have better performance compared to single antigens, and potential antigens have been identified for HIV co-infected patients [55].

ESAT-6 (6 kDa Early Secretory Antigen Target) and CFP-10 (10 kDa Culture Filtrate Protein) are among several immunogenic, early secreted, culture filtrate proteins obtained from *M.tuberculosis* complex. They are encoded within Region of Difference 1 (RD1) which is absent from BCG strains and other environmental mycobacteria [56]. They have been used for cell mediated-based tests. These antigens have been evaluated extensively in the diagnosis of latent TB. Recently, the potential of these two antigens for the diagnosis of active TB has been evaluated by a few studies. A study in Denmark has evaluated the performance of these antigens using whole blood to measure cytokine level (IFN-γ) and found a sensitivity of 85% and specificity of 60% [57].

On the other hand, a few studies were carried out to evaluate the humoral immune response and serodiagnostic efficacy of ESAT-6 and CFP-10 antigens in addition to other antigens. One of these studies was done in Poland and reported a sensitivity of 64.9% and specificity of 89.9% for rESAT-6 and sensitivity of 66% and specificity of 85.2% for rCFP-10 [58]. Another study done in China where investigators used rESAT-6-CFP-10 as a fusion antigen reported a sensitivity and specificity of 73.2% and 73.8%, respectively [59]. However, these studies used healthy controls and this might have lead to a higher specificity than would be expected from clinically suspected non-TB patients [49].

The other antigens reported to be immuno-dominant are MPT-83 and α-crystallin. MPT-83 is an immunologically active cell wall associated protein [60] found in *M.tuberculosis*. α-crystallin is a 16 kDa regulatory cell wall protein. It is a predominant stationary phase protein and it is said to be essential for the long term survival of *M.tuberculosis* [48,61]. MPT-83 combined with other antigens has been shown to confer protection against pulmonary TB in a mouse model [62].

The performance of α-crystallin and MPT-83 antigens was assessed separately for the diagnosis of pulmonary TB by some studies. A study from United States reported a sensitivity and specificity for r16 kDa as 17% and 95%, respectively whereas the sensitivity and specificity of rMPT-83 in the same study were 9% and 84%, respectively [63]. Similarly, another study from United States reported the sensitivity and specificity of r16 kDa antigen for the IgG isotope as 62% and 100%, respectively [64]. A recent study in Guinea comparing the immune response between pulmonary TB patients and healthy controls in a cohort study

showed that there is no difference between the two groups with regard to their immune response to rMPT-83 antigen [65].

Although a number of potential antigens are evaluated, the performance of these tests varied significantly. Variation from population to population for the same antigen has been reported [66]. This variation might be due to differences in the genetics of the population studied, HIV prevalence, stage of disease, the prevalence of non-mycobacterium infections, variation in the antigens of different strains, the degree of repeated infections, the prevalence of nutritional deficiencies as well as prevalence of parasitic infections. This implies that serologic tests for TB should be evaluated at different settings with different population subgroups and different levels of TB infection.

Many of the studies done had methodological weaknesses. Some of the weaknesses include "inappropriate composition of study groups; failure to analyse test performance in pertinent subgroups and bias in the selection of patients`` [67]. Many of the studies are limited to in-house evaluation of the tests or field evaluation at tertiary level excluding primary health care levels. Moreover, only a few studies assessed the accuracy of these tests in Africa [68-70] where such tests could have been more important than anywhere else.

Evaluating a combination of different antigens at different settings with improved methodology is essential to accurately assess the performance of such antigens. Therefore, we evaluated the sero-diagnostic potential of two fusion recombinant antigens: rCFP-10-ESAT-6 and rα-crystallin-MPT-83 (hereafter called serology study) in the diagnosis of active pulmonary TB in an endemic setting. Moreover, we assessed delay in the diagnosis and treatment of TB patients (hereafter called delay study) in two health facilities in Afar Region where such a study has never been reported.

1.3. Research Question, Hypothesis and Objectives

Research Questions

1. Could ELISA using rESAT-6/CFP-10 and rα-crystallin-MPT-83 antigens have better performance than smear microscopy in identifying active TB cases in health facilities of the study area, taking culture as a reference test?

2. What is the duration of patients', health system's and total delay among TB patients in the study area?

3. What are the predictors of delay in the diagnosis and treatment of TB patients in the study area?

Hypothesis

1. rESAT-6-CFP-10 and rα-crystallin-MPT-83 antigens have better performance in the diagnosis of active pulmonary TB than smear microscopy in selected health facilities in the study area.

2. There is a long patients' as well as health system's delay in the diagnosis and treatment of TB patients and socio-demographic & health service related factors are the major predictors of delay.

General objective:

To assess diagnostic delay among TB patients and evaluate the potential of two recombinant fusion antigens in the diagnosis of active pulmonary TB in North-eastern Ethiopia.

Specific objectives:

1. To determine the sensitivity, specificity and likelihood ratios of rESAT-6/CFP-10 and rα-crystallin-MPT-83 antigens among pulmonary TB suspects in the study area, taking culture as a reference test
2. To measure the length of delay in the diagnosis and treatment of TB
3. To identify factors associated with delay in the diagnosis and treatment of TB

Chapter 2: Methodology

2.1. Study area

The study was conducted primarily in the Afar Regional State, northeast Ethiopia. Moreover, since the Afar people frequently visit health institutions in Dessie, two private hospitals and a clinic located in Dessie were included in the study. Dessie Town, with a population of 120,000, is located 400 km Northeast of Addis Ababa in Amhara Regional State. Four hospitals (3 of which are privately-owned) and a number of privately owned clinics are found in the town and with its attractive climate, it remains the most frequently visited town by the Afar people outside of Afar Region.

The Afar Region is one of the 9 administrative regions of Ethiopia with estimated area of 100,000 square km [3]. Semera, capital of the Region, is about 600 km northeast of Addis Ababa on the main road to Djibouti. The Region shares boundary with four national regional states (Tigray in the northwest, Amhara in the southwest, Oromia in the south and Somalia in the southeast) and two international boundaries (Djibouti in the east and Eritrea in the northeast) [71].

Administratively, the Region is divided into 5 zones and 30 districts ("Woredas"). In 2007, the population size of the Region was about 1.4 million with annual growth rate of 2.2%. About 87% of the population were in the rural areas of the Region and the male to female ratio was 1.23 [2]. The Region is mainly lowland with 87% of the land being below 900m above sea level; it is mainly arid and semiarid with annual mean temperature above 27°c and annual mean rainfall between 500-100mm [71].

The livelihood of the Afar people is based on livestock production with limited crop production. Animals are used as a source of food, income and transport. In 2006, it was estimated that there were 10,000,000 livestock in the Region, of which 41.93% were goats and the rest included sheep, cattle and camel. Seasonal migration in search of water and pasture for their animals is a major coping strategy during the dry season [71].

Infrastructure development is poor with limited access to telecommunication, electricity, postal and road services. Moreover, only 28% of the population has potable water supply [71]. In terms of health infrastructure, the Region has 2 hospitals (only one was functioning

during the present study), 42 health centers, 45 health stations, 154 health posts and 6 private clinics. There were 10 medical doctors, 16 health officers, 208 nurses, 7 laboratory technicians, and 12 pharmacy technicians [6]. The health service coverage was 40% in 2005 [71]. The infant and under-five mortality rates in the Region were reported to be 61 and 123 per 1000 live births, respectively. HIV prevalence in the region was 1.9% [6].

Although the prevalence of TB in the Region is not known, it is an endemic area with a notification rate of 103 per 100,000 populations in 2006 [6]. Their life style like their intimacy with their cattle, their dependency for their food on animal products especially raw milk, and the repeated stress related to migration they face is expected to put them at a greater risk for TB with high transmission. The population constantly moves from place to place in search of pasture and water for their cattle and this compromises health service utilization. Moreover, the health service coverage is low with the majority of health facilities found on the main roads. Early and rapid diagnosis at the lowest health facility level would have a tremendous impact on TB control programmes in such areas.

2.2. Study design

A quantitative, facility based cross-sectional study was employed to achieve the aforementioned objectives. For the serology study, pulmonary TB suspected patients visiting selected health facilities were recruited in a consecutive series and examined at one point in time and sample was collected. Thus, all samples from pulmonary TB suspects were tested for TB using the reference standard (culture) and smear microscopy (done at the respective health facilities). Furthermore, 5 ml blood was withdrawn from these individuals for running ELISA using rESAT-6-CFP-10 and rα-crystallin-MPT-83 antigens. ELISA was run at Aklilu Lemma Institute of Pathobiology (ALIPB), Addis Ababa University laboratory. Since suspects were recruited consecutively with no differential selection of cases and controls, comparability of study participants was ensured. In this way, the selected study design overcomes the limitations of diagnostic case-control study design [72].

For delay study, because of poor infrastructure and security threats in the Region, we selected Dubti hospital and Asayta health center, both located in Zone one where nearly a third of the Region's population lives [2]. We included all newly diagnosed TB patients coming to these two health facilities from September 2009 to March 2010.

2.3. Study population

TB management in Ethiopia is currently based on passive case detection in which patients consult health care providers by their own initiative. Subsequently, TB suspects are managed or referred to the next higher level depending on the presence of TB management services. Therefore, the study population for the serology study was all pulmonary TB suspects who came to the following health facilities: Dubti Hospital and Awash Health Center in Afar Region and Selam Hospital, Bati Hospital and Amir Higher Clinic in Dessie Town. Similarly, for the delay study, TB patients just diagnosed and came to the DOTS clinics of the two health facilities were included consecutively in the study period.

2.4. Inclusion and exclusion criteria

Study participants were selected from the study population according to the following inclusion and exclusion criteria.

Inclusion criteria:

Patients fulfilling all of the following were included in the study:

- Patients suspected of having pulmonary TB as judged by the respective health workers in selected health institutions (for serology study).
- Patients just diagnosed as new TB cases and sent for treatment to the DOTS clinic (for delay study)
- Age $\geq$18 years
- Volunteered to participate in the study

Exclusion criteria:

Patients with either of the following were excluded

- Critically ill patients requiring urgent intervention
- Patients with proven coagulation problems (for serology study)
- TB patients who were already on treatment

2.5. Sampling method and sample size

For the serology study, all participants fulfilling the inclusion criteria were recruited until the desired sample size was attained.

Taking a sensitivity of 73% from a previous study (59) with a 95% confidence interval of 10%, using the following formula [73]

$$n \geq \frac{(1.96)^2[\, p\,(1-p)]}{(CI)^2},$$

where p is the sensitivity, n is sample size for the cases and CI is the confidence interval, approximately 76 true cases were needed as determined by the reference test. At the beginning, assuming a 15% prevalence of culture positive pulmonary TB among pulmonary TB suspects, the calculated sample size for the suspects to be included in our study was 507. However, we were able to identify 101 culture positives from 328 pulmonary TB suspects and therefore, sample collection was concluded.

For the assessment of delay, we recruited newly diagnosed TB patients from two DOTS clinics in a consecutive manner over the study period. They were interviewed with structured questionnaire.

2.6. Reference standard for the serology study

The best diagnostic test available for TB is culture and therefore it was used as a reference standard in all patients suspected of pulmonary TB. Smear microscopy on all sputum samples was done at health facilities and this allowed us to determine the sensitivity of the antigens among those who were smear negative but culture positive pulmonary TB cases.

2.7. Sample collection, transport, storage and processing

Our project site was far from the laboratory center for culture and serology. Therefore, appropriate collection, storage and transport of samples were essential steps to ensure the reliability of the results. Serum samples were kept at -20 °C and sputum samples were stored at 4 °C at study sites until transported to ALIPB laboratory within 5-10 days. Sputum and serum were transported to ALIPB laboratory under the necessary precautions to maintain the quality of samples. Subsequently, serum samples were kept at -20 °C until ELISA was run. Sputum samples were immediately inoculated into culture media.

2.7.1. Sputum collection, transport and storage

Sterile sputum cups, approximately 10ml, were used for sputum collection. Participants suspected of pulmonary TB were given sputum cups after explaining how to produce a quality (mucoid or mucopurulent) and adequate (3-5 ml) sputum [74]. Sputum cups were labelled properly with a unique identification number which corresponds to every study participant.

It is a routine practice to collect 3 sputum samples for the diagnosis of pulmonary TB. A leak proof wooden box with ice pack was used to transport containers from study site to ALIPB laboratory.

2.7.2. Blood

Laboratory technicians/technologists working in selected health facilities were responsible for collecting serum samples. About 5 ml of blood was drawn by venipuncture under aseptic technique (70% alcohol was used to clean the overlying skin and a sterile needle and syringe was used to draw blood) from participants. After allowing the blood to clot for 10-30 minutes, it was centrifuged and serum was separated, divided into two aliquots in cryotubes and stored as described above.

2.7.3. Smear microscopy

Smear preparation, staining and examination was done according to WHO guideline [75]. Briefly, new, clean, unscratched slides were labelled with the corresponding patient identity number and specimen were transferred, spread and allowed to air dry for 15 minutes. Then fixing was made by passing the slides through a flame 3 times. The slides were flooded with Ziehl- Neelsen carbolfuchsin. Gentle heating for 3 to 5 minutes was done to facilitate staining. After rinsing the slides with clean water, slides were flooded with 25% sulphuric acid for 3 minutes as a decolourizing agent. Subsequently, slides were rinsed with clean water and methyl blue was used as a counter stain for 1 minute. The slides were then air dried. Using 100x oil immersion objective, smears were examined by laboratory technicians at the respective health facilities.

2.7.4. Bacterial culture

Specimen were processed and cultured according to WHO guideline [74]. Briefly, sputum sample were homogenised and decontaminated using Petroff method. One unit volume of sputum was mixed with equal volume of 4% [1]NaOH and shaken. After letting stand for 15 minutes at room temperature with occasional shaking, it was centrifuged at 3,000 rpm for 15 minutes. The supernatant was poured off while the sediment was neutralized with 0.1N [2]HCL using phenol red as an indicator. Neutralization was achieved when the colour of the solution was changed from purple to yellow. After removing the condensed moisture in the slant of culture media, 0.2-0.4ml (2-4 drops or 2-4 loopfuls) of the centrifuged sediment was inoculated and distributed over the slant surface. Four slopes of Lowenstein-Jensen media were inoculated per specimen, two of them containing pyruvate to isolate *M.bovis.* Culture media were kept in slant position for the first 24 hrs and then upright position at 37°c.

Subsequently, inspection of media was done 72 hrs after inoculation to see the fluid content and then after one week to see for the growth of rapidly growing mycobacteria to differentiate them from *M.tuberculosis.* Subsequent inspection of media after 3-4 weeks was done to look for growth of *M.tuberculosis* or other slow growing contaminants. Those without growth after 8 weeks were discarded and reported as negatives. Liquefied or completely contaminated media were discarded. Partially contaminated media were kept until growth of *M.tuberculosis* is seen or until the 8^{th} week. In late contamination, Acid Fast Bacilli (AFB) staining was done to see for growth of *M.tuberculosis*.

2.7.5. ELISA

Antigens were donated by Statens Serum Institut, Denmark and optimization was done at Norwegian Institute of Public Health. Nunc Maxisorb ELISA plates (flat bottom, Nunc Maxisorp, Roskilde, Denmark) were coated with rESAT-6-CFP10 and rα-crystallin-MPT-83 antigen at 4 μg/ml (0.4 μg antigen per well) in fresh 10 mM [3]PBS (pH 7.4). The plates were then incubated for two days at 4 °C before use. After washing 5 times with PBS containing

[1] NaOH= sodium hydroxide
[2] HCL= Hydrochloric acid
[3] PBS= Phosphate buffered saline

0.05% Tween20™, 100 µl of PBS containing 2% [4]BSA was added to each well as a blocking solution and then incubated at 37°C for 1 hour. After washing as described above, 100 µl of patient serum diluted at 1:75 in PBS containing Tween20™ 0.05% and 2% BSA was added in each well and plates were incubated at 37 °C for 1 hour. Plates were washed as above and 100 µl of sigma product A3187 anti-human IgG-alkaline phosphatase diluted at 1:5000 in PBS containing Tween20™ 0.05% and 2% BSA was added in each well. The plates were incubated at 37°C for 1 hour. ELISA was developed using sigma product S0942 alkaline phosphatase-substrate dissolved in 10% dietanolamine buffer (pH=9.8). Two tablets of sigma product S0942 were dissolved per 10 ml dietanolamine buffer and 100 µl of this solution was added per well. Signals were recorded at 405 nm, at 30 minutes. Samples were run as duplicates and mean value of the two Optical Density (OD) readings were taken for analysis.

Positive and negative control serums were used in every plate. The negative control serum was from an apparently healthy individual who had no chest x-ray abnormality. Besides, the patient was TST and interferon gamma negative. On the other hand, the positive control serum was from culture confirmed pulmonary TB patient with high OD values for both antigens.

2.7.6. HIV test

HIV testing was performed since the performance of the test might be affected by the HIV status of participants. It was done according to the existing national algorithm used to diagnose HIV. The algorithm utilizes three rapid HIV test kits: KHB test (Shanghi Kehua Bio-engineering, Ltd, China), HIV1/2 STAT-PAK® ASSAY (CHEMBIO Diagnostic systems, Inc, USA) and Uni-Gold TM (Trinity Biotech, USA). KHB was used as a screening test whereas STAT-PAK was required to confirm the initial HIV diagnosis made with KHB. Uni-Gold was utilized as a tie-breaker when the screening and confirmatory test results are discordant. Those patients with HIV infection were counselled and referred to antiretroviral therapy clinics for further intervention.

[4] BSA=Bovine serum albumin

2.8. Management of patients with TB

The management of patients with TB in Ethiopia is based on the national guidelines. Smear microscopy is the standard diagnostic procedure for pulmonary TB. Generally, smear positive pulmonary TB is confirmed when a patient has 2 AFB smear positive results or one AFB smear positive result with radiological abnormalities consistent with pulmonary TB (76). For smear negative patients, chest X-ray (if available) and clinical findings were used to establish diagnosis. Culture is not routinely used for diagnostic purposes. Patients with TB are treated under the DOTS programme adopted from WHO guideline. The management of patients who participated in our study was based on the existing national guidelines. Local health workers were responsible for patient management as usual and patients were not delayed until our results were ready. However, we provided culture results to the respective health institutions for further patient management decisions. Serology result was not used in making decision in patient management.

2.9. Operational definitions of terms and variables

Dependent variables:

Pulmonary TB suspects: pulmonary TB suspects were defined according to the national guidelines: patients having cough for 3 weeks or more are pulmonary TB suspects

Presence or absence of disease (final diagnosis): true cases or non-cases as determined by culture and smear microscopy

Test result: positive or negative result as determined by rESAT-6/CFP-10 and rα-crystallin-MPT-83 recombinant fusion antigens

Patients' delay: The time interval between onset of symptoms of TB and first presentation to a professional health provider.

Health system's delay: The time interval between date of first presentation of patients to a professional health provider and initiation of treatment.

Treatment delay: The time interval between date of diagnosis and initiation of treatment

Total delay: The time interval between onset of symptoms of TB and initiation of treatment.

Independent variable:

Non-formal (Informal) health providers: These include traditional health providers, local injectors and drug retail outlets

Formal health providers: Professional health providers working in modern health facilities i.e. hospitals, health centers, clinics owned by government or private sector

Drug retail outlets: includes pharmacies, drug stores, drug vendors and open market drug sellers

Traditional health provides: includes traditional healers and religious healers

Traditional healers: health providers who use mainly herbs to treat human ailments

Local injectors: health providers with no professional training who use drugs to treat their clients. They mainly give injections to their clients.

Pastoralists: People whose source of livelihood is livestock with which they move seasonally in search of pasture and water.

Agro-pastoralists: People whose main source of livelihood is livestock, but also practice farming to some extent.

Distance to health facility: Distance in kilo meter from patient's residence to the nearest health facility, at the time when patient took the decision of seeking medical care.

No education: no formal (school) education

Primary education: 1 to 6 years of formal education

Post-primary education: More than 6 years of formal education

2.10. Definitions of statistical terms used to assess test performance

Prevalence: proportion of TB cases identified by culture and/or smear microscopy among all suspects

Sensitivity: proportion of true positives that are correctly identified by each antigen

Specificity: proportion of true negatives that are correctly identified by each antigen

Likelihood Ratio of a positive test (LR+): How much more likely is a positive test to be found in a person with the condition than in a person without it? It is simply the ratio of sensitivity to 1-specificity

Likelihood Ratio of a negative test (LR-): How much more likely is a negative test to be found in a person without the condition than in a person with it? It is simply the ratio of 1-sensitivity to specificity

2.11. Data collection forms

A questionnaire was used to interview and collect data on basic socio-demographic and clinical aspects of pulmonary TB suspects (Appendix 3). Moreover, diagnostic delay was

assessed using a structured questionnaire (appendix 4). The questionnaire for assessing diagnostic delay addressed socio-demographic characteristics, health related issues like self-treatment, distance from the nearest health facility, knowledge on cause of TB, its seriousness and treatment, symptoms they are suffering from, time interval between onset of symptoms and first visit to a health facility, time interval between first visit of a health facility and diagnosis, and time interval between diagnosis and treatment.

