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Risk factors for tuberculous infection among child contacts of pulmonary tuberculosis cases

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ABBREVIATIONS

AFB	:	**Acid-fast bacillus**
AIDS	:	**Acquired immune deficiency syndrome**
ARTI	:	**Annual risk of tuberculosis infection**
BCG	:	**Bacille Calmette-Guérin**
CFP-10	:	**Culture filtrate protein 10**
DALYs	:	**Disability adjusted life years**
DOTS	:	**Directly observed treatment, short – course**
DTH	:	**Delayed type hypersensitivity**
ESAT-6	:	**Antigen target 6**
GDP	:	**Gross domestic product**
GEE	:	**Generalized estimating equation**
HIV	:	**Immunodeficiency virus**
IDU	:	**Intravenous Drug User**
INF ϒ	:	**Interferon gamma**
IUALTD	:	**International Union Against Tuberculosis and Lung Diseases**
LTBI	:	**Latent tuberculosis infection**
M.TB	:	***Mycobacterium tuberculosis***
MDR-TB	:	**Multidrug -resistant tuberculosis**
MOH	:	**Ministry of Health**
NAOH-NALC	:	**Sodium hydroxide -N-Acetyl -L-Cystein.**

NHPs	:	**National health programs**
NIHE	:	**National Institute of Hygiene and Epidemiology**
NTB	:	**National tuberculosis program**
NTM	:	**Nontuberculous mycobacterium**
OR	:	**Odds ratio**
PBS	:	**Phosphate buffer saline**
PPD	:	**Purified protein derivative**
SPSS	:	**Statistical package for the social science**
TB	:	**Tuberculosis**
TST	:	**Tuberculin skin test**
UIO	:	**University of Oslo**
UNICEF	:	**United Nations International Children's Emergency Fund**
USAID	:	**United States Agency for International Development**
USD	:	**United States dollar**
WHO	:	**World Health Organization**

ABSTRACT

Background: Tuberculosis is one of the leading causes of mortality and morbidity in the world, especially in developing countries. Tuberculosis can transmit from person to person through the air. Thus, household contacts are considered as the high risk group of being infected. To prevent and control tuberculosis, screening of high risk population is widely recommended. However, it is seldom practiced in developing countries because of lacking resources. In Vietnam, National Tuberculosis Program (NTP) focuses on detection and treatment of new tuberculosis cases aiming at exceeding the targets of World Health Organization (WHO) in controlling tuberculosis. Despite these efforts, Vietnam still ranks 13th out of 22 countries with highest burden tuberculosis worldwide. And there is no evidence of decreasing in the number of new tuberculosis detected cases in any reports from NTP. Epidemiology of tuberculous infection among children has not been emphasized by Vietnam National Tuberculosis Program. Additionally, all strategies of NTP have focused on detection and treatment of new cases rather than identifying risk factors for tuberculous infection among contacts.

Tuberculin skin test , the only method of diagnosing tuberculous infection in Vietnam still has many drawbacks because of cross-reaction with BCG vaccination as well as environmental mycobacterium. The whole blood interferon gamma assay has been shown to be more specific than tuberculin skin test and can be used in diagnosing tuberculous infection by numerous studies. Almost all studies have been conducted in low incidence countries and rarely in high burden countries.

Better understanding the epidemiology of tuberculosis among children contacts as well as having the best method of diagnosing tuberculous infection could be a valuable distribution to the evaluation of undergoing TB transmission in the community. We, therefore, conducted a study in three provinces in northern Vietnam in order to identify risk factors for TB infection among children contacts.

Objectives: The study is to compare the whole blood interferon gamma assay with tuberculin skin test in diagnosing TB infection and to identify risk factors for TB infection among children of smear positive tuberculosis cases.

Method and materials: A cross sectional study was performed. 128 smear positive tuberculosis cases and 208 children contacts under fifteen of age between July 2007 and January 2009 were recruited into study. Clinical data of tuberculosis cases were collected based on hospital records. All children contacts underwent tuberculin skin test and

interferon gamma assay from 2 to 15 weeks after detection. Face to face interviews based on the questionnaire were conducted with mothers on the day of admission.