2.12. Significance of the study

The first aim of the study was to evaluate the potential of rESAT-6/CFP-10 and rα-crystallin-MPT-83 antigens for the diagnosis of pulmonary TB. Since this was a preliminary test evaluation, there was no any direct and immediate benefit from the test per se for the participants as well as the community they belong. However, some patients benefited from culture results. Moreover, as part of the global initiative to find new rapid tests for the diagnosis of TB, we believe that this study has added scientific knowledge in the field. Ultimately, identifying simple and rapid tests will have a positive impact on TB control.

We have also addressed delay in the diagnosis and treatment of TB in Afar Region where such information is non-existent. This has generated important information on the contribution of patients and the health system to the total delay as well as factors associated with delay in the study area.

2.13. Data management

Data has been handled confidentially. Data quality was assured through pretesting of questionnaires, training and supervision of Interviewers, use of standard procedures for sample collection & storage, and use of quality control during running the laboratory tests. Names were replaced with a unique identity number and subsequently data was entered into EpiData version 3.1 & rechecked for errors, consistency, missing values and outliers. Subsequently it was exported into SPSS for Windows version 16 for further analysis.

Simple frequency distribution with measures of central tendency (mean, median) and dispersion (standard deviation, range, inter-quartile range (IQR)) were run. Since data from serology study as well as delay study was not normally distributed, non-parametric tests (Mann-Whitney/Kruskal Wallis) were used in evaluating group difference. Chi-square test

was used for categorical variables. Multivariable logistic regression was done to identify independent predictors of dependent variables. In some studies, experts agreed 30 days as acceptable cut-off for delay [33,34] whereas the majority used median value of the observed data as a cut-off [35-37] and we adopted the later.

Awareness regarding TB was assessed using questions addressing their knowledge about treatment of TB and the seriousness of the disease. In relation to treatment, three questions were posed: whether TB is curable or not, fee for treatment and duration of treatment. In relation to seriousness of the disease, participants were asked about causes of TB, risk for patients and people around them before treatment. For each question, a value of 1 was given if correctly answered and 0 if not. Thereafter, a total score was calculated by adding the values for the six questions and subsequently, inter-quartile scores were calculated. Participants that fall into the third quartile were considered as having high knowledge and those below were considered as having low knowledge.

The receiver operating characteristic (ROC) curve for the OD values of the antigens was plotted using STATA version11 and the area under the curve (AUC) and 95% CI were calculated. The best discrimination limit (cut-off value) for each antigen was determined using the ROC curve. Serum samples were identified as positive for the specific antibody response when the OD is greater or equal to the cut-off value. Sensitivity, specificity, and likelihood ratios along with their 95% CI were calculated. In all cases, level of significance was set at 95 % ($p < 0.05$) and all tests were two-tailed.

2.14. Communication of Results

Results of the study will be presented as a thesis at Department of General Practice and Community Medicine, Institute for health and Society, University of Oslo. Moreover, it will be communicated to the Afar Regional Health Bureau. Article/s will be submitted to peer-reviewed journals for publication.

2.15. Ethical consideration

This project has been ethically cleared by the Norwegian Ethical Committee and Ethiopian National Ethics Committee before commencing the study. Patients who fulfilled the inclusion criteria were invited to participate in the study. Both the interview and consent process were

carried out in a private room by health workers. Study participants were informed on the purpose of the study as well as risks and benefits associated with it. Participants were granted their freedom to decline any time during the study and they were assured that their decision not to participate in the study have no impact on the routine health care they get (Appendix 1).

Local health workers speaking the local language were involved in obtaining consent to reduce the power structure. Interpreters were used whenever health workers speaking the local language were not available. Every effort was made to maintain the neutral position of these health workers through short training, follow up and support. After ensuring that the potential subject has understood the information, health workers sought the potential participant's freely-given informed consent. Since the majority of the study participants were illiterates, the informed consent form was read and explained to them using their language and a witnessed consent was taken (Appendix 2).

Chapter 3: Results

This chapter consists of 2 parts: Under 3.1, the results for the evaluation of the diagnostic performance of the two antigens (rESAT-6-CFP-10 and rα-crystallin-MPT-83) have been presented. Under part 3.2, the results of our assessment related to delay in diagnosis and treatment of TB is presented. Accordingly, we have two groups of study participants. The details of each group of participants have been described under the respective part.

3.1. ELISA-based evaluation of the potential of rESAT-6-CFP-10 and rα-crystallin-MPT-83 antigens for the diagnosis of pulmonary TB

A total of 328 participants who were identified as pulmonary TB suspects and subsequently sent to laboratories for smear microscopy in selected health facilities were included in this study. Five of the 328 participants were excluded because of the poor quality of sputum. Therefore, a total of 323 participants were included for further analysis.

3.1.1. Socio-demographic characteristics of study participants

The socio-demographic characteristic of study participants has been summarized in Table 1.The mean age of study participants was 35± 14.5 whereas the median was 30 years with the minimum and maximum of 18 and 80 years respectively. However, about 75 % of the study participants were below 45 years. The number of males was higher than females with a ratio of 1.4 to 1.The majority of the study participants were Muslims (73.1%) and the number of participants with no education (63.5 %) exceeded those with education.

Table 1. Socio-demographic characteristics of pulmonary TB suspects in Northeast Ethiopia

Variable	Count (n=323)	Percent (%)
Age		
18-24	79	24.5
25-44	163	50.4
>44	81	25.1
Sex		
Male	188	58.2
Female	135	41.8
Marital status		
Single	107	33.1
Married	192	59.5
Widowed	10	3.1
Divorced	14	4.3
Residence		
Urban	183	56.7
Rural	140	43.3
Religion		
Muslim	236	73.1
Christian	87	26.9
Ethnicity		
Afar	145	44.9
Amhara	126	39.0
Oromo	52	16.1
Formal education		
None	205	63.5
Primary (1-6)	77	23.8
Post-primary (>6)	41	12.7
Occupation		
Pastoralist	147	45.5
Non-pastoralist	176	54.5

Among the 323 patients, a total of 101 (31.3 %) were identified as pulmonary TB patients based on culture results. Among the 101 culture positives, 41 (40.6 %) were already diagnosed as smear positive pulmonary TB patients based on direct smear microscopy done at health facilities. Six more suspects who were reported as smear positive turned out to be culture negative. Therefore, the proportion of smear positive pulmonary TB patients among all suspects was 47 (14.6 %). Based on bacteriology result (direct smear and culture), a total of 107 (33.1 %) suspects were diagnosed as pulmonary TB patients. Moreover, an additional 50 patients were diagnosed as pulmonary TB cases based on chest x-ray and clinical judgment even though they were both culture and smear negative. After culture result, the overall proportion of pulmonary TB patients among pulmonary TB suspects in the current study was 157 (48.6 %).

However, from now on, the phrase “pulmonary TB patients” refers to those who were culture positive and or smear positive (bacterium positive) patients, unless specifically stated. This might result in some degree of misclassification of pulmonary TB patients as non-TB patients.

3.1.2. Major symptoms reported and their association with pulmonary TB

Participants were interviewed about the different symptoms they were suffering from. Cough was reported in all patients and fever was the second most frequently reported symptom (94.4%). Moreover, about 20% of patients reported haemoptysis.

On bivariate analysis, a higher proportion of pulmonary TB patients reported fever, weight loss and loss of appetite compared to non-TB patients. However, none of them were significantly associated with pulmonary TB. Nearly 73% of pulmonary TB patients reported fatigue &weakness compared to 62% of non- pulmonary TB patients but the difference failed to reach statistical significance. Differences in the presenting symptoms among pulmonary TB and non-TB patients is summarized in Table 2.

Table 2. Major symptoms reported by pulmonary TB suspects and their association with pulmonary TB in Northeast Ethiopia

Symptom	Pulmonary TB (n=107)	Non-TB (n=216)	χ^2	p-value
Cough				
Haemoptysis	21 (19.6)	47 (21.8)	0.20	0.66
No Haemoptysis	86 (80.4)	169 (78.2)		
Fever				
Yes	101 (94.4)	200 (92.6)	0.37	0.55
No	6 (5.6)	16 (7.4)		
Loss of appetite				
Yes	82 (76.6)	158 (73.1)	0.46	0.50
No	25 (23.4)	58 (26.9)		
Weight loss				
Yes	83 (77.6)	150 (69.4)	2.35	0.13
No	24 (22.4)	66 (30.6)		
Night sweating				
Yes	80 (74.8)	163 (75.5)	0.02	0.89
No	27 (25.2)	53 (24.5)		
Fatigue and weakness				
Yes	78 (72.9)	134 (62.0)	3.74	0.05
No	29 (27.1)	82 (38.0)		

3.1.3. Pulmonary TB and its association with socio-demographic, cultural and health-related factors

Bivariate analysis was done to evaluate the association between socio-demographic factors and other selected variables with pulmonary TB (see Table 3). Approximately 81% of pulmonary TB patients were below 45 years of age. The male to female ratio among pulmonary TB patients was approximately 2 to 1 in contrast to a 1.2 to 1 ratio in non-TB patients. The proportion of pulmonary TB among males (37.8%) was significantly higher than the proportion in females (26.7%) (χ^2= 4.37, p=0.04).

Surprisingly, the proportion of pulmonary TB patients among those who did not consume raw milk regularly was significantly higher (39.0%) compared to the proportion among those who consume raw milk regularly (27.8%) (χ^2=4.52, p=0.03). Moreover, there was a significant association between educational status and prevalence of pulmonary TB (χ^2=7.29, p=0.02); the proportion of pulmonary TB was lowest among suspects with no formal education and highest among those with post-primary education.

On multivariable logistic regression, all but raw milk consumption lost statistical significance. After controlling for socio-demographic variables, those who did not consume raw milk regularly were 1.75 times more likely to have pulmonary TB compared to those who consume raw milk (Adjusted Odds Ratio (ORadj.)=1.75, CI 1.06-2.91).

Table 3. Association of pulmonary TB with socio-demographic, cultural and health-related factors among pulmonary TB suspects in Northeast Ethiopia

Variable	Pulmonary TB (n=107)	Non-TB (n=216)	Total, n (%)	χ^2	p-value
Age					
18-24	32 (40.5)	47 (59.5)	79 (24.4)	4.57	0.10
25-44	55 (33.7)	108 (66.3)	163 (50.5)		
>44	20 (24.7)	61(75.3)	81 (25.1)		
Sex					
Male	71 (37.8)	117 (62.2)	188 (58.2)	4.37	0.04*
Female	36 (26.7)	99 (73.3)	135 (41.8)		
Residence					
Urban	65 (35.5)	118 (64.5)	183 (56.7)	1.09	0.30
Rural	42 (30.0)	98 (70.0)	140 (43.3)		
Formal education					
None	59 (28.8)	146 (71.2)	205 (63.5)	7.29	0.02*
Primary (1-6)	27 (35.1)	50 (64.9)	77 (23.8)		
Post-primary (>6)	21 (51.2)	20 (48.8)	41 (12.7)		
Religion					
Muslim	71 (30.1)	165 (69.9)	236 (73.1)	3.66	0.06
Christian	36 (41.4)	51 (58.6)	87 (26.9)		
Ethnicity					
Afar	40 (27.6)	105 (72.4)	145 (44.9)	3.67	0.16
Amhara	47 (37.3)	79 (62.7)	126 (39.0)		
Other	20 (38.5)	32 (61.5)	52 (16.1)		
Occupation					
Pastoralist	44 (29.9)	103 (70.1)	147 (45.5)	1.24	0.27
Non-pastoralist	63 (35.8)	113 (64.2)	176 (54.5)		
Contact with TB patients					
Yes	47 (39.2)	73 (60.8)	120 (37.2)	3.14	0.08
No	60 (29.6)	143 (70.4)	203 (62.8)		
Raw milk ingestion					
Yes	47 (27.8)	122 (72.2)	169 (52.3)	4.52	0.03*
No	60 (39.0)	94 (61.0)	154 (47.7)		
BCG vaccination					
Yes	22 (34.9)	41 (65.1)	63 (19.5)	0.11	0.74
No	85 (32.7)	175 (67.3)	260 (80.5)		

* Significant at p=0.05

3.1.4. Associations of HIV infection with socio-demographic variables

Out of 323 participants, a total of 293 participants were tested for HIV infection and 82 (28%) were positive. The prevalence was lower in males (26.1%) compared to the prevalence in females (30.5%) although this difference was not found to be statistically significant. The peak HIV prevalence in females occurs at younger age compared to males. With regard to females, the peak HIV prevalence was among those between 18 and 24 years old and nearly 72% of HIV infection was found in those between 18 and 34 years of age. On the other hand, the peak HIV prevalence among males was between 35 and 44 years of age and approximately 77% of HIV infection occurred in those between 25 and 44 years of age.

The associations of HIV infection with socio-demographic variables has been assessed using bivariate and multivariate analysis (logistic regression) and summarized in Table 4. On bivariate analysis, age, residence, occupation, literacy and ethnicity were found to be significantly associated with HIV infection. However, multivariable logistic regression indicated that Afar ethnicity was independently associated with low HIV infection (ORadj.=0.28, CI 0.10-0.79); whereas primary education was independently associated with higher HIV infection (ORadj.=2.83, CI 1.28- 6.29) taking no education as a reference group.

Table 4. Associations between HIV infection and socio-demographic variables among pulmonary TB suspects in Northeast Ethiopia

Variable	HIV positive (n=82)	HIV negative (n=211)	ORc (CI)	OR adj.(CI)
Age				
18-24	17	52	1.00	1.00
25-44	55	95	1.77 (0.93-3.36)	2.32 (1.08-5.00) *
>44	10	64	0.48 (0.18-1.22)	0.96 (0.34-2.73)
Sex				
Male	43	122	1.00	1.00
Female	39	89	1.24 (0.72-2.14)	1.63 (0.87-3.09)
Residence				
Rural	18	105	1.00	1.00
Urban	64	106	3.52 (1.89-6.63) *	1.04 (0.45-2.41)
Formal education				
None	28	140	1.00	1.00
Primary (1-6)	41	49	4.18 (2.25-7.80) *	2.86 (1.29-6.35)*
Post-primary (>6)	13	22	2.95 (1.24-7.03) *	1.27 (0.46-3.48)
Ethnicity				
Others	17	31	1.00	1.00
Afar	11	119	0.17 (0.07-0.43) *	0.29 (0.10-0.80)*
Amhara	54	61	1.61 (0.76-3.44)	2.07 (0.96-4.45)
Religion				
Christian	32	48	1.00	1.00
Muslim	50	163	0.46 (0.26-0.83) *	1.58 (0.78-3.18)
Occupation				
Non-pastoralist	67	99	1.00	1.00
Pastoralist	15	112	0.20 (0.10-0.38) *	0.54 (0.23-1.25)

* Significant at p=0.05

3.1.5. HIV infection and TB

The associations between HIV infection with pulmonary TB has been analyzed in relation to smear microscopy, culture and clinical diagnosis. The influence of HIV infection on the diagnostic potential of the two antigens has been analyzed as well (Table 5 summarizes the findings).

The proportion of HIV infection among 101 pulmonary TB patients was 36.6% whereas the corresponding proportion among 192 non-TB patients was 23.3%. Pulmonary TB patients were 1.9 times more likely to have HIV infection compared to non-TB patients (OR=1.9, CI 1.09-3.32). Similarly, the proportion of pulmonary TB among 82 HIV infected pulmonary TB

suspects was 45.1% whereas the corresponding proportion among 211 HIV negative pulmonary TB suspects was 30.3%. HIV infected pulmonary TB suspects were 1.89 times more likely to be pulmonary TB patients than HIV negative pulmonary TB suspects (OR=1.89, CI 1.08-3.30).

Taking all patients diagnosed as pulmonary TB based on smear microscopy, culture and clinical judgement, the proportion of pulmonary TB patients among 82 HIV infected suspects was 59.8% whereas the proportion of pulmonary TB patients among 211 HIV negatives was 43.1%. HIV infected suspects were 1.96 times more likely to be pulmonary TB patients than HIV negative suspects (OR=1.96, CI 1.13-3.40).

The influence of HIV infection on the results of smear microscopy has been assessed among 95 culture positive pulmonary TB patients. It has been found that 30 (48.4%) of the 62 HIV negative pulmonary TB patients were smear positive compared to only 8(24.2%) of the 33 HIV positive pulmonary TB patients. There is a 66% reduction in smear positivity among HIV infected pulmonary TB patients compared to HIV negative pulmonary TB patients (OR=0.34, CI 0.12-0.95).

Table 5. Association of HIV infection with pulmonary TB according to the different diagnostic methods in Northeast Ethiopia

Variable	HIV Positive	HIV Negative	OR (CI)
Smear microscopy result (n=293)			
Negative	70	179	1.00
Positive	12	32	0.96 (0.44-2.07)
Smear microscopy or culture (n=293)			
Negative	45	147	1.00
Positive	37	64	1.90 (1.09-3.32)*
Smear microscopy result among culture positives (n=95)			
Negative	25	32	1.00
Positive	8	30	0.34 (0.12-0.95) *
Final diagnosis (n=293) ‡			
Non-TB	33	120	1.00
Pulmonary TB	49	91	1.96 (1.13-3.40) *

‡ Diagnosis based on smear microscopy, culture, chest x-ray and clinical judgment

*Significant at p=0.05

3.1.6. Accuracy of smear microscopy and physician's diagnosis

The overall accuracy of smear microscopy has been assessed using culture as a reference. It was found that smear microscopy has a low sensitivity (40.6%) with a specificity of 97.3%. The positive and negative predictive values were 87.2% and 78.3% respectively (table 6). Furthermore, the accuracy of smear microscopy in government (Table 7) and private health facilities (Table 8) has been assessed separately. It was found out that there is a significant difference (χ^2=7.86, p=0.005) in the sensitivity of smear microscopy in the two groups of health facilities: 52.6% in government compared to only 25% in private health facilities. However, the corresponding specificities of smear microscopy were comparable: 100% in private health facilities versus 96% in government health facilities.

Table 6. Accuracy of smear microscopy for the diagnosis of pulmonary TB in Northeast Ethiopia

		Culture		
		Positive	**Negative**	**Total**
AFB	**Positive**	41	6	**47**
	Negative	60	216	**276**
	Total	**101**	**222**	**323**

Sensitivity = 40.6% Specificity = 97.3%

LR positive=15.03 LR negative= 0.61

Table 7. Accuracy of smear microscopy for the diagnosis of pulmonary TB at Government health facilities in Northeast Ethiopia

		Culture		
		Positive	**Negative**	**Total**
AFB	**Positive**	30	6	**37**
	Negative	27	144	**171**
	Total	**57**	**150**	**207**

Sensitivity =52.6% Specificity = 96.0%

LR positive= 13.15 LR negative= 0.49

Table 8. Accuracy of smear microscopy for the diagnosis of pulmonary TB at private health facilities in Northeast Ethiopia

		Culture		
		Positive	**Negative**	**Total**
AFB	**Positive**	11	0	**11**
	Negative	33	72	**105**
	Total	**44**	**72**	**116**

Sensitivity =25% Specificity =100%

LR positive= Infinitive LR negative= 0.75

Accuracy of physician's diagnosis based on clinical, direct smear microscopy, and or chest x-ray results was assessed for its accuracy against culture (Table 9). The overall sensitivity and specificity of physician's diagnosis was 66.3% and 74.8%, respectively. The specificity of physician's diagnosis is low compared to smear microscopy. About 72% of suspects were accurately diagnosed as TB or non-TB patients.

We further analyzed the impact of HIV infection on physician's diagnostic accuracy. We found that HIV infection influences physician's diagnostic accuracy (Table 10 & 11). The sensitivity in HIV negative pulmonary TB patients was 72.6% compared to 54.5% in HIV positive pulmonary TB patients, although this difference failed to reach statistical significance (χ^2=3.14, p=0.08). Similarly, the specificity in HIV negatives was 80.5% compared to 65.3% in HIV positive non-TB patients and this difference was found to be statistically significant (χ^2=4.80, p=0.03).

Table 9. Overall accuracy of physician's diagnosis of pulmonary TB in Northeast Ethiopia

		Culture		
		Positive	**Negative**	**Total**
Physician's Diagnosis	**TB**	67	56	**123**
	Non-TB	34	166	**200**
	Total	**101**	**222**	**323**

Sensitivity = 66.3% Specificity = 74.8%

LR positive=2.63 LR negative =0.45

Table 10. Accuracy of physician's diagnosis of pulmonary TB among HIV negative suspects in Northeast Ethiopia

		Culture		
		Positive	**Negative**	**Total**
Physician's Diagnosis	**TB**	45	29	**74**
	Non-TB	17	120	**137**
	Total	**62**	**149**	**211**

Sensitivity =72.6% Specificity = 80.5%

LR positive= 3.72 LR negative= 0.34

Table 11. Accuracy of physician's diagnosis of pulmonary TB among HIV positive suspects in Northeast Ethiopia

		Culture		
		Positive	**Negative**	**Total**
Physician's Diagnosis	**TB**	18	17	**35**
	Non-TB	15	32	**47**
	Total	**33**	**49**	**82**

Sensitivity =54.5% Specificity =65.3%

LR positive= 1.57 LR negative=0.70

3.1.7. Accuracy of rESAT-6-CFP-10 and rα-crystallin-MPT-83 antigens

Taking culture as a reference, 89 serum samples from 101 culture positives and 115 serum samples from 216 culture and smear negatives were randomly selected and ELISA was run on these samples. The 6 smear positive but culture negative patients were excluded from the ELISA test.

Summary statistics has been done for the OD values of the two antigens and their mean values are higher than the corresponding median values in both antigens indicating skewed distribution of OD values (Table 12).

Table 12. Summary statistics of OD values for rESAT-6-CFP-10 and rα-crystallin-MPT-83 in culture positive and negative patients in Northeast Ethiopia

	Summary statistics	Culture negatives†	Culture positives	p-value
N		115	89	
Age	Median (10^{th}-90^{th} percentile)	35(20-60)	30(19-55)	0.11
rESAT-6-CFP-10	Mean± SD	0.34±0.31	0.73±0.86	
	5^{th} percentile	0.14	0.18	
	10^{th} percentile	0.15	0.21	
	25^{th} percentile	0.17	0.24	
	Median	0.23	0.35	<0.001*
	75^{th} percentile	0.35	0.88	
	90^{th} percentile	0.71	2.34	
	95^{th} percentile	0.91	3.27	
rα-crystallin-MPT-83	Mean ± SD	0.99±0.55	1.124±0.70	
	5^{th} percentile	0.40	0.37	
	10^{th} percentile	0.45	0.45	
	25^{th} percentile	0.55	0.64	
	Median	0.81	0.91	0.29
	75^{th} percentile	1.28	1.30	
	90^{th} percentile	1.79	2.43	
	95^{th} percentile	2.21	2.67	

* Significant at p=0.05 † Culture and smear negatives

Histogram of OD values has been constructed for both antigens and the distribution is skewed to the right with extreme values (see Figure 1 & 2). One sample Kolmogorov-Smirnov test was done as well and p-value for both is below 0.001 indicating that the distribution of OD values for both antigens is far from normal distribution. Log transformation was done and logOD values for rα-crystallin-MPT-83 became normally distributed; however, the logOD

values of rESAT-6-CFP-10 remained skewed. Therefore, non-parametric method was used for further analysis.

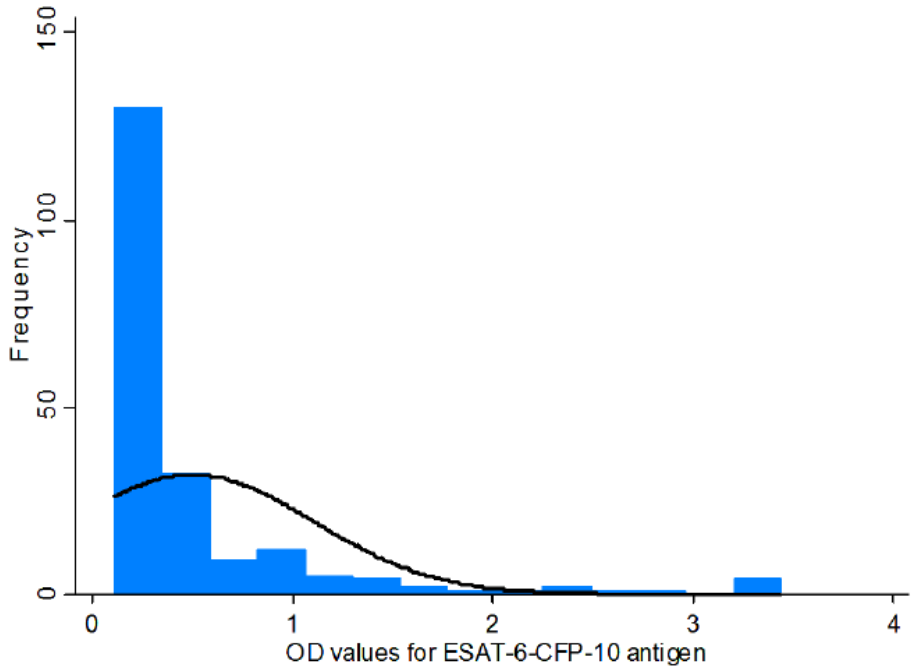


Figure 1. Frequency distribution of OD values for rESAT-6-CFP-10 in Northeast Ethiopia

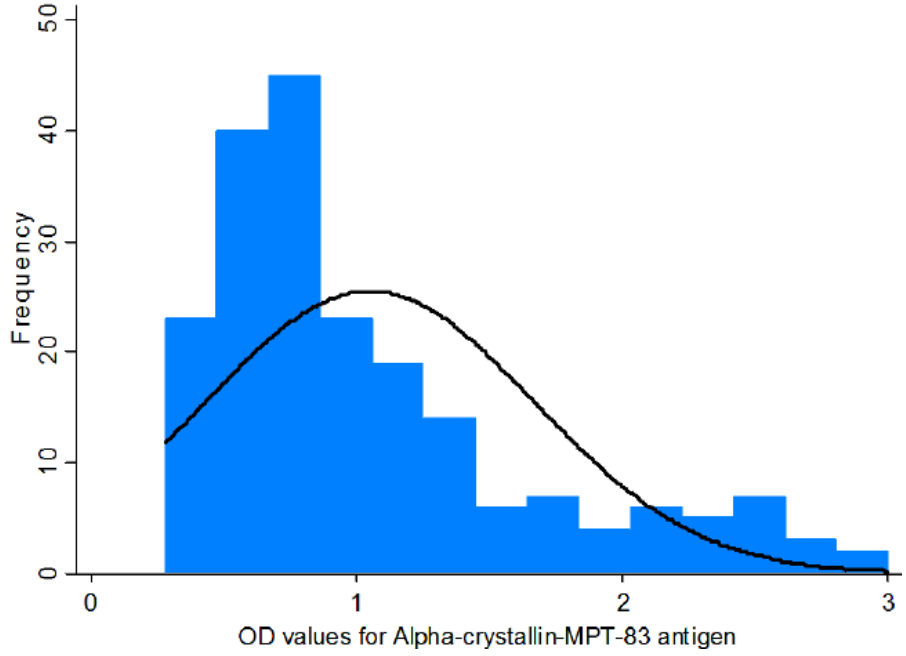


Figure 2. Frequency distribution of OD values for rα-crystallin-MPT-83 antigen in Northeast Ethiopia

Possible association of some selected variables with OD values of pulmonary TB suspects has been evaluated using Mann-Whitney (for two groups) and Kruskal-Wallis (for three or more groups) tests. The result is summarized in Table 13. For rESAT-6-CFP-10 antigen, vaccination with BCG has been associated with higher median OD values compared to the non-vaccinated group (median of 0.44 in vaccinated versus 0.26 in non-vaccinated) and this difference has been found to be statistically significant (p=0.004). Similarly, the median OD values for rα-crystallin-MPT-83 antigen among vaccinated and non-vaccinated groups has been found to be 1.02 and 0.82 respectively; however, this difference did not reach statistical significance (p=0.052).

On the other hand, there was no statistically significant association of median OD values for the two antigens with sex, age, ethnicity, ingestion of raw milk, contact with TB patients, being pastoralist, smear result of culture positive pulmonary TB patients and HIV infection (all having p value above 0.05).

Table 13. Associations of socio-demographic, cultural and health-related factors with OD values for the two antigens in Northeast Ethiopia

Variable	count	Median (IQR) †	p-value†	Median(IQR) ‡	p-value‡
Age (n=204)					
18-24	46	0.33 (0.21-0.71)	0.27	0.83 (0.59-1.26)	0.97
25-44	103	0.26 (0.19-0.45)		0.83 (0.63-1.32)	
>44	55	0.27 (0.18-0.45)		0.85 (0.59-1.18)	
Sex (n=204)					
Male	116	0.28 (0.20-0.48)	0.93	0.82 (0.59-1.29)	0.72
Female	88	0.27 (0.19-0.51)		0.85 (0.65-1.29)	
Ethnicity (n=204)					
Afar	91	0.26 (0.18-0.58)	0.48	0.82 (0.61-1.42)	0.96
Amhara	83	0.29 (0.21-0.45)		0.85 (0.65-1.21)	
Others	30	0.32 (0.20-0.52)		0.82 (0.54-1.41)	
Occupation (n=204)					
Pastoralist	99	0.28 (0.18-0.52)	0.75	0.90 (0.65-1.44)	0.08
Non-pastoralist	105	0.28 (0.21-0.47)		0.78 (0.57-1.20)	
Raw milk ingestion					
(n=204)	99	0.27 (0.19-0.55)	0.91	0.88 (0.63-1.44)	0.11
Yes	105	0.29 (0.20-0.46)		0.78 (0.60-1.20)	
No					
Contact history (n=204)					
Yes	74	0.27 (0.19-0.54)	0.60	0.84 (0.65-1.29)	0.54
No	130	0.29 (0.20-0.48)		0.83 (0.58-1.29)	
BCG vaccination					
(n=204)	30	0.44 (0.24-0.81)	0.004*	1.02 (0.66-1.87)	0.05
Yes	174	0.26 (0.19-0.41)		0.82 (0.60-1.24)	
No					
Smear result (n=89)					
Positive	37	0.32 (0.24-1.23)	0.73	0.76 (0.55-1.71)	0.31
Negative	52	0.36 (0.24-0.68)		0.94 (0.70-1.27)	
HIV status (n=201)					
Positive	55	0.29 (0.21-0.46)	0.56	0.90 (0.70-1.18)	0.51
Negative	146	0.27 (0.19-0.51)		0.82 (0.55-1.34)	

† rESAT-6-CFP-10 ‡rα-Crystallin-MPT-83 *Significant at p=0.05

For rESAT-6-CFP-10 antigen, the median OD values of smear positives (n=37) and negatives (n=52) among culture positive pulmonary TB patients (n=89) was compared using Mann-Whitney test and there is no statistically significant difference between them (p=0.733). Similarly, there is no statistically significant difference in the median OD values of smear positives (n=37) and negatives (n=52) among culture positives (n=89) for rα-crystallin-MPT-83 antigen (p=0.306).

For both antigens, OD values of culture positive and negative patients were compared to see if there is a difference. Summary statistics (table 12) and box plots (Figures 3 and 4) show that there is a difference between culture positive and culture negative patients in their median OD values for both antigens; however, the difference for rESAT-6-CFP-10 antigen was more striking where culture positive patients having higher OD values compared to culture negative patients. Moreover, from the box plot, we can see that OD values for rESAT-6-CFP-10 in culture positives was more skewed and spread compared to culture negatives. However, for rα-crystallin-MPT-83 antigen, there was no much difference in skeweness and spread of OD values of culture positive and negative patients.

Mann-Whitney test has been done to see if the difference in the OD values for culture positive and negative patients was statistically significant. For rESAT-6-CFP-10, the median OD value (0.34) of culture positives was significantly higher than the median OD value (0.23) of culture negatives (Mann-Whitney, $p<0.001$) indicating a stronger IgG specific immune response in culture positive pulmonary TB patients. However, for rα-crystallin-MPT-83 antigen, there was no statistically significant difference in the median values of culture positives (0.88) and culture negatives (0.81) (Mann-Whitney, $p=0.29$).

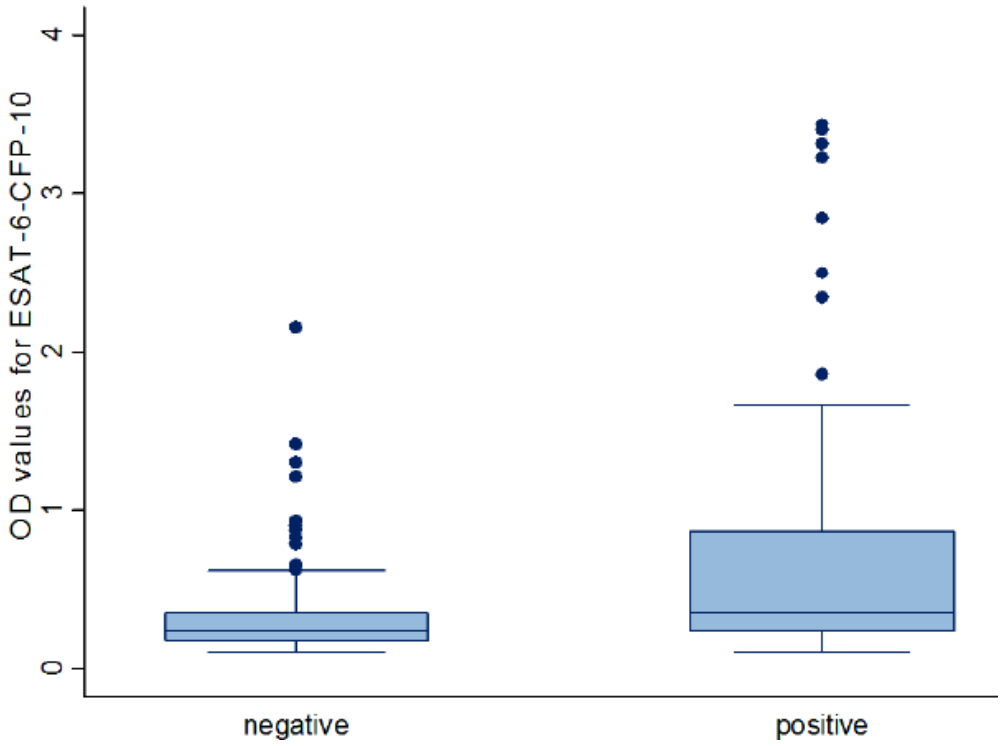


Figure 3. OD values of culture positive and culture negative patients to rESAT-6-CFP-10 antigen in Northeast Ethiopia

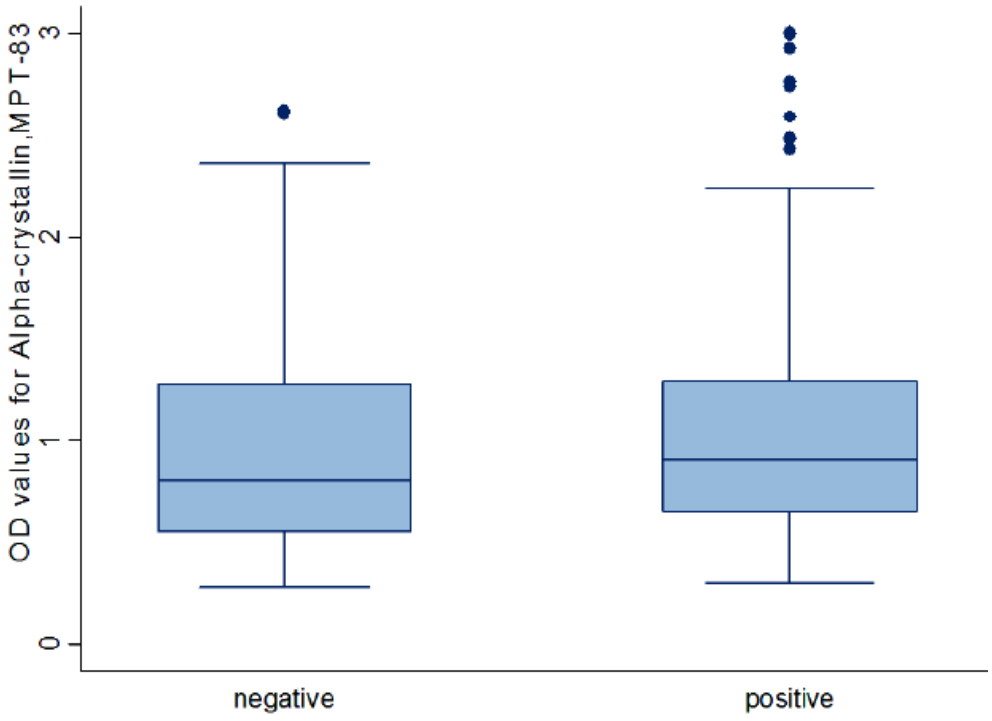


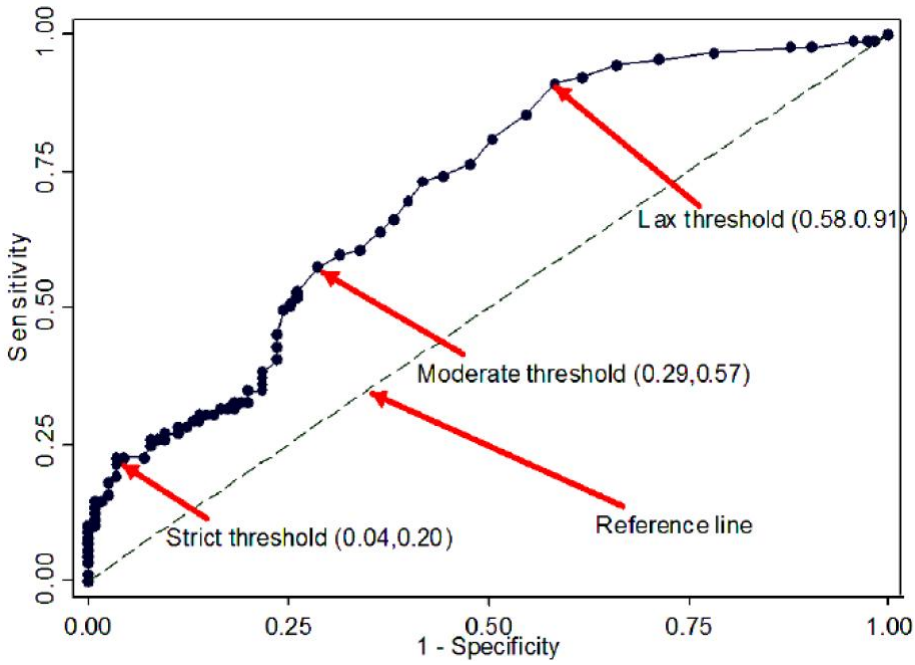
Figure 4. OD values of culture positive and culture negative patients for rα-Crystallin-MPT-83 antigen in Northeast Ethiopia

Non-parametric ROC curve for the two antigens has been constructed as sensitivity versus 1-specificity taking each OD value as a possible cut-off point. AUC for a ROC curve of a test is a summary measure of the overall performance of the test. The higher the value the better the test performance is. AUC for rESAT-6-CFP-10 antigen was 0.708 (CI 0.625- 0.768). This means a randomly picked culture positive patient had a probability of 0.708 to have a higher OD values for rESAT-6-CFP-10 antigen compared to a randomly picked culture negative patient. On the other hand, AUC for rα-crystallin-MPT-83 antigen was 0.543 (CI 0.463-0.623) indicating that this antigen had low discriminating ability. The probability of a randomly selected culture positive patient to have a higher OD values compared to a randomly picked culture negative patient is just 0.543 which is very close to 0.5, a value that is expected on tossing a coin.