Results: A total of 128 tuberculosis cases and 208 children contacts in Ha Noi, Ha Tay and Thai Binh provinces were recruited into study. Children contacts were under 15 (of age), 38% of which was under 5.

The overall, agreement between TST positive (cut off point of 10 mm) and INF gamma assay positive was substantial (Kappa: 0.62; 95% CI: 0.45-0.75).

In multivariate analysis, using generalized estimating equation model:

Factors remained association with TST positive, namely *have low income (OR=3.526; 95% CI 1.598-7.779; p=0.002); have parent with tuberculosis (OR= 7.913; 95% CI 2.886-21.696; p<0.001); exposed to sputum smear grade of 3+ (OR=3.098; 95% CI 1.134-8.461; p=0.027); exposed to female with tuberculosis (OR= 0.309; 95% CI 0.126-0.758; p=0.01).*

Factors remained association with INF gamma assay positive was: *Duration of cough before treatment (OR=6.9; 95% CI 1.9-25.07; p =0.003), have low income (OR=6.142; 95% CI 2.055-18.358; p = 0.001); have parent with tuberculosis (OR= 4.458 95% CI 1.041-19.093; p=0.044) and exposed to sputum smear grade of 3+ (OR=11.313; 95%CI 1.972-64.903); p=0.006).*

Conclusion: In this study, we found that the proportion of TB infection among children contacts was similar with both two tests. The agreement between TST and INF gamma assay was substantial. The results from study suggested that the children contacts with AFB smear positive tuberculosis cases had high proportion of TB infection as determined by both two tests. Have low income; closed contacts with tuberculosis cases; having parents with tuberculosis; delay in tuberculosis diagnosis and the density of bacteria in sputum seem to contribute to the spread of *M. tuberculosis* infection. We recommend an awareness program to prevent TB infection among children contacts from tuberculosis cases in family. Contact investigation should be considered as part of National TB program.

Key words: Tuberculous infection, risk factors, epidemiology, interferon gamma assay, children under fifteen of age, rural areas, Vietnam.

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INTRODUCTION

Despite increased level of awareness and improved diagnostic facilities as well as medical technologies, tuberculosis still accounts for 2.5 million deaths worldwide every year and becomes global health problem (1). It is estimated that at least 180 million children under the age of 15 were infected and about 170.000 children died of tuberculosis each year (2;3). TB infection and disease among children are much more prevalent in developing countries, where resources for TB control are scarce, than in developed countries (1). However, despite the importance of the disease with the public health, TB is rarely investigated in children, as the diagnosis is difficult in the young age groups and children are usually not infectious. Additionally, contact following is rarely done in developing countries because of lacking resources.

To control Tuberculosis, Vietnam particularly emphasized on DOTS (Directly Observed Therapy Sort Course) as well as Expanded Program for Immunization (EPI) including BCG vaccination. According to the evaluation of WHO, Vietnam is one of the countries that achieved the successfulness in DOTS, detected 82% estimated number of new infectious cases for the period of 6 years from 1997 to 2002, high above the WHO target of 70% and cured more than 89% of cases exceeding the WHO target of 85% (4-7).

Despite these achievements, Vietnam still ranks 13th out of the 22 countries with the highest burden tuberculosis worldwide. In the Western Pacific Region, Vietnam is the third after China and Philippines (4). It is estimated that in 2007, there were 220 total TB cases per 100,000 people and 171 newly diagnosed cases per 100,000 people. It is estimated that about 95,000 people in Vietnam were diagnosed with tuberculosis in 2007, the largest number of people to be diagnosed in a single year since 2000. Cases of multi-drug resistant TB increased from 2.3% of recorded cases between 1996 and 1997 to 2.7% of recorded cases between 2005 and 2006 (8).