Based on the ROC curve, the optimum sensitivity and specificity of rESAT-6-CFP-10 were determined to be 57.3% and 71.3% respectively. Based on these values, the cut-off value was determined from STATA output to be 0.32. This cut-off resulted in a moderate specificity but

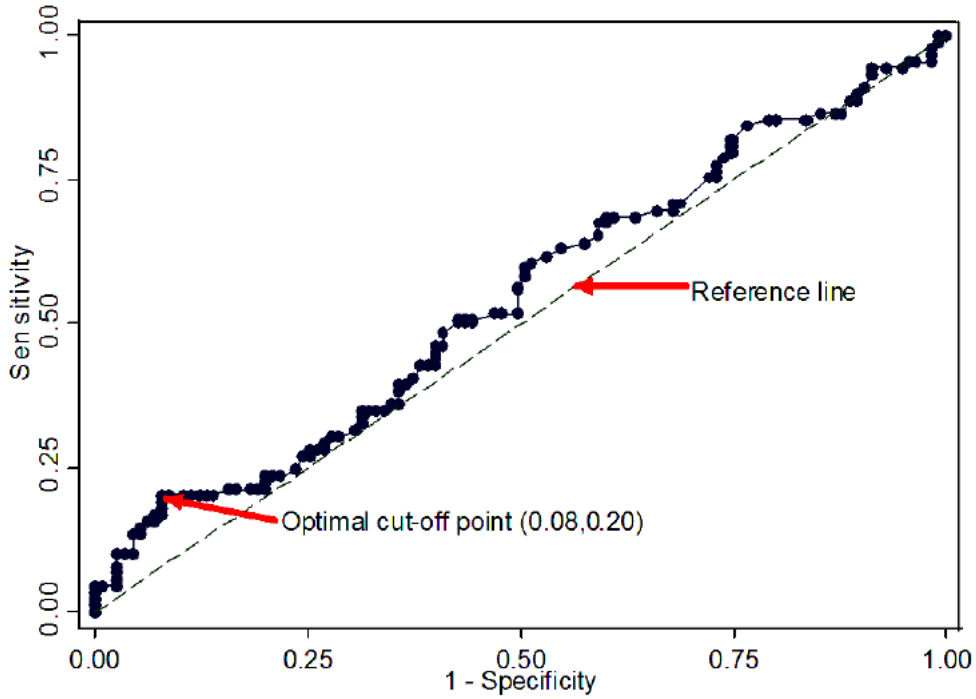
low sensitivity. Alternatively, a lax threshold would result in a higher sensitivity (91.2%) but a lower specificity (42.2%). On the other hand, a strict threshold would result in a low sensitivity (20%) and a high specificity (96%). Figure 5 below shows three possibilities of sensitivity and 1-specificity taking three different threshold (cut-off) values.

Likewise, the optimum sensitivity and specificity of rα-crystallin-MPT-83 from the ROC analysis were 20% and 92% respectively and the corresponding cut-off value was found to be 1.85 (Figure 6).



AUC=0.708 (0.625-0.768), Se=0.036

Figure 5. ROC curve of OD values for rESAT-6-CFP-10 antigen among pulmonary TB suspects in Northeast Ethiopia



AUC=0.543 (CI 0.463-0.623), Se=0.041

Figure 6. ROC curve for OD values of rα-crystallin-MPT-83 antigens among pulmonary TB suspects in Northeast Ethiopia

Following determination of cut-off values for the two antigens, those patients evaluated by the ELISA test were dichotomized as negative if their OD values are less than the cut-off value or positive if their OD values are greater or equal to the cut-off values (0.32 for rESAT-6-CFP-10 and 1.85 for rα-crystallin-MPT-83). Subsequently, 2 by 2 tables were constructed and sensitivity, specificity, predictive values and likelihood ratios were determined.

The sensitivity and specificity of rESAT-6-CFP-10 were 57.3% and 71.3%, respectively. About 65% of the suspects were correctly classified as pulmonary TB or non-pulmonary TB patients. The positive and negative predictive values were 61.7% and 68%, respectively, whereas the positive and negative likelihood ratios were 2.00 and 0.60, respectively (Table 14). Accuracy of the combination of smear microscopy and ELISA using rESAT-6-CFP-10 antigen was assessed. In a scenario in which a subject was considered to have a negative result when both smear microscopy and ELISA for rESAT-6-CFP-10 results were negative, the sensitivity was 75.3% and the specificity was 68.7%. Although both tests combined achieved a higher sensitivity compared to either of them alone, the specificity was very low

compared to the specificity of smear microscopy and remained similar with the specificity for rESAT-6-CFP-10 antigen alone.

Subgroup analysis was done to see the accuracy of this antigen among smear negative but culture positive pulmonary TB as well as HIV-TB co-infected patients. The sensitivity of this antigen among smear negative but culture positive pulmonary TB patients (57.7%) was not significantly different (χ^2=0.01, p=0.93) from the sensitivity among smear positive, culture positive pulmonary TB patients (56.8%). The sensitivity and specificity of this antigen among HIV positives were 50% and 73.9%, respectively whereas the sensitivity and specificity among HIV negatives were (61.4%) and (70.8%), respectively; however, there was no statistically significant difference in the sensitivity (χ^2=1.09, p=0.30) and specificity (χ^2=0.09, p=0.78) of the antigen among HIV positives and negatives.

Table 14. Accuracy of rESAT-6-CFP-10 antigen in the diagnosis of pulmonary TB in Northeast Ethiopia

		Culture	
	Positive	**Negative**	**Total**
rESAT-6-CFP-10 **Positive**	51	33	**84**
Negative	38	82	**120**
Total	**89**	**115**	**204**

Sensitivity (CI) =57.3% (46.9%- 67.1%)

Specificity (CI) =71.3% (62.5%- 78.8%)

LR^+ =2.00 (1.42-2.80) LR^- =0.60 (0.46-0.78)

Accuracy=65.2%

The sensitivity and specificity of rα-Crystallin-MPT-83 were 20.2% and 92.2%, respectively. The positive and negative predictive values were 66.7% and 59.9%, respectively whereas the positive and negative likelihood ratios were 2.58 and 0.87, respectively. About 60% of the

patients were accurately picked as pulmonary and non-pulmonary TB patients (Table 15). The accuracy of this antigen combined with smear microscopy was assessed. In a scenario where a subject is considered negative when smear and ELISA using rα-crystallin-MPT-83 antigen results are negative, the sensitivity was 51.7% and the specificity was 87%. Although the sensitivity of smear microscopy has increased by nearly 11%, there is a concomitant drop in its specificity when combined with ELISA results of this antigen.

Further subgroup analysis was done and accordingly, the sensitivity of this antigen among smear negative but culture positive pulmonary TB patients was 17.3% compared to the sensitivity among smear positives (24.3%); however, this difference was not found to be statistically significant (χ^2=0.58, p=0.46). The sensitivity and specificity of this antigen among HIV infected and non-infected pulmonary TB suspects were not significantly different (p>0.05). Among HIV infected pulmonary TB patients, the sensitivity and specificity of this antigen were 18.8% and 95.7%, respectively whereas among non-HIV infected pulmonary TB patients, the sensitivity and specificity were 21.1% and 91%, respectively.

Table 15. Accuracy of rα-Crystallin-MPT-83 antigen in the diagnosis of pulmonary TB in Northeast Ethiopia

		Culture		
		Positive	**Negative**	**Total**
α-Crystallin-MPT-83	**Positive**	18	9	**27**
	Negative	71	106	**177**
	Total	**89**	**115**	**204**

Sensitivity (CI) =20.2% (13% -30%)

Specificity (CI) = 92.2% (86% -96%)

LR^+ = 2.58 (1.22-1.48) LR^- = 0.87 (0.77-0.97)

Accuracy= 60.8%

3.2. Assessment of delay among TB patients in Afar Region, Ethiopia

3.2.1. Sample distribution

A total of 216 patients diagnosed with different forms of TB and came to DOTS clinics of two government health facilities in the Afar Regional State were interviewed using a structured, pretested questionnaire. Socio-demographic characteristics of the study population are summarized in Table 16. The mean age of study participants was 32.73 $\pm$12.29 with a median of 30 years and range of 18 to 88 years. The vast majority (83.8%) of study participants were under 45 years of age.

Among study participants, the number of males was found to be higher than females with a ratio of 1.67:1. The proportion of those with no education (64.8%) exceeded those with education (35.2%) and pastoralists accounted for 42.1% of study participants. Moreover, 90.1% of pastoralists did not have education compared to just 46.4% among non-pastoralists.

About 36.6% of study participants lived in a typical Afar traditional house locally named as "*Debora*" which can easily be moved from place to place. The mean distance of participants' dwelling from health facilities was 14.5 km with a median of 3 km. The majority (75%) of study participants reported to live within 10 km radius from health facilities. However, when the study participants were grouped as pastoralists and non-pastoralists, the mean distance for pastoralists became 24.2 km with a median of 13.3 km whereas for non-pastoralists the respective values were 7.5 km and 2.5 km. The majority (61.5%) of pastoralist lived more than 10 km from the nearest health facility during their initial visit compared to only 14.4% among non-pastoralists. Regarding the biomedical knowledge of participants on the causes, treatment and outcome of TB, 84.3% of participants had high knowledge.

Table 16. Socio-demographic characteristics of study participants in Afar Region, Ethiopia

Variable	Frequency	Percent (%)
Age		
18-24	54	25.0
25-44	127	58.8
>44	35	16.2
Sex		
Male	135	62.5
Female	81	37.5
Marital status		
Single	68	31.5
Married	119	55.1
Widowed	14	6.5
Divorced	15	6.9
Residence		
Urban	143	66.2
Rural	73	33.8
Religion		
Muslim	175	81.0
Christian	41	19.0
Formal education		
None	140	64.8
Primary	53	24.5
Post-primary	23	10.7
Occupation		
Pastoralist	91	42.1
Non-pastoralist	125	57.9
Type of house		
Debora	79	36.6
Thatched house	46	21.3
Corrugated iron sheet	91	42.1
Number of rooms		
One	161	74.5
Two	41	19.0
Three	14	6.5
Number of people in one house		
One to three	78	36.1
Four to six	88	40.7
Seven and above	50	23.2

Regarding the forms of TB, 137 (63.4%) had pulmonary TB and the rest (36.6%) were identified as extra-pulmonary TB patients. Among pulmonary TB patients, the majority (61.3%) of them were smear negative with low smear positivity rate (38.7%) comparable to the serology study presented above.

Participants were asked about the symptoms they were suffering from. Patients with pulmonary TB reported persistent cough (100%), fever (93.4%), weight loss (92%), loss of appetite (89.1%), night sweating (84.7%), and haemoptysis (26.3%). On the other hand,

patients with extra-pulmonary TB reported fever (89.9%), swelling (mainly peripheral lymph nodes) (73.4%), loss of appetite (77.2%), weight loss (77.2%), night sweating (64.6%), chest pain (25.3%), and cough (17.7%).

Differences between pastoralists and non-pastoralists in relation to socio-demographic characteristics, form of TB, distance to the nearest health facility and first health seeking action were investigated. The two groups did not differ significantly with regard to age, form of TB and first health seeking action. However, the proportion of males, those with no education, Muslims and those living more than 10 km from health facilities were significantly higher among pastoralists compared to their respective proportions among non-pastoralists (p-values for all less than 0.001). Table 17 below shows differences between pastoralists and non-pastoralists.

Table 17. Comparison of pastoralists with non-pastoralists with respect to some characteristics in Afar Region, Ethiopia

Variable	Pastoralists n (%)	Non-pastoralists n (%)	Total n (%)	χ^2	p-value
Age					
18-24	18 (19.8)	36 (28.8)	54 (25.0)	4.96	0.084
25-44	53 (58.2)	74 (59.2)	127 (58.8)		
>44	20 (22.0)	15 (12.0)	35 (16.2)		
Sex					
Male	71 (78.0)	64 (51.2)	135 (62.5)	16.17	<0.001*
Female	20 (22.0)	61 (48.8)	81(37.5)		
Formal education					
None	82 (90.1)	58 (46.4)	140 (64.8)	46.02	<0.001*
Primary	9 (9.9)	44 (35.2)	53 (24.5)		
Post-primary	0 (0.0)	23 (18.4)	23 (10.7)		
Religion					
Muslim	87 (95.6)	88 (70.4)	175 (81.0)	21.75	<0.001*
Christian	4 (4.4)	37 (29.6)	41 (19.0)		
Family size					
One to three	13 (14.3)	65 (52.0)	78 (36.1)	33.43	<0.001*
Four to six	47 (51.6)	41(32.8)	88 (40.7)		
Seven and above	31(34.1)	19 (15.2)	50 (23.2)		
Patient category					
Pulmonary TB	56 (61.5)	81(64.8)	137 (63.4)	0.241	0.623
Extra-pulmonary TB	35 (38.5)	44(35.2)	79 (36.6)		
Biomedical knowledge					
Low	18 (19.8)	16 (12.8)	34 (15.7)	1.94	0.164
High	73 (80.2)	109 (87.2)	182 (84.3)		
Distance to health facility					
<=10 km	51 (56.0)	112 (89.6)	163 (75.5)	32.03	<0.001*
>10 km	40 (44.0)	13 (10.4)	53 (24.5)		
First health seeking action					
Formal	77 (84.6)	114 (91.2)	191(88.4)	2.23	0.135
#Non-formal	14 (15.4)	11 (8.8)	25 (11.6)		

Includes traditional healers and drug dispensers *Significant at p=0.05

3.2.2. Health seeking action of study participants

Patients were asked about their first health seeking action. Among all participants, 149 (69%) consulted someone in their social circle before seeking help from health providers. Over two-third of participants visited government health facilities initially. Close to 20% went to

private health facilities whereas 11.6% went to non-formal health providers mainly traditional healers and drug outlets. Figure 7 shows the different health providers visited initially.

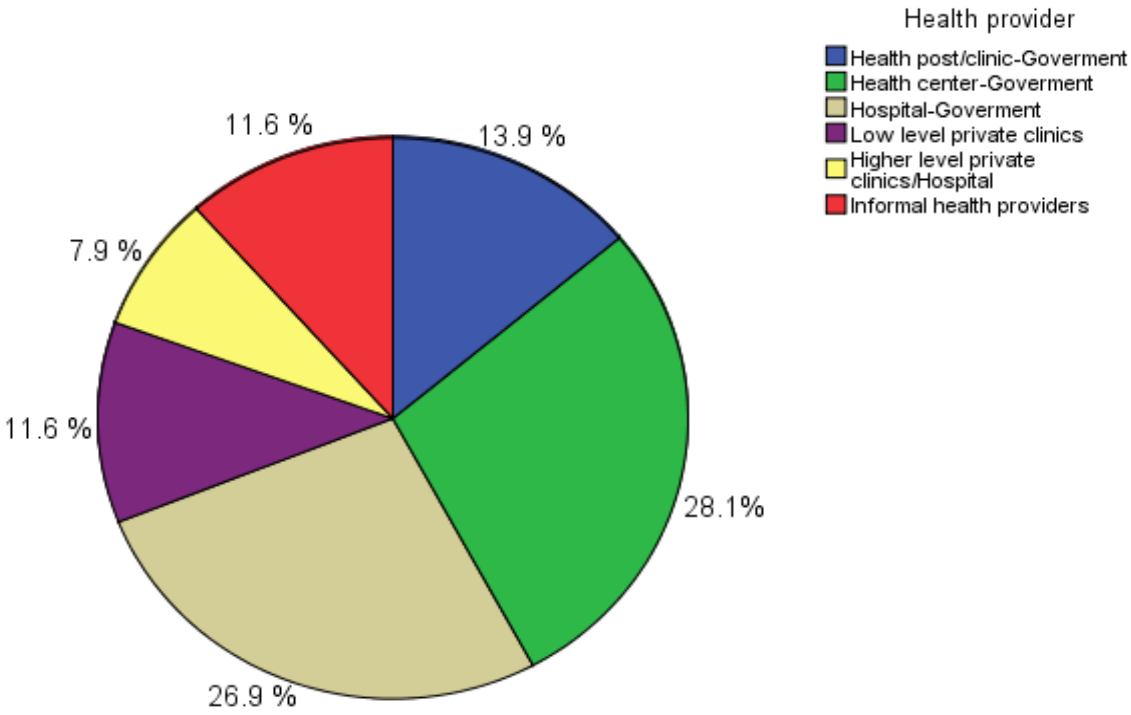


Figure 7. Type of health providers first consulted by study participants in Afar Region, Ethiopia

First health seeking action of study participants and associated factors were further investigated after categorizing health providers as non-formal and formal. A total of 191(88.4%) of study participants sought help from formal health providers first and the rest 25 (11.6%) sought help from non-formal health providers first: 7 (3.2%) sought help from local injectors, 9 (4.2%) went to drug outlets and 9 (4.2%) consulted traditional healers. Data on group differences in relation to first health seeking action was summarized in Table 18. There was no significant difference with regard to participants' first health action by age, sex and form of TB.

The proportion of those with no education, pastoralists and those who live at a distance of more than 10 km from health facilities was higher among those who sought non-formal health providers' help first compared to the proportion among those who sought formal health providers' help first although these differences did not reach statistical significance. However, the proportion of rural dwellers (56%) and those with self-treatment with home remedies (36%) were significantly higher among participants who sought non-formal health providers' help first compared to their respective proportions (30.9% and 11.0%) among those who sought formal health care providers' help ($p<0.05$ for both). Those who treated themselves reported to use butter, milk, honey, steam inhalation, massage with plant leaves, and drinking juice produced from plant leaves.

Table 18. Group differences with regard to first health seeking action of study participants in Afar Region, Ethiopia

Variable	Formal health providers n (%)	Non-formal health providers n (%)	Total n (%)	χ^2	p-value
Age					
18-24	45 (23.6)	9 (36.0)	54 (25.0)	4.13	0.127
25-44	117 (61.2)	10 (40.0)	127 (58.8)		
>44	29 (15.2)	6 (24.0)	35 (16.2)		
Sex					
Male	120 (62.8)	15 (60.0)	135 (62.5)	0.075	0.784
Female	71 (37.2)	10 (40.0)	81 (37.5)		
Residence					
Urban	132 (69.1)	11 (44.0)	143 (66.2)	6.23	0.013*
Rural	59 (30.9)	14 (56.0)	73 (33.8)		
Formal education					
None	120 (62.8)	20 (80.0)	140 (64.8)	3.02	0.221
Primary	49 (25.7)	4 (16.0)	53 (24.5)		
Post-primary	22 (11.5)	1 (4.0)	23 (10.7)		
Occupation					
Non-pastoralist	114 (59.7)	11 (44.0)	125 (57.9)	2.231	0.135
Pastoralist	77 (40.3)	14 (56.0)	91 (42.1)		
Distance					
<=10 km	148 (77.5)	15 (60.0)	163 (75.5)	3.651	0.056
>10 km	43 (22.5)	10 (40.0)	53 (24.5)		
Self-treatment					
No	170 (89.0)	16 (64.0)	186 (86.1)		
Yes	21 (11.0)	9 (36.0)	30 (13.9)	+	0.003*
Forms of TB					
Pulmonary TB	118 (61.8)	19 (76.0)	137 (63.4)	1.927	0.165
Extra-pulmonary TB	73 (38.2)	6 (24.0)	79 (36.6)		

* Significant at p=0.05 + Fisher's exact test was done because of violation of Chi-square assumptions

3.2.3. Lengths of different delays and associated factors

In this section, the different types of delay and their association with socio-demographic as well as health service related factors are presented. Figure 8 depicts the different components of total delay. This approach helps to identify the contribution of the different components of delay to the total delay.

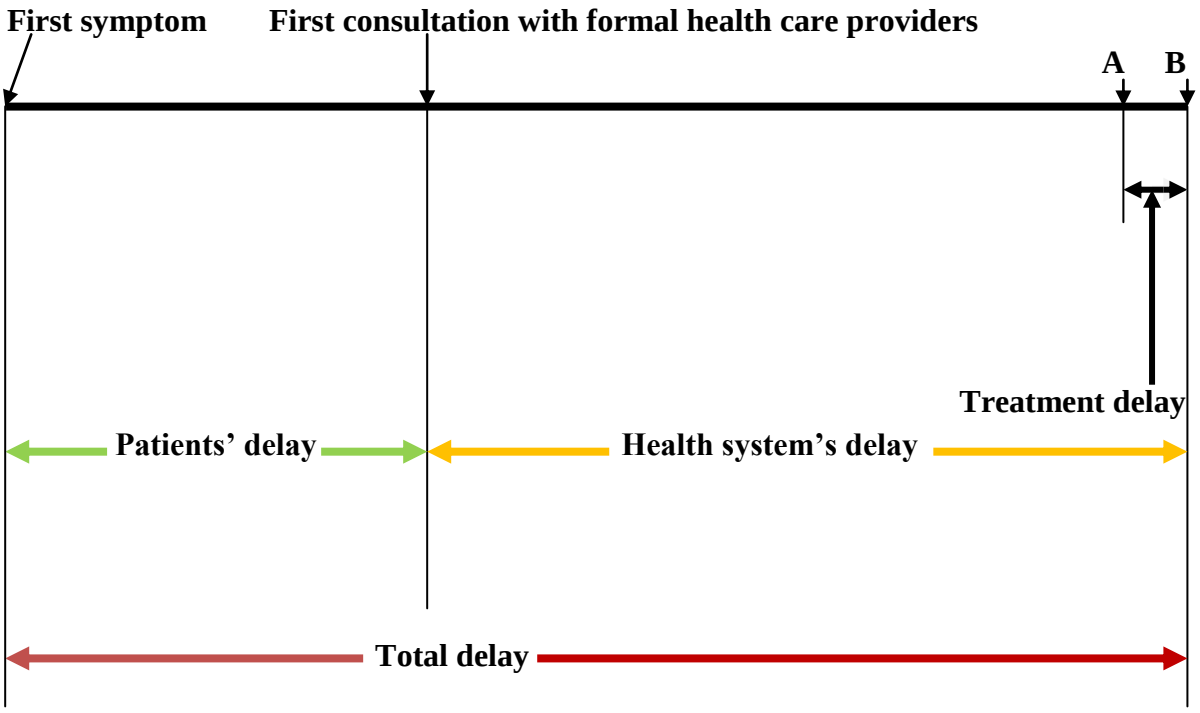


Figure 8. Relationship among the different types of delay

3.2.3.1. Patients' delay

Patient delay was defined as the time between onset of TB symptoms and the first consultation with a professional health provider.

In this study, the median patients' delay was 20 days with IQR of 8 to 60 days. Nearly 54% of patients were able to report to formal health providers within 20 days and about 76% of participants sought help within one month. The longest patient delay, 1456 days (nearly 4 years), was reported in one patient. Except 3 patients, the rest reported within one year. Figure 9 below shows cumulative distribution of patient delay in seeking help from formal health providers.

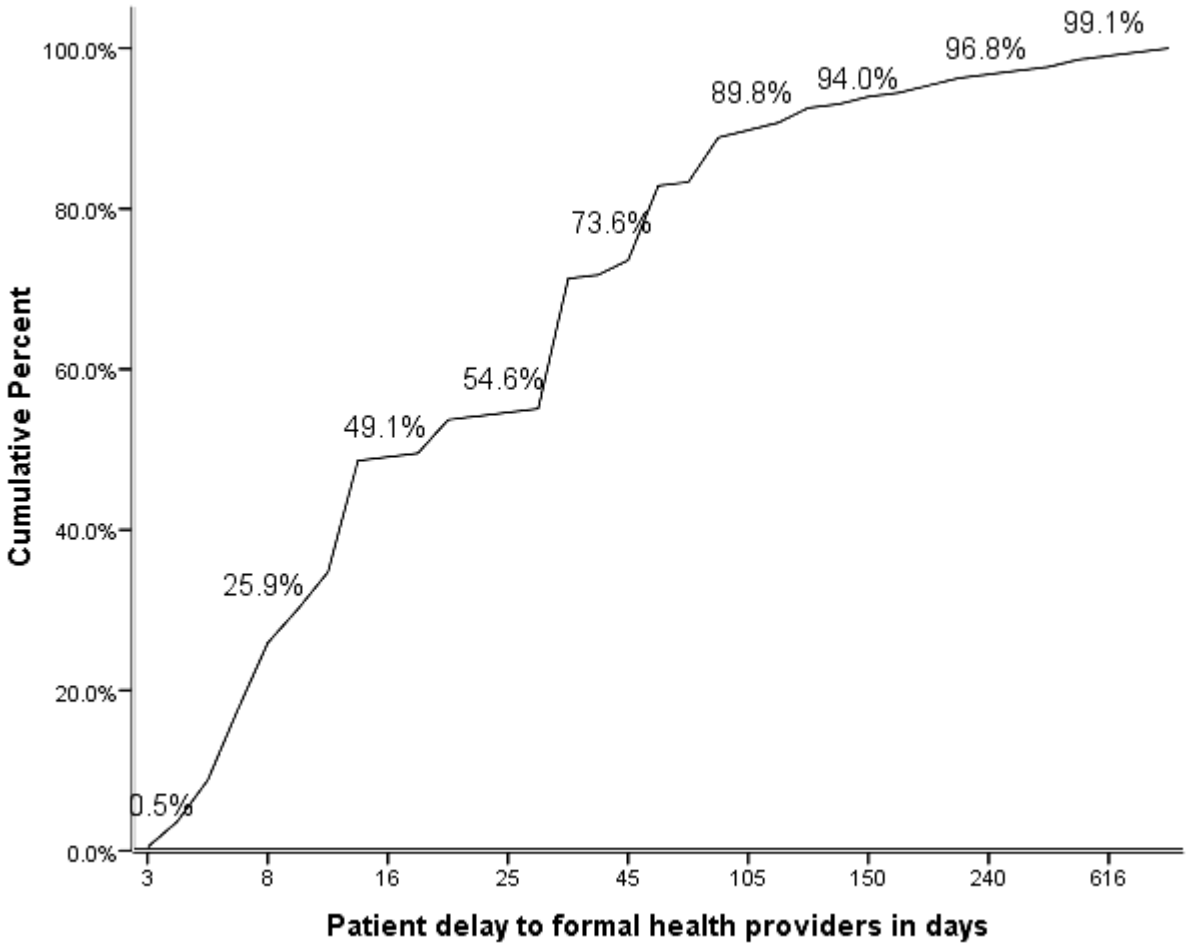


Figure 9. Cumulative distribution of patients' delay in seeking help from formal health providers among study participants in Afar Region, Ethiopia

The median delay for those patients who visited non-formal health providers initially was very long (60 days) compared to only 15 days for those who were first seeking help from formal health providers and this difference was statistically significant (Mann Whitney, P<0.001). For those who initially consulted non-formal health providers, the median time between onset of symptoms and first consultation of non-formal health providers, however, was just 10 days. Similarly, patients who treated themselves initially reported a longer delay (52.5 days) compared to those who didn't treat themselves (15 days) and this difference was statistically significant (Mann Whitney, p<0.001).

Moreover, the median delay for pastoralists was twice that of non-pastoralists (30 days versus 15 days) and this difference was found to be statistically significant (Mann Whitney,

p<0.001). After dichotomizing participants' knowledge on causes, outcomes and treatment of TB as low and high, the median delay for those with high knowledge was 15 days as opposed to a 30 days delay for those with low knowledge. However, this difference did not reach statistical significance (Mann Whitney, p=0.058).

Group differences in patient delays' have been further evaluated using the median as the cut-off point for comparison. Table 19 below presents summary of the findings (both bivariate and multivariable logistic regression).

There was no significant difference with regard to patient delay by sex, marital status and form of TB in bivariate analysis. On the other hand, those with pastoralist identity (Crude Odds Ratio (OR_C) = 2.72, CI 1.56-4.74), those who treated themselves (OR_C =4.65, CI 1.90-11.38), those who live over 10 km distance from health facilities (OR_C = 2.13, CI 1.13-4.00), and those who sought help from non-formal health providers first (OR_C =7.44, CI 2.46-22.53) were more likely to be delayed compared to their counterparts. Similarly, those participants older than 44 years (OR_C =2.58, CI 1.07-6.24) were more likely to be delayed compared to those who are below 24 years of age. On the other hand, those with post-primary education were less likely to be delayed compared to those with no education (OR_C =0.24, CI 0.09-0.69).

However, on multivariable logistic regression, self-treatment (ORadj. = 3.99, CI 1.50-10.59), and first health-seeking action from non-formal health providers (ORadj. = 6.18, CI 1.84-20.76) remained as predictors of patients' delay.

Table 19. Association of socio-demographic and health service factors with patients' delay in Afar Region, Ethiopia

Variable	Delay (>20days)	No delay (<=20 days)	ORc (CI)	ORadj. (CI)
Age				
18-24	23	31	1.00	1.00
25-44	54	73	1.00 (0.52-1.90)	1.20 (0.52-2.78)
>44	23	12	2.58 (1.07-6.24) *	3.00 (0.99-9.12)
Sex				
Male	65	70	1.00	1.00
Female	35	46	0.81 (0.47-1.43)	1.01 (0.48-2.11)
Marital status				
Single	29	39	1.00	1.00
Married	59	60	1.32 (0.73-2.41)	0.83 (0.37-1.86)
Widowed	5	9	0.75 (0.23-2.47)	0.48 (0.10-2.21)
Divorced	7	8	1.18 (0.38-3.62)	1.22 (0.33-4.48)
Occupation				
Non-pastoralist	45	80	1.00	1.00
Pastoralist	55	36	2.72 (1.56-4.74) *	1.91 (0.90-4.05)
Formal education				
None	75	65	1.00	1.00
Primary	20	33	0.53 (0.28-1.00)	0.82 (0.37-1.83)
Post-primary	5	18	0.24 (0.09-0.69) *	0.52 (0.15-1.79)
Biomedical knowledge				
Low	19	15	1.00	1.00
High	81	101	0.63 (0.30-1.32)	0.78 (0.30-1.86)
Distance to facility				
<=10 km	68	95	1.00	1.00
>10 km	32	21	2.13 (1.13-4.00) *	1.33 (0.62-2.86)
Form of TB				
Pulmonary TB	60	77	1.00	1.00
Extra-pulmonary TB	49	39	1.32 (0.76-2.29)	1.52 (0.80-2.87)
Self treatment				
No	77	109	1.00	1.00
Yes	23	7	4.65 (1.90-11.38) *	3.99 (1.50-10.59)*
First health action				
Formal health provider	79	112	1.00	1.00
Non-formal provider	21	4	7.44 (2.46-22.53) *	6.18 (1.84-20.76) *

*Significant at p=0.05

3.2.3.2. Health system's delay

This is the time from first contact with the health system till the commencement of treatment for TB. In our study, 142 (65.7%) of patients were diagnosed at government health facilities and the rest 34.3% were diagnosed at private health facilities.

The mean system's delay was 68.70 days with a median of 33.5 days ranging from a minimum of 2 days to a maximum of 712 days. Only 23.1% of patients were started on treatment within 15 days after the first contact with the formal health providers. Forty-four percent of the patients were started on treatment within 1 month whereas it took nearly 3 months for 75% of the patients to be diagnosed and start treatment after consulting a formal health provider; the rest 25% were diagnosed and started on treatment after 3 months. Figure 10 below indicates the cumulative distribution of health system's delay in the study area.

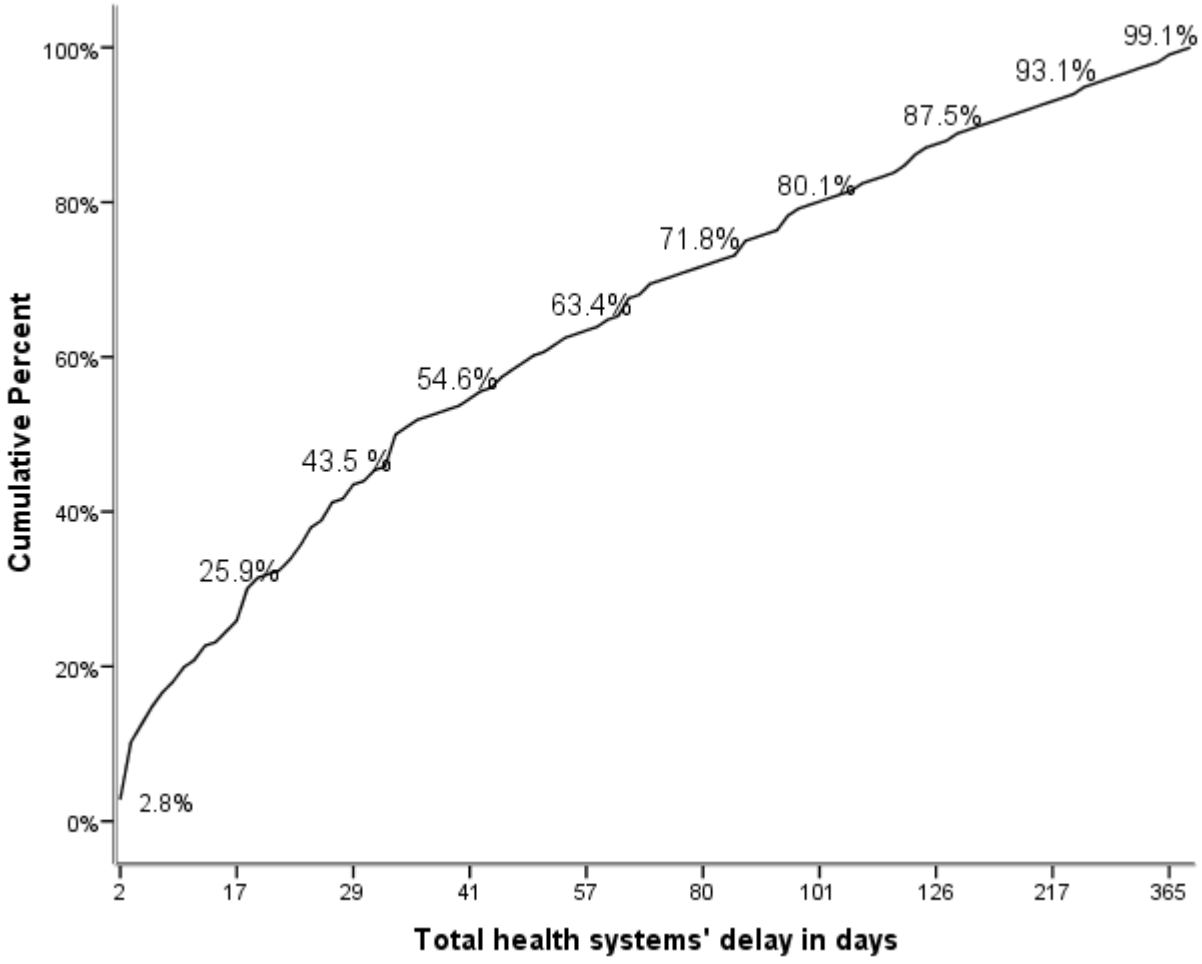


Figure 10. Cumulative distribution of health system's delay among TB patients in Afar Region, Ethiopia

The median number of health facilities visited before diagnosis was 2. Out of the 216 patients, about 23% visited only one health facility until diagnosis was made. However, roughly 70% of patients had to visit 2 or 3 health facilities until they were notified to have TB. The rest (7%) of patients visited 4 health facilities until diagnosed.

The median number of visits made before diagnosis was 2. Patients had up to 6 visits before they were diagnosed. Only 14.8% of them received their diagnosis in the first visit. About 70% of patients were diagnosed during the second or the third visit. Figure 11 shows the distribution of number of visits.

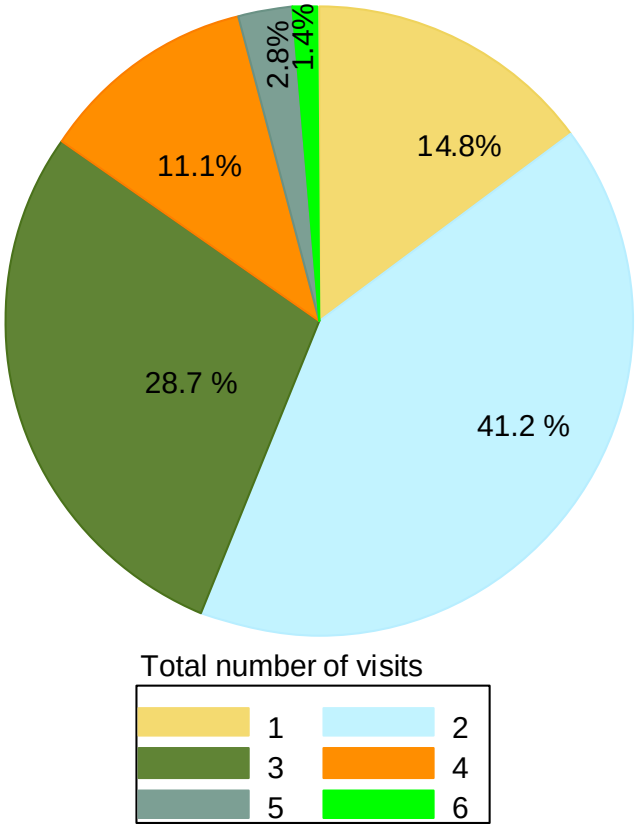


Figure 11. Distribution of number of visits until diagnosis was reached among TB patients in Afar Region, Ethiopia

We examined the possible association between patient-related and health system-related delay. Interestingly, we found an inverse relationship i.e. patients who consulted the health

system earlier experienced a longer health system's delay. Spearman's correlation coefficient was -0.31 ($p<0.001$).

Patients were divided into delayed and non-delayed using the median patient delay (20 days) as a cut-off point. Subsequently Mann-Whitney test was done to see if there is a significant health system's delay for those patients who came earlier assuming that diagnosis might be more challenging in those who presented early compared to those who come after having a more advanced TB. The median health system's delay for those who consulted the health system within 20 days of the onset of their illness was 49 days (IQR 25-88 days). However, the median health system's delay for those who consulted the health system after 20 days from the onset of their illness was 29 days (IQR 5-70) and this difference was statistically significant (Mann-Whitney, $p<0.001$).

Those who sought help first from non-formal health providers had a shorter median health system's delay (20 days) compared to those who sought help first from formal health providers (35 days); however, this difference was not statistically significant (Mann Whitney, $p=0.12$).

Factors which could influence health system's delay were examined using both bivariate analysis and multivariate logistic regression (median health system's delay was taken as a cut-off to dichotomize the data). On bivariate analysis, socio-demographic factors do not seem to influence health system's delay. However, form of TB and the type of health facility initially visited were found to be significantly associated with health system's delay. Table 20 summarizes the result.

Accordingly, those with extra-pulmonary TB had a significantly higher health system's delay compared to those with pulmonary TB on bivariate analysis and this difference persisted in multivariable logistic regression (ORadj=2.08, CI 1.08- 4.04).

Moreover, the type of health facility visited initially has been found to influence health system's delay. In bivariate analysis, it was found that those who visited government health posts/clinics, health centers and private clinics/hospitals had a significantly longer health system's delay compared to those who visited government hospitals. On multivariable logistic regression, taking government hospital as a reference, first visit to government

clinics/health post (ORadj=19.70, CI 6.18-62.79), health centers (ORadj. =4.83, CI 2.23-10.43), and private clinics/hospitals (ORadj. = 2.49, CI 1.07-5.84) were independent predictors of longer delay.

Table 20. Association of socio-demographic and health-related factors with health system's delay in Afar Region, Ethiopia

Variable	Delay (>33.5 days)	No delay (<=33.5 days)	ORc	ORadj.
Age				
18-24	27	27	1.00	1.00
25-44	69	58	1.19 (0.60-2.36)	1.24 (0.58-2.62)
>44	12	23	0.52 (0.20-1.37)	0.64 (0.22-1.83)
Sex				
Male	65	70	1.00	1.00
Female	43	38	1.22 (0.70-2.12)	1.33 (0.67-2.62)
Formal education				
None	67	73	1.00	1.00
Primary	29	24	1.32 (0.70-2.48)	1.68 (0.74-3.84)
Post-primary	12	11	1.19 (0.49-2.87)	1.67 (0.54-5.18)
Occupation				
Non-pastoralists	61	64	1.00	1.00
Pastoralists	47	44	1.12 (0.65-1.92)	1.68 (0.77-3.68)
Distance				
<=10 Km	82	81	1.00	1.00
>10 km	26	27	0.95 (0.51-1.77)	0.78 (0.35-1.74)
Form of TB				
Pulmonary TB	60	77	1.00	1.00
Extra-pulmonary TB	48	31	1.99 (1.13-3.49)*	2.08 (1.08-4.04) *
Self-treatment				
No	98	88	1.00	1.00
Yes	10	20	0.45 (0.20-1.01)	0.51 (0.20-1.32)
Health provider				
Hospital	16	52	1.00	1.00
Health centre	44	25	5.72 (2.72-12.05)*	4.83 (2.33-10.43) *
Clinic/health post	27	5	17.55 (5.80-53.07) *	19.70 (6.18-62.79) *
Private clinics	21	26	2.63 (1.18-5.86) *	2.49 (1.07-5.84) *

* Significant at p=0.05

Treatment delay: As a component of health system's delay, the time taken from diagnosis till initiation of treatment has been analyzed. Accordingly, the mean treatment delay was 2.58

days with a median of 1 day (IQR 1-4 days). Approximately 60% of patients were initiated with treatment within 2 days following diagnosis and over 90% of TB patients were started on treatment within 5 days. All except one patient were started on treatment in 10 days.

After grouping the health facilities where diagnosis was made into private and government owned, analysis has been done to see if there is a difference in treatment delay. Accordingly, the treatment delay was longer at private health facilities with median of 4 days. The corresponding median values for government health facilities was 1 days respectively and this difference was found statistically significant (Mann-Whitney test, $p<0.001$). Ninety-eight percent of patients diagnosed at government health facilities were started on treatment within 5 days; however, almost 22% of patients diagnosed at private health facilities were started on treatment after 5 days. Figure 12 below illustrates the difference in treatment delay between private and government health facilities.