Facing with this situation, the targets of Vietnam National Tuberculosis Program are to reduce the number of TB cases and deaths through 2011 by focusing on preventing MDR-TB, improving the quality and accessibility to health care services, and implementing a collaborative strategy for public and private health care providers in 12 provinces and cities.

Tuberculosis infection among children can be used as a marker of recent ongoing transmission in the communities (4). Therefore information on prevalence of tuberculosis infection among children is important to evaluate tuberculosis transmission in communities. Having knowledge of the risk factors for tuberculosis infection in

children is important to evaluate the level of ongoing transmission of infection and to help adapt activities within national TB control programs. But to my knowledge, most studies conducted in Vietnam have not analyzed risk factors for tuberculosis transmission among children contacts as well as focused on investigating latent tuberculosis infection in contacts. On the other hand, those studies mostly focused on cases in term of tuberculosis treatment, MDR prevention and some of them concentrated on molecular epidemiology of pathogen. The aim of this study was to compare the whole blood interferon gamma assay with tuberculin skin test in diagnosing TB infection and to identify risk factors for TB infection among children of smear positive tuberculosis cases

CHAPTER 1
LITERATURE REVIEW

1.1. DEFINITION OF TUBERCULOSIS INFECTION:

TB is a chronic infection caused by a bacterial microorganism, the tubercle bacillus or *M.TB*. It is a potentially fatal contagious disease that can affect almost any part of the body but mainly involve the lungs.

Tuberculosis is defined as two – step process. The first step is acquisition of infection and the second is developing to disease. The definition of tuberculosis depends on the stage of infection and it can be divided into 2 forms: latent infection and active tuberculosis. Infected individuals are not ill and not infectious but may develop to active tuberculosis. Infectious individuals can transmit infection and require TB treatment for cure.

1.1.1 Latent infection: When a person inhales air that contains droplets, the smaller droplet nuclei may reach the small air sacs of the lung (the alveoli) and it is called initial infection. Approximately two to eight weeks after infected with M. tuberculosis, host immune system springs into action. Macrophages — specialized white blood cells that ingest harmful organisms — begin to surround and "wall off" the tuberculosis bacteria in the lung as a scab forming over a wound. If the macrophages are successful, the bacteria may remain within these walls for years — alive, but in a dormant state. In this case, infected person have positive on the tuberculin skin test but do not develop symptoms or physical evidence of active disease, their x-rays remain negative. It is called latent infection. Latent infected person are not contagious; however, they do form a pool of infected patients who may get sick at a later date and then transmit tuberculosis to the others.

1.1.2. Active tuberculosis: In case of host immune system fail in fighting with bacteria, *Mycobacterium tuberculosis* actually begin to multiply, exploit macrophages for their own survival causing primary disease. In the other cases, if the immune system become weakens, fail in keeping *M. tuberculosis* under control, the bacteria multiply inside the granulomas, which eventually may enlarge into noncancerous tumor-like nodules. The centers of these nodules have the consistency of soft, crumbly cheese. Over time, the centers can liquefy and break through the granulomatous wall surrounding them, spilling bacteria into your lungs' airways and causing large air spaces (cavities) to form (active TB). The bacteria may then spread from the cavities to the rest of the lung as well as to other parts of body and cause active tuberculosis. According the investigation of WHO,

around 5% of infected persons get sick within 12-24 months of being infected. Another 5% heal initially but, after years or lifetime, develop active tuberculosis either in the lungs or elsewhere in the body. This form of active tuberculosis is called post-primary disease.

1.2. TRANSMISSION ROUTES:

In natural circumstances, *Mycobacterium tuberculosis* is transmitted by expulsion of exhaled droplets from an active form of tuberculosis to an uninfected one. Air-born droplets generated when tuberculosis patients cough, sneeze, speak, sing etc. These droplets that contained bacilli are able to penetrate to the alveoli of the respiratory tract of the uninfected individual causing infection (9;10). Although tuberculosis is contagious, it's not especially easy to catch. In general, it needs a prolonged exposure to an infected person to become infected. Thus the risk of becoming infected is largely exogenous in nature, determined by the characteristics of the source case, environment, number and duration of exposure, virulence of *M. tuberculosis* and also the immune system of individual (11-15) while, the risk of developing TB given that infection has occurred, is largely endogenous, determined by the integrity of the cellular immune system. Sometimes active TB can develop years after the initial infection. This occurs when the immune system can't keep dormant TB bacteria under control, and the walled-off germs become active. Overall, 10% of infected people will develop clinical TB sometime during their life, haft of them during the first 2 years following infection (16;17).