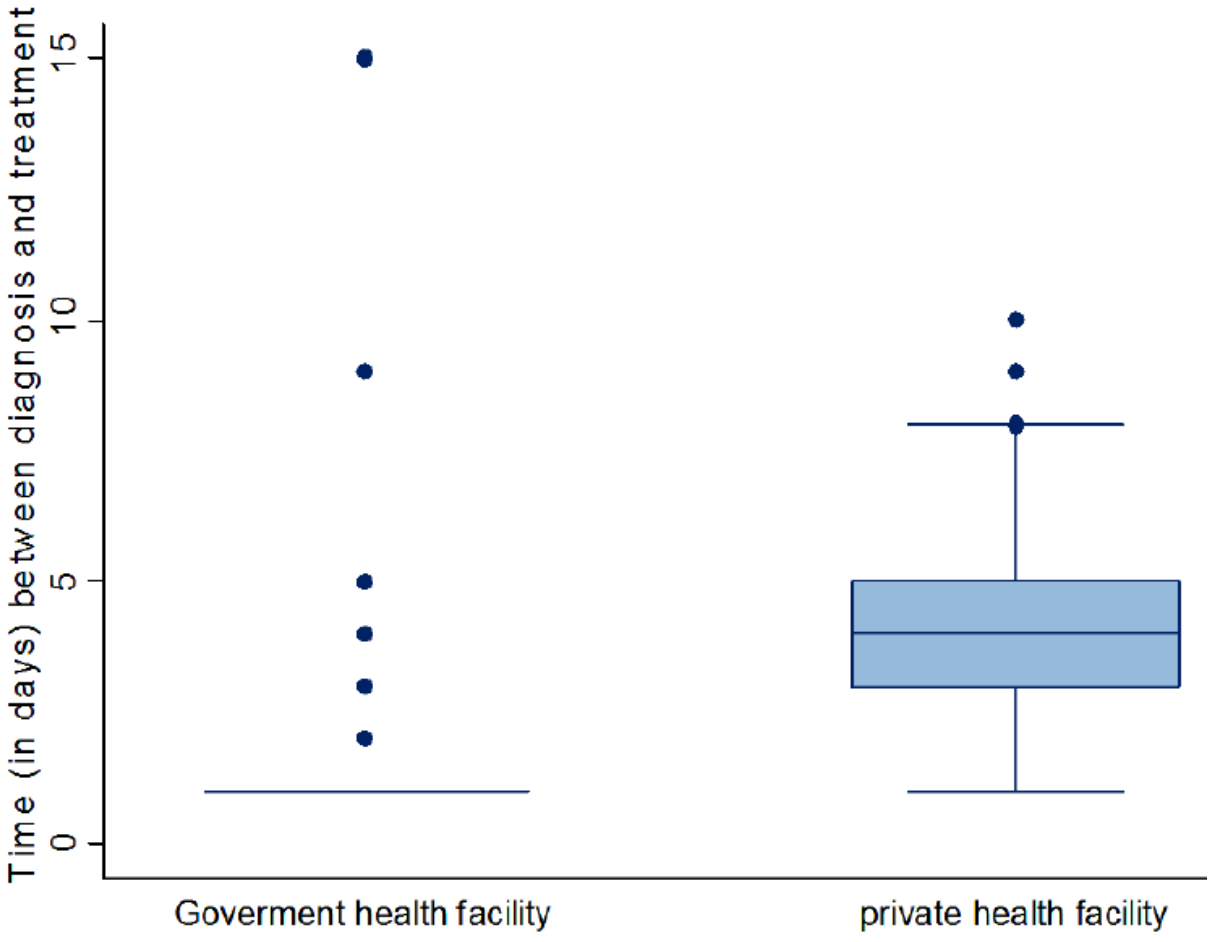


Figure 12. Group differences (private versus government owned facilities) with regard to treatment delay in Afar Region, Ethiopia

3.2.3.3. Total delay

Total delay is the time which extends from the onset of symptoms of TB until the initiation of anti-TB drugs. It is simply the sum of patients' delay and health system's delay. In our study the median total delay was 70.5 days with IQR of 37 to 126.75 days. Only 5.1% of patients were started on treatment within one month period following onset of symptoms. Three-fourth of patients were started on treatment within 126 days (roughly 4 months).The maximum delay reported was 1464 days. Figure 13 below shows the cumulative distribution of total delay among study participants.

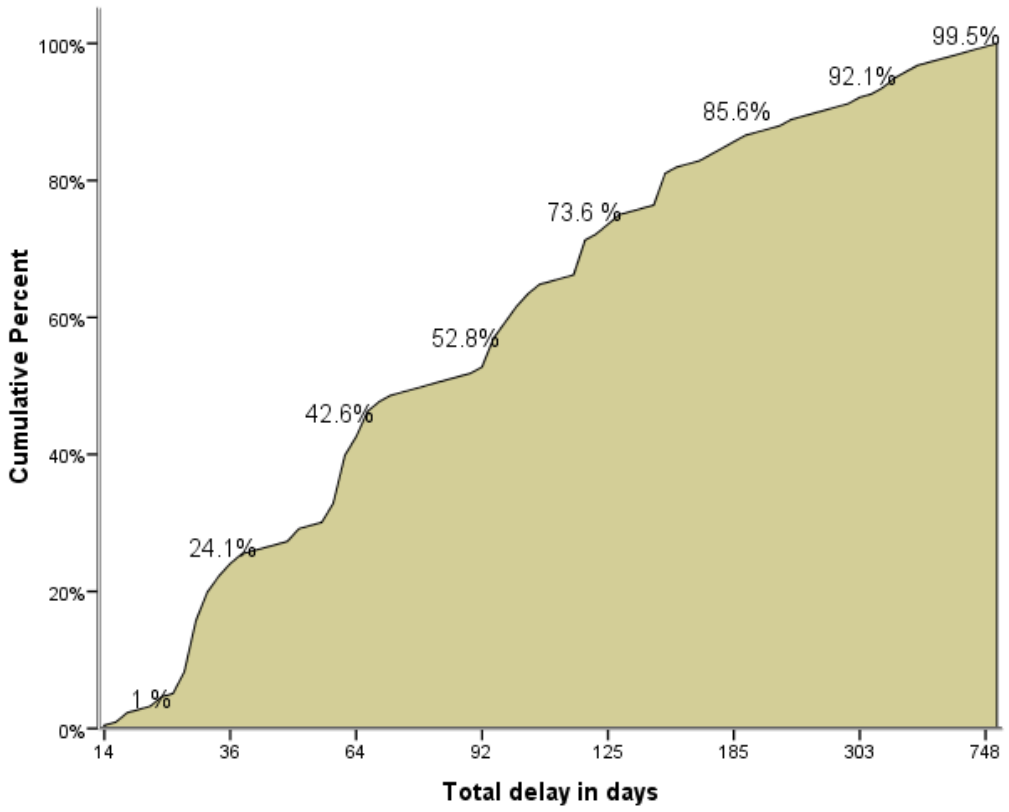


Figure 13. Cumulative distribution of total delay among study participants in Afar Region, Ethiopia

Health system's delay has contributed a greater proportion to the total delay. Health system's delay was longer than patients' delay for 149 (69.0%) patients whereas patients' delay was

longer than health system's delay in only 65 (30.1%) patients and 2 (0.9%) patients had equal patient's and health system's delay.

The association of total delay with socio-demographic and health-related factors was examined using bivariate analysis (Mann-Whitney for two independent groups or Kruskal-Wallis for more than two groups) and the result has been summarized in table 21. Total delay was not significantly associated with age, sex, marital status and educational status of study participants (all having $p>0.05$). Although it did fail to reach statistical significance ($p=0.09$), those who lived more than 10 km distance from health facilities had a longer median total delay (93 days) compared to those who lived within 10 km radius (67 days).

On the other hand, first health seeking action was found to significantly influence total delay (Kruskal-Wallis, $p<0.03$). Moreover, patients with pastoralist identity had a significantly longer total delay compared to non-pastoralists (Mann-Whitney, $P=0.01$). Similarly, patients with extra-pulmonary TB had a significantly longer total delay compared to those who had been diagnosed with pulmonary TB (Mann-Whitney, $p=0.002$). This difference has been further compared using line graph (Figure 14) and from the graph we can see that for a given number of days, the percentage of patients initiated on treatment is higher among those with pulmonary TB compared to those with extra-pulmonary TB and this difference was more pronounced for those who started treatment after about a month and before 10 months following onset of TB symptoms.

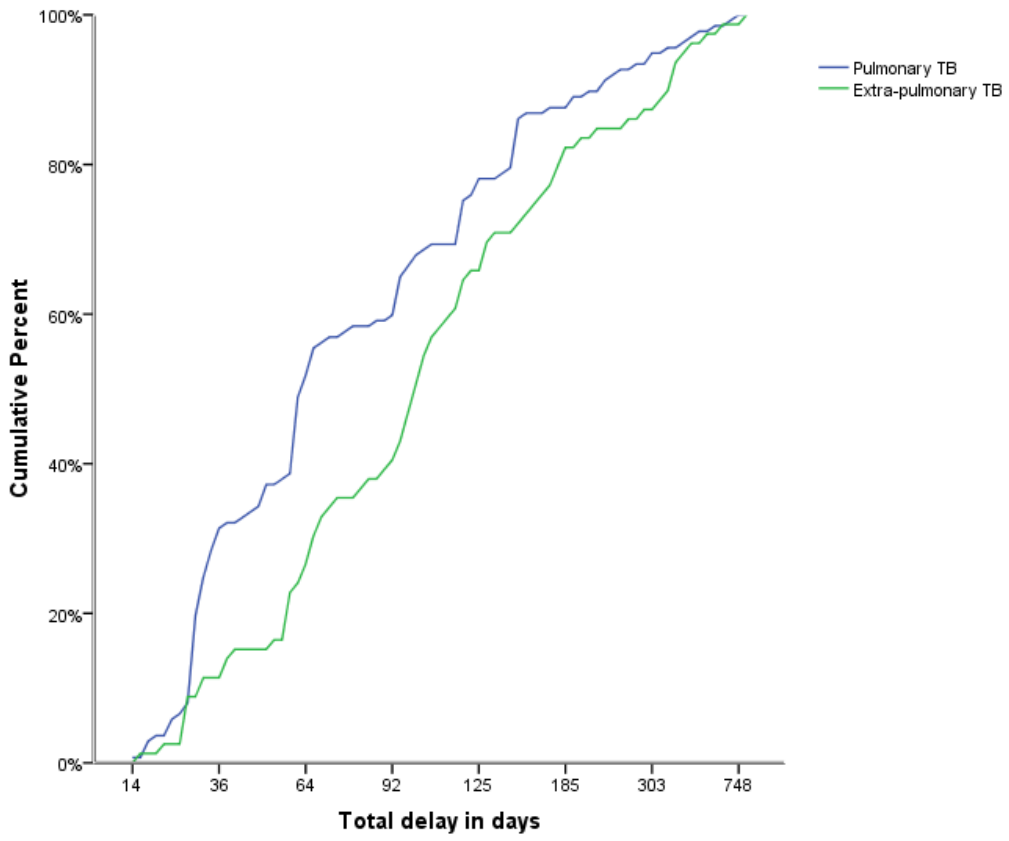


Figure 14. Cumulative distribution of total delay comparing patients with pulmonary and extra-pulmonary TB among study participants in Afar Region, Ethiopia

Table 21. Group differences with regard to median total delay among study participants in Afar Region, Ethiopia

Variable	Count	Median (IQR)	p-value
Age			
18-24	54	68.5 (35.75-133.25)	
25-44	127	93 (37-126)	0.25ǂ
>44	35	65 (39- 153)	
Sex			
Male	135	70(39-126)	0.82*
Female	81	71 (35.5-153)	
Marital status			
Single	68	73.5 (36-152.75)	0.45ǂ
Married	119	78 (38-127)	
Widowed	14	56 (35.5-132.75)	
Divorced	15	93 (63-123)	
Residence			
Urban	143	67 (35-126)	0.13*
Rural	73	93 (62-153)	
Formal education			
None	140	92 (48-153)	0.14ǂ
Primary	53	69 (35-123)	
Post-primary	23	54 (34-125)	
Occupation			
Non-pastoralist	125	65 (34-123)	0.01*
Pastoralist	91	93 (63-153)	
Distance			
<=10 km	163	67 (35-126)	0.09*
>10 km	53	93 (63-140.5)	
Form of TB			
Pulmonary TB	137	64 (34.5-123-5)	
Extra-pulmonary TB	79	95 (64-158)	0.002*
Self-treatment			
No	186	68.5 (36-125.25)	
Yes	30	94.5 (59.25-162.25)	0.16*
1st Health seeking action			
Clinic/health post	32	123 (63-153)	
Health center	69	93 (62-183)	<0.03ǂ
Hospital	68	64 (33-123)	
Private clinics	47	65 (36-125)	

* Mann-Whitney test ǂ Kruskal-Wallis test

Multivariable logistic regression analysis was done using the median value as a cut-off to categorize participants as delayed and not delayed. The result (Table 22) shows that having extra-pulmonary TB (ORadj.= 2.56, CI 1.39-4.73) is an independent predictor for longer total

delay. Moreover, first visit to government health posts/clinics (ORadj. =2.55, CI 1.01-6.03) is an independent predictor of longer total delay.

Table 22. The association of socio-demographic and health related factors with total delay among TB patients in Afar Region, Ethiopia

Variable	Delay, n >70.5 days	No delay, n ≤ 70.5 days	OR_{adj} (CI)
Age			
18-24	26	28	1.00
25-44	67	60	1.16 (0.58-2.35)
>44	15	20	0.87 (0.34-2.26)
Sex			
Male	67	68	1.00
Female	41	40	1.04 (0.56-1.95)
Formal education			
None	74	66	1.00
Primary	26	27	1.14 (0.53-2.43)
Post-primary	8	15	0.70 (0.24-2.04)
Occupation			
Non-pastoralist	57	68	1.00
Pastoralist	51	40	1.40 (0.69-2.86)
Distance			
<=10 km	78	85	1.00
>10 km	30	23	1.11 (0.53-2.31)
Form of TB			
Pulmonary TB	57	80	1.00
Extra-pulmonary TB	51	28	2.56 (1.39-4.73) *
Self-treatment			
No	91	95	1.00
Yes	17	13	1.42 (0.61-3.29)
1st Health seeking action			
Hospital	31	37	1.00
Health center	38	31	1.28 (0.63-2.61)
Clinic/health post	21	11	2.55 (1.01-6.03) *
Private clinics/hospitals	18	29	0.82 (0.37-1.82)

* Significant at p=0.05

In general, in this study, patient-related delay (median of 20 days) was shorter than health system-related delay (median of 33.5 days) and therefore, a greater proportion of the total delay was related to the health system. Therefore, delay in TB diagnosis and treatment in the current study was more attributable to the health system than to the patients.

Chapter 4: Discussion, conclusion and recommendations

Early detection and effective treatment are key strategies to control TB. Early detection would be possible if a high quality diagnostic test is accessible thereby shortening health system's delay. Moreover, TB patients need to be aware of their illness and seek help within a reasonable time. However, at this point in time, diagnostic delay remains a formidable challenge [38] and the currently available conventional diagnostic tools for TB leave millions undiagnosed and untreated. Smear microscopy has been the predominant diagnostic tool in high burden countries; however, it has unacceptably low sensitivity (20-80%) [41,42]. In countries like Ethiopia where the first contact of TB patients with the health system is mainly at the lowest level, diagnosis of TB is challenged as a result of lack of accurate, simple and point-of- care diagnostic tests. Serological tests based on antibody detection have the potential to meet this demand.

We, therefore, evaluated the potential of two fused recombinant antigens in the diagnosis of pulmonary TB; moreover, we have assessed delay in the diagnosis and treatment of TB in the study area where such information is non-existent. First, we discussed the findings related to the serology study and subsequently, we discussed the results of delay study.

4.1. ELISA-based evaluation of the potential of rESAT-6-CFP-10 and rα-crystallin-MPT-83 antigens for the diagnosis of pulmonary TB

A total of 323 pulmonary TB suspects were included to assess the performance of two recombinant fusion antigens.

4.1.1. Distribution of the sample

In our study, the proportion of males exceeded that of females among pulmonary TB patients in a ratio of about 2 to1 which is consistent with a similar study in Ethiopia [77] as well as a review on sex differences in the epidemiology of TB [78]. In nearly all countries, the notification rate among males is higher than in females [16]. Possible explanations for the dominance of males among TB patients might be related to differences in health seeking behaviour or different level of health workers' awareness resulting in under-notification in females, biological differences or differential exposure to *M.tuberculosis* [77-79].

The distribution of pulmonary TB by age showed that the vast majority (81.3%) of pulmonary TB patients were in the productive age group (<45 years) and similar findings have been reported in previous studies from Ethiopia [36,37,77,80]. Moreover, it has been documented that notification rate peaks in young adults in low income countries as opposed to high income countries [16].

Interestingly, in our study, raw milk consumption was found to be associated with low risk of having pulmonary TB. A study in Sweden evaluated the relationship between human TB and TB infection in cattle before the era of pasteurization and chemotherapy. The investigators reported that there was a positive correlation between infection in man and cattle. However, the correlation between TB-related morbidity and mortality in man and TB infection in cattle was negative [81]. Infection by *M.bovis* may be conferring long-term protection against *M.tuberculosis.* Since the reason for such difference is not obvious from the current study, further study is required.

4.1.2. HIV infection among pulmonary TB suspects

HIV remains a serious health challenge in low income countries and TB is a major opportunistic infection among HIV infected individuals. In Ethiopia, HIV prevalence in the general population was estimated to be 2.1% with high urban-rural gradient: 7.7% in urban areas and 0.9% in rural areas [5]. In our pulmonary TB suspects, those from urban areas had a higher HIV prevalence (37.4%) compared to those from rural areas (14.6%), a result consistent with a study from Ethiopia [82] and this is a reflection of the prevalence difference between urban and rural residents in the general population in the country [5].

In our pulmonary TB suspects, the age group from 25 to 44 has the highest HIV prevalence. Moreover, the peak HIV prevalence in females occurs at younger age group compared to males, consistent with the results reported in two studies [77,83] from Ethiopia.

Analysis of the association between socio-demographic factors and HIV infection showed that primary education was independently associated with a higher prevalence of HIV infection taking those with no education as a reference group. This is in agreement with a study done among army recruits in Ethiopia [84]; on the other hand, a more recent study among Ethiopian voluntary counselling and testing clients found higher education as

protective factor for HIV infection [83]. Generally, during the early years of the HIV epidemic, higher education was associated with greater risk of HIV infection in Africa but with time higher education is becoming protective although the findings are not consistent [85].

In this study, surprisingly, being Afar was independently associated with low HIV prevalence. Although, we do not have concrete evidence to support our hypothesis, the low HIV prevalence in Ethnic Afars might be related to their geographical and cultural inaccessibility to the rest of the country.

We found that HIV is significantly associated with pulmonary TB. Moreover, significantly higher proportion of HIV positives were diagnosed clinically with pulmonary TB compared to HIV negatives. These findings are consistent with a study done in Ethiopia [77]. This is not surprising since HIV is known to be a strong predictor of progression from infection to active TB [86].

In our study, HIV prevalence among pulmonary TB patients was 36.6% which is comparable with the national HIV prevalence estimate (41%) among TB patients [5]. A study done at a teaching referral hospital in Addis Ababa, Ethiopia [77] reported a higher prevalence (57.1%) among pulmonary TB patients. On the other hand, another study from South Ethiopia reported a lower prevalence among pulmonary TB patients compared to ours [82]. This difference could be explained by the difference in study population. The vast majority of participants in the study done in Addis Ababa were from urban areas mainly the capital where HIV prevalence is high in the general population. Contrary to this, a significant proportion (43%) of our study participants were from rural areas; besides, patients who come to a tertiary hospital are likely to be those with difficult diagnosis such as HIV/AIDS complicated with opportunistic infections resulting in a higher HIV prevalence. On the other hand, participants in the study from South Ethiopia were mainly (69%) rural residents, possibly resulting in lower HIV prevalence.

In this study, we have found a higher HIV prevalence among non-TB patients compared to the general population and this is consistent with the study from Ethiopia [77], indicating the need for screening non-TB patients presenting with respiratory symptoms for HIV infection.

4.1.3. Accuracy of smear microscopy and physician's diagnosis

Based on culture results, the prevalence of pulmonary TB among pulmonary TB suspects in our study was 31.3% which is very close to the prevalence (33%) reported in a study from Ethiopia [77]. Currently used means of diagnosis of TB in the study area were compared with culture as a reference to see their accuracy.