The etiology for progression from TB infection to disease is not well understood. However, risk factors for the progression to infectious tuberculosis have been indentified:

- Aging
- Drug or alcohol abuse
- Malnutrition
- Medical conditions: chemotherapy, prolonged use of prescription medications such as corticosteroids
- Infection with the human immunodeficiency virus (HIV) and IDU.

1.3. RISK FACTORS FOR TUBERCULOSIS INFECTION

1.3.1 Geographic factors: As tuberculosis is spread by respiratory droplets, concentration of airborne bacilli and duration of exposure to active TB cases are

considered as two key factors in transmission of tuberculosis infection. Many studies have established that the TB infection prevalence is higher in household contacts compare to the general population (15;16;18) The prevalence is highest for those who are sharing activities and room air with sputum smear positive cases (14-16;18-20). Therefore, proximity and persistence of contacts are major determinants of risk of Mycobacterium tuberculosis transmission. Moreover, investigation on TB prevalence among household contacts also indicated that children especially infants are at both increase risk of latent infection and active tuberculosis(14).

1.3.2 Socio-economic factors: Some studies have shown that the socio-economic factors, such as poor housing, crowded conditions, poorly ventilated spaces, low income, lack of access to medical care, lack of knowledge of TB prevention are associated with tuberculous infection (14;21-25). In on way, poverty can be understood as roof cause of tuberculosis. Reported from WHO, 2002 cited that "*While TB is not exclusively a disease of the poor, the association between poverty and TB is well established and widespread"*.

1.3.3 Malnutrition: malnutrition impacts on cell-mediated immunity which is the principal host defence against tuberculosis. Thus it is an important risk factor for the infection and development of tuberculosis. In addition, some observations on risk factors for tuberculosis infection show that there is no significant difference in prevalence of positive tuberculin skin test among malnourished compared to normal children (24;26;27). However this results should be re-considered in term of the depressed effect of severe malnutrition on the hypersensitivity response to tuberculin.

1.3.4 Immunodeficiency: Some studies indicated that in HIV-infected persons tuberculosis most often results from the reactivation of latent TB infection (26;28;29) but there is no strong evidence that HIV-seropositive persons are more likely to acquire tuberculous infection than HIV-seronegative individuals, given the same degree of exposure (26;30). However, once infection does occur, the risk of developing disease is much greater among persons with HIV infection, because HIV impairs the host's ability to contain new tuberculous infection. Thus immunodeficiency is not a only direct risk for TB infection but also a risk factor for progression to active tuberculosis.

***1.3.5 Pathogen related factors*:** People with high bacterial density in sputum, untreated TB, including MDR-TB are highly contagious and can transmit this serious type of TB to others. Moreover, the changing in genotype can lead to increasing virulence making bacteria more sensibilities to be transmission and become dominant strains (11).

1.3.6 Genetic factors: The questions that "why all the infected individuals do not acquire active disease?" had been investigated for many years. The effect of genetic factors on TB reactivation has been described by detecting of mutation in interferon gamma receptor-1 gene (INF-ϒ-R1) in child suffering from BCG infection (31). The hyper-susceptibility to mycobacterial infection related to genetic factors has been described in many studies (32;33). It was demonstrated that the mutation at locus 395 of interferon gamma receptor 1 gene leading to dysfunction of the protein in the cell membrane. This makes the individual more susceptible to mycobacterial infection (34).