Diagnosis of TB in the study area mainly depends on smear microscopy, clinical judgement and/or radiological findings. In this study, the role of different symptoms in the diagnosis of pulmonary TB has been assessed and none of the common symptoms were significantly associated with pulmonary TB compared to non-TB patients. Other studies have reported one or more of the common symptoms in pulmonary TB as predictors of the disease. For example, a study in Ethiopia [77] identified fever as a predictor for pulmonary TB. Generally, the role of symptoms in predicting pulmonary TB is limited because of their poor specificity to TB [87].

Based on direct smear microscopy, 14.5% suspects were smear positive pulmonary TB patients. This is relatively lower compared to a hospital-based study done in Ethiopia [77] which reported a smear positivity rate of 19.8% and this might be related to the low sensitivity of smear microscopy in our study. In our study, the overall sensitivity of direct smear microscopy at health facilities was found to be 40.6%. However, the sensitivity of smear microscopy at government health facilities (52.6%) was comparable to the sensitivity (54.2%) reported by the study done in Ethiopia [77]. In general, the sensitivity of direct smear microscopy reported by different studies is variable, some reporting sensitivity as high as 80% and still others reporting as low as 20%. For example, a review paper on studies comparing direct and processed smear [42] reported a sensitivity ranging from 31% to 80%.

Furthermore, among culture positive pulmonary TB patients, the sensitivity of smear microscopy was found significantly lower in HIV infected patients compared to those without HIV infection. This is in good agreement with the study done in Ethiopia [77] and elsewhere [88]. Generally, pulmonary TB patients with HIV infection tend to have decreased cavitations and paucity of pulmonary inflammation with increased rate of smear negativity [88].

The sensitivity of smear microscopy in private health facilities was significantly lower than the sensitivity at government health facilities. Similarly, a report on summative evaluation of pilot private health sites indicated that the proportion of smear positives (21.2%) is lower than the national averages. Private health facilities were included in the management of TB in Ethiopia quite recently. Piloting was started in 2006 [89] and subsequent scale-up has been done in 2009. It is possible that health providers especially laboratory technicians may not be well trained in this short period. This might in turn have affected the competency in smear preparation and reading. Moreover, it was observed that patients coming from rural areas especially Afar Region needs their result in a single day and there is a tendency to do a single smear examination which obviously reduces the sensitivity of smear microscopy result. There is a need to closely monitor private clinics apart from training and technical support. Moreover, research needs to be done to explore possible factors contributing for such a significant difference in the sensitivity of smear microscopy between the two groups of health facilities so that evidence-based action could be mounted to improve the contribution of private clinics in the fight against TB.

Before culture result was ready, based on physician's diagnosis, about 72% of suspects were accurately diagnosed as TB and non-TB in our study. In HIV infected patients, both sensitivity and specificity of physician's diagnosis were lower, consistent with the report from Ethiopia [77]. This is partly a reflection of the low sensitivity of smear microscopy as well as chest x-ray in HIV infected patients [77]. This could indicate that a significant proportion of pulmonary TB patients are misdiagnosed in situations where HIV prevalence is high. This could lead to significant delay with a higher risk of transmission, greater morbidity and mortality. In this regard, the consequences of late diagnosis in HIV co-infected TB patients is far more grave compared to HIV negative TB patients [90,91] warranting an early and accurate diagnosis of TB in these group of patients. Moreover, increased awareness of health workers and better diagnostic tools for HIV-related pulmonary infections other than TB is essential.

4.1.4. Diagnostic performance of rESAT-6-CFP-10 and rα-crystallin-MPT-83 antigens

A total of 204 serum samples (89 from culture verified pulmonary TB patients and 115 culture negative non-TB patients) were included in running ELISA using two recombinant fusion antigens to assess their performance in the diagnosis of active pulmonary TB. Our

ELISA result showed a significantly higher OD values for rESTA-6-CFP-10 antigen among pulmonary TB patients compared to non-TB patients. In line with this, a study done in Ethiopia [92] reported a significant difference in the IgG specific immune response to rESAT-6 and rCFP-10 antigens between TB contacts and TB patients.

However, the difference in IgG-specific immune response between TB and non-TB patients for rα-crystallin-MPT-83 antigen in the present study was not statistically significant.

We found sensitivities of 57.3% and 20.2% for rESAT-6-CFP-10 and rα-crystallin-MPT-83 antigens, respectively. The corresponding specificities were 71.3% and 92.2% for rESAT-6-CFP-10 and rα-crystallin-MPT-83 antigens, respectively. In a review addressing the performance of purified antigens for the diagnosis of pulmonary TB [55], sensitivity ranging from 2 to 100% and specificity ranging from 44 to 100% was reported. Similarly, another review [49] addressed the performance of commercial serological tests and reported a sensitivity ranging from 10 to 90% and a specificity ranging from 47 to 100%. A study done by WHO/TDR [40] on the performance of 19 commercially available rapid diagnostic tests for pulmonary TB reported sensitivity as low as 0.97% and as high as 59.7%. The specificity of each test was relatively higher than the corresponding sensitivity reported and it ranged from 53 to 98.7%. This is consistent with our finding where for each antigen, the specificity is higher than the corresponding sensitivity.

Studies specifically addressing the performance of rESAT-6 and rCFP-10 antigens (through measuring OD values for IgG) in the sero-diagnosis of pulmonary TB either as a separate or fusion antigen have reported varying results. A study done in China [59] reported the performance of three different antigens among which rESAT-6-CFP-10 was one. The sensitivity and specificity of rESAT-CFP-10 among TB patients and healthy controls were 60.4% and 73.8%, respectively and this is in good agreement with our finding for rESAT-6-CFP-10 antigen.

Another study recently reported from China [93] evaluated 17 antigens including rESAT-6-CFP-10. The sensitivity and specificity of rESAT-6-CFP-10 antigen were 66.7% and 81.8%, respectively. Similarly, a study from Poland reported a sensitivity and specificity of 64.9% and 88.9% for rESAT-6 and 66% and 85.2% for rCFP-10 antigen, respectively [58]. The

sensitivity and specificity of rESAT-6-CFP-10 antigen in our study is lower compared to the reports from these two studies. In these studies, they used healthy controls to determine specificity which might have resulted in a higher specificity [55]. On the other hand, a study from South Korea [94] reported a lower sensitivity for rESAT-6 (37%) and rCFP-10 (46%). However, in this study, the cut-off value was determined on the ROC curve when the specificity is 100% for each antigen. Obviously, when we increase specificity, usually, there is a concomitant loss of sensitivity which is most probably the case in the Korean study.

To our knowledge, there are no studies which evaluated the diagnostic potential of α-crystallin-MPT-83 as a recombinant fusion or cocktail antigen. However, some studies have evaluated the sero-diagnostic potential of these two antigens separately. A study from Guinea compared the immune response of pulmonary TB patients and healthy controls to different antigens in a cohort study and they found that there was no significant difference between the two groups with regard to their immune response to MPT 83 antigen [65] which is consistent with our finding for the fusion antigen. Another study from the United States assessed the performance of different antigens together with smear microscopy in screening active pulmonary TB. This study reported a sensitivity and specificity of 17% and 95%, respectively for the 16 kDa recombinant antigen whereas the sensitivity and specificity of rMPT 83 were 9% and 84%, respectively [63]. Our finding of high specificity with very low sensitivity for the fused antigen is consistent with this report.

On the other hand, two studies reported a higher sensitivity for the 16 kDa recombinant antigen compared to ours. The first study was from the United States which assessed the performance of this antigen through measuring IgG, IgA and IgM; they reported a sensitivity and specificity of 62% and 100%, respectively for the IgG isotope [64]. Similarly, a study from China has reported a sensitivity of 65.7% and specificity of 91.1% [93]. Moreover, the later study reported a significant difference in the immune response between healthy controls and pulmonary TB patients. Our finding does not support the findings of these two studies which reported a much higher sensitivity. Apart from differences in the antigen used, the composition of the study population in our study was different compared to the two studies and this might have contributed for the difference in the sensitivity. For example, more than 50% of pulmonary TB patients in the Chinese study were both smear and culture negative

and the controls were all healthy; this might have influenced the cut-off point and hence the sensitivity and specificity of the antigen.

Generally, the difference in the sensitivity and specificity of the two antigens in our study compared to others might be explained by the difference in the type and source of the antigen used as well as differences in the study population composition. Moreover, it has been reported that the performance of an antigen varies across different populations [66,95] and this could be an additional possible reason for the differences observed.

There are reports indicating that different antigens have higher sensitivity in smear positive patients compared to smear negative patients [51-54,96,97] and in HIV negatives than HIV positives [54]. However, we did not find any significant difference in the IgG-specific immune response to both antigens between smear negative and smear positive patients as well as HIV positive and HIV negative pulmonary TB patients. A study in Denmark [66] showed a higher sensitivity of three antigens (TB 9.7,TB 15.3 and TB16.3) in smear negatives compared to the overall sensitivities of these antigens in pulmonary TB patients and one antigen (TB 9.7) had a higher sensitivity in HIV positives compared to HIV negatives. This difference could be due to different immune response to different antigens, stage of the disease (TB as well as HIV) and differences in study population.

Interestingly, we found a significant difference in the IgG-specific immune response to rESAT-6-CFP-10 antigen between BCG vaccinated and non-vaccinated patients, those vaccinated having higher median OD values. This is consistent with the findings of the study from China [93]. The Chinese study reported a significant difference in the immune response to α-crystallin (16 kDa) antigen between vaccinated and non-vaccinated group, vaccinated group having higher mean OD values. In our study, the difference in the immune response to rα-crystallin-MPT-83 did not reach statistical significant (p=0.052); however, the median OD values were higher among vaccinated group compared to non-vaccinated group. Generally, the RD-1 which codes ESAT-6 and CFP-10 is absent from BCG strains [98] and it is not clear why such differences in the IgG-specific immune response between BCG vaccinated and non-vaccinated was observed.

4.2. Delay in the diagnosis and treatment of TB patients

A total of 216 TB patients who were sent to DOTS clinic of two health facilities in Afar Region were interviewed using pretested, structured questionnaire to assess the delay in diagnosis and treatment of TB.

4.2.1. Distribution of the sample

In this study, the vast majority (83.5%) were below 45 years of age and this is consistent with previous reports from Ethiopia [36,37,80]. Moreover, the number of males outweighs that of females and these findings are consistent with the national report [6] as well as the global trend [16].

Regarding the form of TB, the proportion of pulmonary TB and extra-pulmonary TB were 63.4% and 36.6%, respectively and this is in good agreement with the 2006 report for the nation [6]. However, the proportion of extra-pulmonary TB for Afar Region in 2006 was low (24.7%) compared to ours as well as the national figure [6]. Among pulmonary TB patients, the proportion of smear positive patients in our study (38.7%) was lower than the regional (51%) as well as the national (46%) report [6] but close to the finding(42%) of a study from Tigray Region [32]. Among pulmonary TB suspects included in our evaluation of the two antigens, the proportion of smear positives was 37.4% which is very close to the proportion for those included in the assessment of delay. The difference from regional and national reports might be related to the fact that we have included a smaller proportion of health facilities and the time was also limited as opposed to a one year period for the reports. However, one cannot rule out the possibility of errors in compiling and reporting data at regional as well as national level. There might also be a bias towards smear positive pulmonary TB during reporting.

The proportion of females was significantly lower among pastoralists compared to non-pastoralists. Generally, the notification rate for males is higher than females in nearly all countries [16]; however, a further significant drop in the notification rate for females among pastoralists might indicate the possibility of lower access to TB diagnostics and treatment services among female pastoralists.

Significantly higher proportions of pastoralist were illiterates and lived at greater distance compared to non-pastoralists. This is expected since the majority (69.2%) of pastoralists were

from rural areas where the literacy rate is low compared to urban dwellers [99]. The proportion of Muslims (95.6%) among pastoralists is significantly higher compared to the proportion among non-pastoralists (70.4%) and this is consistent with the regional census result [2].

4.2.2. Health seeking action and patients' delay

In our study, a surprisingly low proportion of TB patients sought help from non-formal health care providers in contrary to previous reports from Ethiopia [32,36,37]. However, unlike in our study, two [32,36] of these studies reported a low knowledge of participants about TB. A recent study in Afar region on knowledge and perception of pastoralist communities about TB reported a high degree of awareness [100] which is consistent with our finding. Although we did not observe a significant difference in knowledge score between those who sought help from non-formal health providers and those who sought help from formal health providers, the high degree of awareness among our study participants about TB might be a possible reason for their preference to formal health care providers. Information is highly valued among the Afar people and they heavily depend on face-to-face communication which is called "*Dagu*". "*Dagu*", a highly valued and integral part of the Afar day-to-day life, is considered as a social capital and traditional heritage [101]. It might be possible that information related to TB might have been channelled through "*Dagu*" resulting in high awareness about the disease.

The median patient delay in our study is relatively short (20 days for all forms of TB, 15 days for pulmonary TB and 21 days for extra-pulmonary TB). The median patient delay for all forms of TB in our study is shorter than those reported from Ethiopia: 60 days in Addis Ababa [80], 30 days in Southern Ethiopia [102], 30 days in Northern Ethiopia [32], 60 days in Somali Region, Ethiopia [36]. Similarly, the median patient delay in our study is shorter than those reported elsewhere: 21 days in Botswana [103], 28 days in South Africa [104], 28 days in Ghana [105], 28 days in Norway [106], 31 days in Thailand [107], 56 days in Nigeria [108] and 120 days in Tanzania [33]. On the other hand, the median patient delay in our study is longer than those reported in Italy (7 days), New Zealand (7 days), Taiwan (7 days), Thailand (11 days), Malaysia (14 days) [38] and Uganda (7 days) [109]. One study from India reported identical median patient delay with that of ours [110]. Compared to the WHO-lead multi-country study in the WHO Mediterranean Region, patient delay in our study was

shorter than those reported for Iran, Iraq, Somali, Syria and Yemen but longer than those reported for Egypt and Pakistan [35].

Patient awareness and early consultation is an important step towards early case detection. In this study, we have found that patients have, in fact, reported to health facilities within a reasonable time: a patient with persistent cough is generally advised to consult formal health care providers in 2 to 3 weeks time and in our study, at least 50% of patients were able to report to health facilities within 3 weeks. In highly mobile population with a relatively low literacy rate and poor infrastructure, it is encouraging that half of TB patients are in fact coming forward and seeking help from formal health providers as early as 20 days following the debut of TB symptoms. The high degree of awareness about TB in the study population might have played a key role in the early health seeking behaviour.

In the present study, we have found that first visit to a non-formal health provider was an independent predictor of patient delay; this is consistent with the reports of two studies from Ethiopia [32,37] and another study from Thailand [107]. Similarly, a study done in Tanzania [33] reported that patients' delay was significantly longer for those who first visited a traditional healer. In our study, those patients who consulted non-formal health providers initially have actually did that early enough (median of 10 days); this is a good opportunity to decrease patient-related delay if attention is given to non-formal health providers' awareness on TB so they could refer TB suspects to formal health providers early.

Similarly, we have found self-treatment as an independent predictor of patient delay which is consistent with a previous report from Ethiopia [37]. Those patients who treated themselves might consult formal health care providers only after the disease gets worsened. In a qualitative study in Kenya, it was reported that treatment steps for TB symptoms are generally sequential and the usual initial response of patients to an illness is self-treatment [111].

4.2.3. Health system's delay

The median health system's delay in our study was estimated to be 33.5 days which is long indicating the inadequacy of the health system to diagnose symptomatic TB patients despite a relatively early consultation made by the majority of patients.

In comparison to previous reports, the median health system's delay in our study is shorter: 63 days in Uganda [109], 35 days in Botswana [103], 35 days in Malaysia [112] and 56 days in Ghana [105]. Moreover, it is shorter than those reported for Iran (42 days) and Pakistan (87 days) in the WHO multi-country study [35]. However, the median health system's delay in our study is longer than those reported from Ethiopia: 6 days in Addis Ababa [80] and Somali region [36] and 21 days in Amhara region [37]. Similarly, a shorter health system's delay has been reported in 5 WHO Eastern Mediterranean Region countries (Egypt, Iraq, Somalia, Yemen and Syria) [35] as well as Thailand [107], Nigeria [108], Tanzania [33], and Malaysia [112] as compared to ours. A study in Norway [106] that included all forms of TB reported health system's median delay of 33 days which is consistent with our finding. The variation in health system's delay across different studies might be mainly related to the efficiency of the diagnostic services available as well as health workers' awareness and skills in the diagnosis of TB.

In the present study, extra-pulmonary TB was found to be an independent predictor for health system's delay which is consistent with previous reports from Ethiopia [36], Norway [106] and London [113]. This is not surprising since diagnosis of extra-pulmonary TB is not as easy as pulmonary TB. Literally all organs could be involved and the clinical presentation is non-specific and hence health workers may not put extra-pulmonary TB as a differential diagnosis of other related illnesses. Even after a patient is suspected of extra-pulmonary TB, confirmation of diagnosis is extremely difficult because of lack of simple and accurate diagnostic tests. This creates a big dilemma whether to start a patient on anti-TB medication based on clinical grounds; therefore, patients might be treated with several doses of antibiotics before commencing on anti-TB medication and this leads to delay in diagnosis and treatment. Although patients with extra-pulmonary TB do not pose a threat to the community in terms of transmission, they could suffer long term disability, high morbidity and mortality as a result of late diagnosis.

Those patients who first visited government health posts/clinics and health centers had a significantly longer delay compared to those who first visited government hospitals and this is consistent with previous reports from Ethiopia [37] and Botswana [103]. According to a review, the main problem with regard to delay seems to be related to "a vicious cycle of repeated visits of the same healthcare level, resulting in non-specific antibiotic treatment and

failure to access specialized TB services" [38]. Government health posts/clinics do not have laboratory facilities for TB; moreover, they are staffed with junior nurses and health assistants who are primarily trained for prevention and promotion activities as well as nursing care. However, they are also allowed to treat minor illnesses and therefore, it is likely that TB patients could easily be confused and treated as simple respiratory infections or other non-specific medical conditions. Poor skills of health professionals together with absence of laboratory services could have led to a significant delay in the diagnosis and treatment of TB patients who presented to health posts/clinics initially. Although health centers do have TB diagnostics as well as treatment services, in remote areas like Afar Region, health professionals with good skills are lacking and therefore, patients will be examined by less experienced health workers mainly nurses. It is likely that TB patients, at least initially, might have been treated as cases of simple respiratory infections or other minor illnesses rather than requesting smear microscopy leading to a significant delay with increased transmission, morbidity and mortality.

First visit to private clinics/hospitals was found to be an independent predictor of longer delay compared with government hospital and this is in agreement with previous studies [37,107,110]. However, in comparison to government health posts and health centers, there is a significantly shorter delay for those patients who initially visited private clinics/hospitals. This study was done after a major shift has taken place in the management of TB in the country beginning 2006: from a purely public health sector duty to Public-Private Mix DOTS. In order to improve the quality of care at private health facilities, USAID Private Sector Program provides technical support including training [89]. Therefore, it is expected that awareness on TB among health workers at private health facilities has increased since their inclusion in the management of TB. Moreover, from our observation, patients from Afar Region tend to visit private clinics and hospitals in Dessie Town where better qualified physicians as well as better diagnostics (chest x-ray and cytology) are available. These factors might have contributed to the early diagnosis of TB patients at private clinics compared to low level government health facilities in our study.

The median treatment delay in our study was 1 day. This is in good agreement with the reports for the 7 countries in WHO Eastern Mediterranean Region (0 for Egypt, Iraq and Yemen; 1 for Iran and Syria; 2 for Pakistan and Somalia) [35]. A study from Ghana reported a median treatment delay of 1.43 days with a range of 0 to 14 days which goes with our

finding [105]. Generally, the treatment delay for patients diagnosed at government health facilities is acceptable. However, the median treatment delay for those patients who were diagnosed at private health facilities was 4 days, much longer than the median delay for those diagnosed at government health facilities. This is mainly due to the fact that private health facilities, mainly found in Dessie, are far from patients' homes and after being notified their diagnosis, patients are sent with a referral sheet to the nearest health facility. In the mean time it is possible that patients report to the government DOTS clinic late for various reasons. Apart from delay in the initiation of treatment, it is possible that patients might be lost. Improved diagnostic services at lower government health facilities coupled with increased awareness of the public on the availability of such services closer to their home could avoid unnecessary delay after diagnosis is made.

4.2.4. Total delay

The median total delay in our study was found to be 70.5 days and this is in good agreement with previous reports from Ethiopia [36,37,80] as well as Nigeria [108] and Thailand [107]. Longer median total delay was reported in Uganda [109], Botswana [103], Tanzania [33], Ghana [105], and Malaysia [112] compared to ours whereas the median total delay in Norway was shorter than ours [106]. In the WHO Eastern Mediterranean Region multi-country study, in 5 of the 7 countries (Egypt, Iraq, Somalia, Syria, Yemen), the median total delay was reported to be shorter than ours and for the rest two countries (Iran and Pakistan), it was longer than ours [35].

In our study, we have found health system's delay as a major contributor to the total delay, consistent with a previous study from Ethiopia [37]. Interestingly, we found an inverse relationship between patient's and health system's delay and this is consistent with the finding from Botswana [103]. Those who visited the health system earlier experienced a longer health system's delay and this may indicate the difficulty in making a diagnosis in those presented early compared to those presented late. In line with this, two studies from Ethiopia [36,80], reported a long median patient delay (60 days) and a short health system's delay (6 days). However, in our study, it could also be related to lack of vigilance from health workers side in suspecting TB in those who presented early. This is an important area that needs further research in order to identify factors involved in delayed diagnosis of those patients who visited health facilities earlier.

In this study, we have found extra-pulmonary TB as independent predictor of longer total delay and as previously pointed out, this might be related to the difficulty in suspecting and subsequently diagnosing TB in this group of patients.

A first visit to government health post/clinic remained an independent predictor of longer total delay and this is possibly related to the poor skills of health workers and absence of TB diagnostic services.

4.3. Strengths and limitations of the study

Strengths

The serology study:

1. A relatively large number of bacteriologically positive pulmonary TB patients were included in evaluating the potential of the two antigens in the diagnosis of pulmonary TB

2. We recruited all pulmonary TB suspects consecutively; moreover, we included all levels of health facilities (a government health center, a government hospital, a private clinic as well as two private hospitals for the serology study). This helped us to control selection bias.

3. To avoid verification bias, we used culture as a reference test in all patients for the evaluation of the two antigens. Moreover, culture is recommended as a diagnostic gold standard [44].

The delay study

1. A relatively large sample size was achieved for the assessment of delay within the study period

2. We included patients with all forms of TB unlike the majority of studies reported so far

Limitations

For the serology study

1. Serum was collected from field sites and transported for 400- 600 km with wet ice. This might have a negative impact on the ELISA result. We tried to avoid exposing the samples to undesirable temperatures.

2. There is no ideal gold standard test for immune-based tests in TB. We used culture which is the best gold standard currently; however, sensitivity of culture is not 100% and hence we might have misclassified some TB cases as non-TB cases.

For the delay study:

1. Selection bias: since we limited our study to TB patients who reported in two government health facilities, we do not have any control on those who did not report to health facilities or who reported to private clinics as well as other government health facilities and therefore, our findings may not be generalized to all TB patients in the region. However, we strongly believe we have generated valuable information pertinent to the two health facilities and TB patients who takes care from these health facilities.

2. Health workers were involved in interviewing participants and it is possible that patients might have reported a shorter time for health system's delay to please the interviewers.

3. We adopted a cross-sectional study design to collect information on exposure and outcome variables. In cross-sectional study design, it is not possible to know whether the exposure variables precede the outcome variables.

4. Recall bias is an inevitable limitation in interview-based studies and in our study, this problem might have been there. We have used religious holidays to facilitate in recalling and hence limit the impact of recall bias.

4.4. Conclusion

In the present study, we have evaluated the potential of two fused recombinant antigens for the diagnosis of active pulmonary TB in a clinical setting. Moreover, we have assessed the delay in the diagnosis and treatment of TB patients in two government health facilities.

The performance of rα-Crystallin-MPT-83 antigen in the diagnosis of pulmonary TB is low. There is no significant difference between pulmonary TB and non-TB patients in their median OD values for this antigen and therefore, it does not seem to be useful in the diagnosis of TB in the study area. On the other hand, the performance of rESAT-6-CFP-10 is relatively good compared to many commercial sero-diagnostic tests for TB. Although its sensitivity is better than smear microscopy, the corresponding specificity is low and therefore, it is not accurate enough to replace or supplement smear microscopy in the diagnosis of active TB in the study area. However, few studies have evaluated these antigens in a well characterized panel of sera and therefore additional work is required before giving sound conclusion.

Assessment of delay in diagnosis and treatment of TB in the two health facilities has revealed that TB patients had experienced long delay. In this regard, the contribution of the health system was found to be considerably high. Interestingly, a significant proportion of patients have reported within three weeks although they experienced a longer delay at the health system compared to those who sought medical attention late. In general, our findings in relation to patients' delay, health system's delay, treatment delay and total delay are comparable to previous reports.

Upon exploring possible predictors of the different types of delay, we have found self-treatment and a first visit to non-formal health providers as independent predictors of patients' delay. On the other hand, extra-pulmonary TB has been found to be an independent predictor of health system's delay as well as total delay. First visit to lower level government health facilities (health posts/clinics and health center) as well as private health facilities were found to be independent predictors of health system's delay which might be related to lack of better qualified health personnel as well as accurate diagnostic services. Moreover, first visit to government health posts/clinics was an independent predictor of total delay.

In this study, we have showed that there is long delay in the diagnosis and treatment of TB patients and this is especially attributable to the health system. The fact that the majority of patients presented themselves to health facilities early enough is an indication that patients' awareness is relatively good; however, the health system's response especially at the lower

level is inadequate. Therefore, the quest for point of care diagnostic tests should remain a priority to make sure that TB control programmes become successful.

4.5. Recommendations

1. The lowest health facilities (clinics/health posts) should be equipped with simple and rapid diagnostic tests to reduce health system's delay. Health workers at the lowest health facilities (mainly nurses and health assistants) are at the front line where most patients seek help. Therefore, efforts to increase the skill and awareness of them could be intensified through continuing medical education on clinical presentation of patients, importance of early diagnosis and treatment of TB patients as well as when and where to refer patients with possible TB. Moreover, continuous support and supervision of these health workers should be in place to make sure they are contributing to the overall TB control programme.

2. The sensitivity of smear microscopy was found to be significantly lower in private health facilities compared to government health facilities. There is a need to increase the skills of laboratory technicians in the diagnosis of TB especially at private health facilities in the study area. This could be done through skill-based training of laboratory technicians on smear preparation and reading. Moreover, close supervision, support and motivation of laboratory workers at all levels would be of paramount importance. Along with improving the skills of laboratory technicians, National TB and Leprosy control Programme (NTLP) should make sure that there are good microscopes as well as continuous supply of laboratory reagents for smear microscopy at TB diagnostic laboratories.

3. The significant health system's delay of patients who had their first visit to private health facilities in the study area needs to be addressed. The country has already launched a Public-Private Mix DOTS programme and therefore, training aiming at increasing private health workers' awareness on TB as well as skills on diagnosis is essential. Moreover, close supervision and continued support of private health facilities is important.

4. Although TB patients in our study report relatively early, nearly half of them reported after 3 weeks. This is also the case in other parts of the country and therefore, further awareness creation using a well organized information, education and communication (IEC) programme could be done. In this regard, the already existing cultural means of communication, "*dagu*",

could be effectively used to disseminate information regarding the causes, symptoms, and treatment of TB as well as where and when a patient with TB symptoms should seek help.

Further research implications:

1. The performance of rESAT-6-CFP-10 antigen is relatively good compared to many commercial TB serodiagnostic tests; however, we measured the OD values for IgG antibody only and it is difficult to give sound conclusion from the present study alone. Therefore, additional work is needed to measure the three major antibodies (IgG, IgM, IgA) in well characterized panel of sera.

2. BCG lacks the gene that encodes ESAT-6 and CFP-10. However, in the present study, BCG vaccinated patients had significantly higher OD values. The reason for such difference is not clear from the current study. Therefore, more work is required to elucidate the effect of BCG on humoral immune response to rESAT-6-CFP-10 antigen.

3. Drinking raw milk seems to be protective against pulmonary TB. However, further study is required to make a sound conclusion on this association as well as to identify the immunological basis for such protection.

4. The prevalence of HIV infection among Afar ethnic pulmonary TB suspects in our study was significantly low compared to other ethnic groups. However, the possible reasons for such difference is not clear from the current study and therefore, more comprehensive (both quantitative and qualitative methods) research is required to confirm our result as well as identify protective factors which may have an important implication to contain the spread of HIV infection in Afar Region.

5. The accuracy of smear microscopy significantly differed between government and private health facilities. Moreover, we have found a significant health system's delay of patients who had their first visit in private facilities compared to those who visited a government hospital. Further research is required to identify possible reasons for this difference and hence a targeted intervention could be designed.

6. We have found an inverse relationship between patients' delay and health system's delay. Possible reasons for such a relation need to be clarified. Even if patients report early enough,

they do not receive their diagnosis (and hence treatment) early enough resulting in late diagnosis and treatment.

7. Since our study related to delay was limited to two health facilities, a study with more representative sample could be envisaged to generalize to all TB patients coming to health facilities in the Region; this would give evidence that could be applicable to the Region as a whole.

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6. Appendices

6.1. Appendix 1

Informed consent

Diagnostic delay and the potential of two fusion antigens for the diagnosis of Tuberculosis in Northeast Ethiopia

TB is one of the major health problems in Ethiopia and the HIV/AIDS epidemic is aggravating the spread of TB. Early and rapid diagnosis of the disease helps to treat patients as well as to control the disease. However, diagnosis of the disease remains a major challenge because of delayed consultation as well as absence of rapid and reliable diagnostic tests. Currently, a number of serological tests are being evaluated; similarly, we are interested to evaluate the potential of one of these diagnostic tests for the diagnosis of TB. The objective of this study is to evaluate the performance of an antigen using blood sample in the diagnosis of TB as well as assess the delay in diagnosis and treatment of TB in the study area.

You are kindly requested to participate in the study. As a TB patient reporting to the DOTS clinic, if you agree, you will participant in an interview about TB for about 30 minutes. You are not required to give any sample and there will be no further appointment related to the study.

On the other hand, if you are pulmonary TB suspect and agree to participate in the study, you will be asked to give the following samples: sputum and about 4 ml blood. You will be interviewed in relation to your disease for about 15 to 30 minutes. HIV testing will be done. You will be provided counselling both before and after testing and you are strongly advised to know your status. However, your decision not to be tested and know your status is respected.

No serious complication is expected as a result of sample collection. However, during blood collection, you may experience some pain and discomfort. Rarely, thrombophlebitis can happen. If you happen to develop any complication, you will be provided treatment in the health institution free of charge and the cost will be covered by the project. If you are found to be a case of TB according to the existing diagnostic protocol of TB in the country, you will be treated in the health facility free of charge which is also the standard treatment even if you do not participate in the research.

As a pulmonary TB suspect, your participation in the study will be for about 2 days which is the average time taken for sputum sample collection, microscopy examination and reporting the result routinely. If you happen to have TB, you will start treatment for your illness. On the other hand, if you are not diagnosed as TB patient but found to be a case of TB after culture result (which will take a maximum of 2 months), you will be provided with treatment and therefore, you are advised to come back to your physician on your appointment. There is no direct benefit as a result of your participation in the study. However, your participation will contribute for the efforts being done to develop a new test for the diagnosis of TB.

Information related to your name will be treated strictly confidential. Names and identifiers will be coded and deleted after data collection and therefore data will be treated anonymously for communication of results. Informed consent forms and questionnaires will be left behind in a locked cabinet in Ethiopia and will be destroyed after a year. Participation in the study is fully voluntary and therefore you may decline from the outset. Your choice not to participate in the study will have no effect at all on the care you will be provided from the health facility and hence you still receive the same treatment as others without any prejudice.

6.2. Appendix 2
Declaration of consent

I confirm that I have been given adequate information about the research project. I have understood that the information I provide will be used for research purposes and the information related to myself will be kept strictly confidential. I am aware that participation in the study is fully voluntary and I can withdraw anytime without giving any reason. Moreover, I am fully aware that non-participation in this project will not subject me to any health service denial from this health facility either now or in the times to come. I confirm that all the information provided to me is very clear and has been conveyed by the language that I fully understand. Finally, I declare that I have agreed to participate in the study.

Name of participant.....

Name of the person obtaining consent.....

Signature of the person obtaining consent.....

Name of the person witnessing the consent……

Date.....

Note: This form has been translated to Amharic language which is the official language in the study areas

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6.3. Appendix 3
Questionnaire for PTB suspects

Title of the study: **Diagnostic delay and the potential of two fusion antigens for the diagnosis of Tuberculosis in Northeast Ethiopia**

Participant name_____ code number_____

Zone_____Woreda_____Kebele_____

Name of the health institution_____

A. General information about the patient

1. Age (in years)_____

2. Sex: 1= Male 2=Female

3. Current marital status 1=Single 2=Married 3=Widowed 4=divorced

4. Place of residence: 1=Urban 2=Rural

5. The highest level of education attained: 1=none 2=primary 3= post-primary

6. Religion: 1=Muslim 2=Christian other_____

7. Ethnicity: 1=Afar 2= Amhara 3= Oromo 4=other

8. Current occupation: 1=Pastoralist 2=Agro- pastoralist

3. Other (describe)_____

9. If pastoralist or agro-pastoralist: Herd type

1=Goat 2=sheep 3= cattle 4=camel 5= mixed

10. Ingestion of raw milk: 1=Yes 2= no

11. Contact with TB patients (patients with cough: 1=Yes 2= no

12. If yes to 10, 1= Family member 2= outside family member

13. History of previous treatment for TB: 1=Yes 2= no

14. If yes to 12, 1=Treatment completed 2= treatment interrupted

15. History of BCG (please look for BCG scar) 1=yes 2=no

B. Current illness

1. When did you start having the present illness (Date of onset for the main complaint):

_____

2. When was your first visit to a health provider (be it formal or informal)_____

3. The first health provider visited

1= informal 2= formal, private 3=formal, government

4. Which of the following symptoms are you suffering from?

Symptoms	**Yes**	**No**
Cough without blood		
Cough with blood (haemoptysis)		
Neck, armpit or groin swelling		
Fever		
Loss of appetite		
Chest pain		

Shortness of breathing		
Weight loss		
Night sweating		
Fatigue and weakness		
Other (please describe)		

5. Sputum collected_____

6. Serum collected_____

7. AFB result_____

8. Culture result_____

9. Diagnosis_____

6.4. Appendix 4:
Questionnaire for the assessment of diagnostic delay

Title of the study: **Diagnostic delay and the potential of two fusion antigens for the diagnosis of Tuberculosis in Northeast Ethiopia**

Participant TB Number_____

Zone_____Woreda_____Kebele_____

Date of interview_____

Name of the interviewer_____Signature_____

A. General information about the patient

1. Patient category: 1=Smear positive PTB 2= Smear negative PTB
   3=Extra pulmonary TB (Type)_____
2. Age (in years)_____
3. Place of residence: 1=Urban 2=Rural
4. Sex: 1= Male 2=Female
5. Current marital status 1=Single 2=Married 3=Widowed 4=divorced
6. The highest level of education attained:
   1=none 2=primary 3= post-primary
7. Religion: 1=Muslim 2=Christian other_____
8. Current occupation: 1=Pastoralist 2=Agro- pastoralist
   3. Other (describe)_____
9. If pastoralist or agro-pastoralist: Herd type

1=Goat 2=sheep 3= cattle 4=camel 5= mixed

10. Distance from your home to the nearest health facility: (either in minutes, hours or km)

_____

11. Type of house used for dwelling: 1= "Debora" 2= Corrugated iron sheet roof

3. Other, please describe_____

12. Number of rooms in the house_____

13. Number of people living in the house_____

B. Current illness and visit to health providers*:*

1. When did you start having the present illness (Date of onset for the main complaint):

_____

2. Which of the following symptoms are you suffering from?

Symptoms	**Yes**	**No**
Cough without blood		
Cough with blood (haemoptysis)		
Neck, armpit or groin swelling		
Fever		
Loss of appetite		
Chest pain		
Shortness of breathing		
Weight loss		
Night sweating		
Fatigue and weakness		

Other: please specify_____

3. Which of the above symptoms most urged you to seek for medical care?

_____

4. What did you think of the type of disease you have? _____

5. Did you consult a person what to do/where to go for help at the onset of the present illness? 1= Yes 2= No

6. If yes to 6, whom did you consult? Please describe_____

7. Did you first try to treat the illness by your own using homemade remedies?

1= Yes 2= No

8. If yes to 8, describe what you used to relieve your symptoms?

_____

9. Which of the following health providers did you visit for the current illness (please order them as 1 for the health provider visited first and 2 for the second and so on)?

No	Public health care facilities	Yes	order	Duration from date of onset of symptoms to visit
9.1	Clinic /health post			
9.2	Health center (Government)			
9.3	Hospital (Government)			
9.4	Lower level clinic (private)			
9.5	Mid level clinic (Private)			
9.6	High level clinic/Hospital (private)			
9.7	Local injectors			
9.8	Pharmacies, drug stores, drug vendors, open market drug sellers			
9.9	Traditional health providers (herbalists, religious healers, holy water, others).			

For 9.8 and 9.9 please underline the specific health provider.

10. How many times have you visited a **government medical provider** for your symptoms before it was confirmed to be TB?

1= Once 2=Twice 3=Three times 4= Four times 5=Five times 6= More than five

11. Were the above visits with the same or different medical providers?

1= Same 2=Different

12. How many times have you visited a **private medical provider** for your symptoms before it was confirmed to be TB?

1= Once 2=twice 3=3 times 4= 4 times 5=5 times 6= more than 5 times

13. Were the above visits with the same or different private medical providers?

1= Same 2= Different

14. Where did it become for the first time clear that the disease is TB?

1= TBMU (TB management unit for government health facilities)

2= Private medical provider

C. If the diagnosis of TB was made at the private medical providers

(NOTE: if diagnosis is not made at private medical providers, please go to D)

1. What did the doctor/ the health worker at the private medical provider do when he/she confirmed that your illness was TB?

1=He/she referred me to the TBMU with slides

2=I was referred without slides

3= I was started on anti-TB drugs and referred

4= other, please describe_____

2. How long did it take from the time you visited the private medical provider till you were told to have TB?_____hrs/days/weeks

3. How long did it take from the time you were referred by the private medical provider till you first reported to the TBMU?_____days/weeks.

4. How long did it take from the time you first reported to the TBMU till you first started Anti-TB drugs? _____days/weeks

5. How long did it take from the time you were referred by a private medical provider till you first started taking the anti TB drugs?_____days/weeks.

D. Diagnosis made at the TBMU (at government health facilities with DOTS clinic as well as AFB service**)**

Note: Go to question 7 if diagnosis is made at private health providers and diagnosis is accepted without further investigation at your TBMU

1. Date of first visit to the TBMU? _____

2. How did you decide to visit the TBMU? _____

1= Referred by HP (health post)/clinic Date Referred_____

2= Self-Referred Date referred_____

3=Referred by private Date referred_____

4. Others, please specify, _____

3. How long did it take since you came to the TBMU till you were first seen by the doctor/ health worker? _____ hrs/days/weeks

4. Date the patient was first seen by the doctor /health worker Checked (from the patient's card)_____

5. How long did it take from the time you were first seen by the doctor/ health worker till you first received the sputum request for AFB/x-ray/FNA? _____hrs/days/ weeks

6. How long did it take from the time you gave sputum or other lab tests for examination till you received the results?_____

7. How long did it take from the time you were notified to have TB (received AFB result/other lab tests) till you started the first Anti- TB regimen? _____hrs/days/weeks

7.1 Date Anti-TB treatments were ordered checked (**from the patient card**)

_____

7.2. Date of registration for treatment (**from district registry book**)

checked_____

8. How long did it take from onset of the present illness till you first started anti TB chemotherapy? _____ (days, weeks, month)

E. Knowledge about TB:

1. Have you heard, known about pulmonary TB? For example, TB causes chronic cough? Haemoptysis? 1= Yes 2= No

1.1. If yes to 1, where has the information come from?

1= Family 2= Neighbors 3= Friend 4=Health workers

5= Media 5= Books (reading)

Other, describe_____

2. If TB is treated, can it be cured? 1= Yes 2= No 3=I do not know

3. What do you think are the causes of TB?

Possible causes	**No**	**yes**	**I do not know**
Witchcraft			
Poverty			
Bacilli			
hard work			
Sexual overindulgence			
Malnutrition			
Unventilated home			
Living together with untreated TB patient			
HIV			
other causes			

4. Do you know any danger if a TB patient is not treated? 1= Yes 2= No

4.1 If yes, what is it?

For the patient, _____

For the people around, _____

5. Do you know that the drugs are available for free? 1=Yes 2=No 3= I don't know

6. How long is TB treated? 1= 1-year 2= 6-8 months 3= I do not know
3=other, please describe_____