1.4. METHOD OF DIAGNOSIS LATENT TUBERCULOSIS INFECTION.

Tuberculosis in children is an indicator for ongoing transmission in the community and is directly related to the incidence of adult. However, the accurate figure for TB in children in the world is not readily available. This is because of the difficulties in diagnosing childhood tuberculosis, inadequate health care systems in developing countries and the lack of interest in childhood tuberculosis by national tuberculosis program.

Diagnosis of latent tuberculosis infection in children is traditionally based on tuberculin skin test but it has many drawbacks concerning specificities. Newer *in vitro* diagnostic methods are immune-based have been used increasingly although they are not widely available especially in developing countries.

1.4.1 The tuberculin skin test (TST):

A positive tuberculin skin test reaction is considered as an indicator for primary infection with *M. tuberculosis* in children. In most children tuberculin reactivity becomes apparent in 3-6 weeks, but in same cases it can take up to 3 months after initial infection. It was also reported that up to 20% of patient with tuberculosis may not react to TST (35). The rate of false negative TST in children with tuberculosis and also HIV infected, is unknown but it is certainly higher than 10% (36;37).

The antigens that are used for TST, purified protein derivative (PPD), is a mixture of many mycobacterial antigens, some of which are shared among pathogenic mycobacteria belonging *M. tuberculosis* complex (*M. tuberculosis*, *M. bovis* and *M. africanum*), environmental nontuberculous mycobacteria (NTM) and the vaccine subtrain *M. bovis* Bacille Calmette-Guerin (BCG) vaccine strains (33). Thus, it is impossible to distinguish between a tuberculin reaction that is caused by tuberculosis infection and one caused by BCG vaccination or NTM exposure. Tuberculin skin test was based on measuring the delayed type hypersensitivity (DTH) response to intradermic inoculation of PPD. If

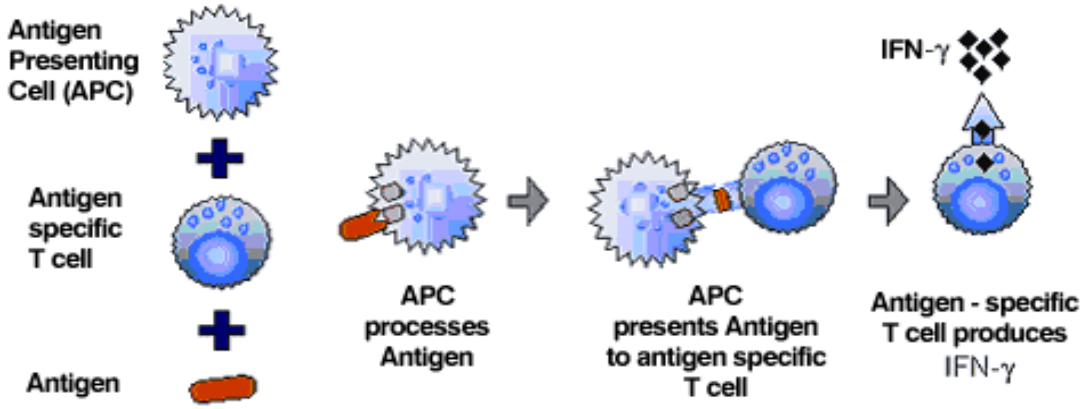
individual has been infected with tuberculosis, swelling and induration at the site of injection will occur after 48-72 hours. This process is described in (39) that "*Approximately 4 hours after injection of antigen, neutrophils rapidly accumulate around the post-capillary venules at the injection site. The neutrophil infiltrate rapidly subsides and by about 12 hours the injection site becomes infiltrated with T cells and blood monocytes and some basophils, also organized in perivenular distribution. The endothelial cells lining these venules swell, show increased biosynthetic organelles and become leaky to plasma macromolecules. Fibrinogen escapes from the blood vessels into the surrounding tissues, where it is converted into fibrin. The deposition of fibrin and to a lesser extent accumulation of T cells and monocytes within the extravascular tissue space around the injection site cause the tissue to swell and become indurated*". Thus, TST reaction is absolutely depended on the present of memory T cells.

1.4.2 Whole-Blood Interferon-Gamma Assay:

Because of many drawbacks of tuberculin skin test that mentioned above, in-vitro T-cell based assays that measure interferon-gamma (IFN-γ) production have been developed for the diagnosis of latent tuberculosis infection. They are the QuantiFERON®-TB Gold In-Tube (Cellestis Limited, Carnegie, Victoria, Australia) and the T-SPOT.*TB*® (Oxford Immunotec, Oxford, UK) assays.

The antigens that used in these tests are a mix of antigen target 6 (ESAT-6) and culture filtrate protein 10-(CFP-10). Genes encoding ESAT 6 and CFP 10 are located in RD1 region which is deleted from BCG trains but presented in *M. tuberculosis* (40;41). Southern blotting of genomic DNA has demonstrated that both ESAT 6 and CFP 10 are present in *M. tuberculosis, M. africanum*, virulence *M. bovis* whereas these two genes could not be demonstrated in any BCG vaccination strains and in NTM, with a few exceptions (*M.kansassi, M. szulgai, M. marinum*). Thus, theoretical, they are highly specific indicators for *M. tuberculosis* infection (42;43). It was also found that interferon gamma assay had been more specific than tuberculin skin test and could be use in diagnosis TB infection (44-47).

Figure1.1. Mechanism of whole blood interferon gamma assay



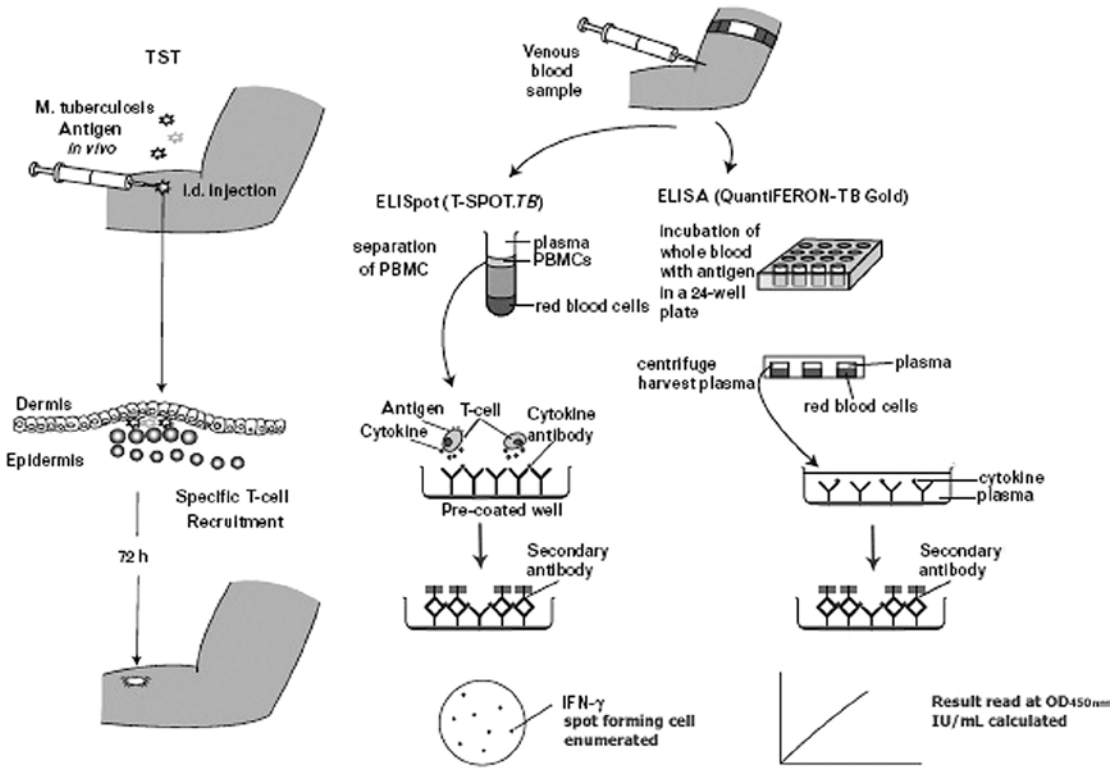
Down load from:

http://www.stanford.edu/class/humbio103/ParaSites2006/TB_Diagnosis/quantiferon

If individuals are infected with *M.TB* they will have specific memory T cells that can recognize mycobacterial antigen in the second infection. This recognition process release interferon gamma, a specific cytokine for cell mediated immune response. The whole blood IFN-γ assays were developed by measuring concentration of IFN-γ.

The diagrammatic representation of TST and whole blood IFN-γ assays are shown in finger 1.2 as bellow:

Figure 1.2: Diagrammatic representation of TST and whole blood IFN-γ assay



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1.5. THE GLOBAL BURDEN OF TUBERCULOSIS

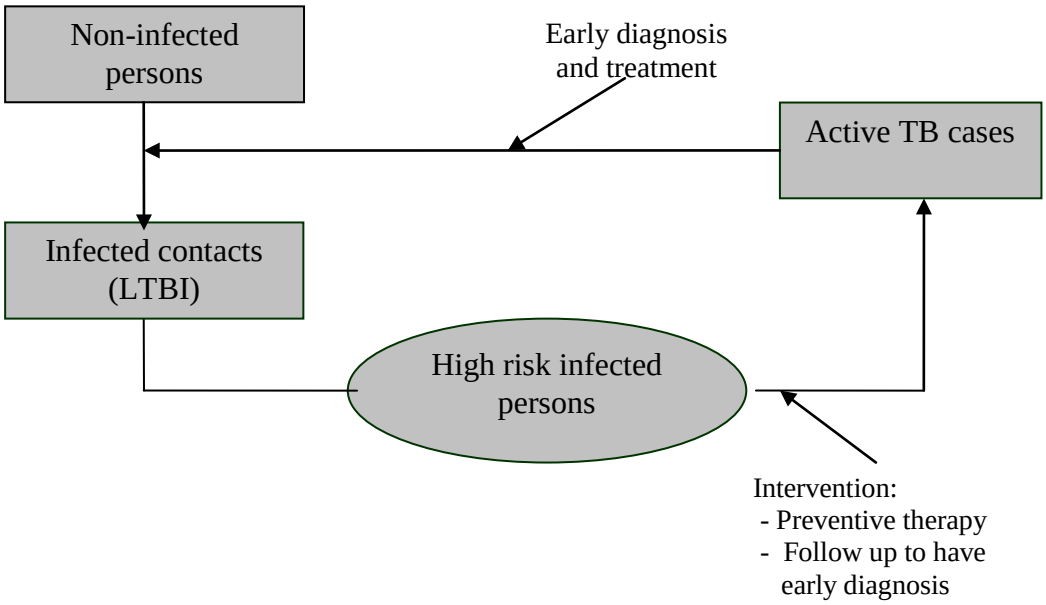
Tuberculosis is global problem, but is especially prevalent in developing countries in condition of poverty, overcrowding, inadequate sanitation, malnutrition and lack of basic health services. More than 90% of TB cases and 98% of TB death occur in the developing countries. The annual risk of TB infection in Sub-Saharan Africa is more than 50 times the rate in Western Europe (49) . WHO stated that between 2000 to 2010 nearly one billion people will be newly infected with *M. tuberculosis* and 200 million people will develop diseases in coming years (49). It was also reported that approximately nine million new cases of tuberculosis were detected worldwide causing 1.78 million death from TB in 2007 (50). It is well known that tuberculosis is one of leading infectious killers of young and middle aged adults in developing countries. Children are particularly vulnerable to TB infection because of frequent family contact, especially when their parent are infectious. It is estimated that at least 180 million children under 15 of age were infected with *M. tuberculosis*, over 250,000 children develop TB and 100,000 children will continue die each year from TB (2).

1.6. PREVENTION AND CONTROL OF TUBERCULOSIS

There are clear evidences that tuberculosis can transmitted from infectious persons to the others through the air. Each individual with active tuberculosis but untreated can infect 10-15 other people per year (49). It was also reported in recent studies that people who with prolonged, frequent, or intense contact with TB cases are at particular high risk of being infected (18;19;24;25;51). Thus, control tuberculosis can be reached by breaking the chain of transmission (identifying and treating infectious disease), preventing infection (BCG vaccination) and preventing disease among infected high risk individuals (preventive therapy) (50).

According the guideline of WHO, 2009 strategies for global tuberculosis control and prevention included: Directly observed therapy (DOTs) implementation, diagnosis and treatment of MDR-TB, collaborate TB/HIV activities, address the needs of poor and vulnerable population, strengthen health care system based on primary health care, engage all care providers, empower people with tuberculosis and communities, and promote research (50). Besides, investigate TB contacts, follow up and give preventive therapy for those who are being infected were also recommended in many countries (51)

Figure1.3: Breaking the cycle of infection:



1.7. COUNTRY PROFILE

1.7.1. Background

Vietnam is located in South-East Asia, possessing nearly 3,400 kilometers of coastline and long internal borders with Hanoi as the capital city. The country has an area of 329,560 square kilometers, the population as of 2007 was 85.195.000 and 73 % of the population lives in rural areas (GSO 2007). The population grow rate for Vietnam is 1.30%. The number of people aging 0-14 years accounts for about 29.4 % of the population, while the proportion of people 5-65 years and over 65 years of age are 65 % and 5.6 %, respectively. Life expectancy of total population is 70.35 years (male 67.86 years and female 73.02 years). The infant mortality rate is 29.88 deaths/1,000 live births *(2004 estimation)* (52).

Ethnically, Vietnam is home of 54 ethnic groups such as Kinh, Tay, Nung, Chinese, Hmong, Thai, etc. Among them the Kinh ethnic group is the majority, form 86% of the population and reside in the lowlands and cities. Geographically, the country of Vietnam can be seen in three different parts: the North, the South, the centre and highland with the total of 61 provinces, 4 centrally administered cities, 631 districts, 10,553 communes and 104,146 villages (54) .

Figure 1.4: ***The map of Vietnam (53)***

Although the country is located in the tropical region, the climate is tropical only in central and southern of Vietnam, with warm and humid weather all year round (22-35^{o}C). There are two seasons in the south: the rainy and the dry season. In the north, there are four distinct seasons: spring, summer, autumn and winter. Vietnam has a tremendous topographical diversity with mountainous, midland and low land areas.

Vietnam is one of the fastest growing economies in the world. The GDP growth rate registered at around 7 % per year in the last decade. Nevertheless, Vietnam is still a poor country struggling to recover from the ravages of war and the rigidities of a centrally-planned economy. The GDP per capital was about US$ 550 in 2004 (55)

1.7.2. Health care system in Vietnam

The health care network of Vietnam has been established from central to local areas. Ministry of Health is assigned to organize and manage health care services all over the country. At local levels, provincial departments of health, district medical centres and commune medical stations are responsible for organizing, managing and providing

health care services to the population in their areas. The structure of health care system can be summarized as follows:

- ***National level***: Ministry of Health (MOH); Medical Colleges; National Research Institutes; national hospitals.
- ***Provincial level***: Department of Health; Provincial hospital; provincial medical schools; specialized medical centres (such as preventive medicine centre, centre for tuberculosis control, etc).
- ***District level***: district centre of health (including district hospital, teams of hygiene and epidemiology), local general clinics.
- ***Commune level***: commune medical station, village health workers; volunteers.

In recent years, Vietnam's health sector strategies have emphasized on active prevention, public service delivery at the "grass roots" level, the expansion of health insurance coverage, the value of traditional medicines, and the active participation of the private sector under the government's leadership (56).

For spending on health-care, Vietnam has achieved remarkable results for a country that has limited public resources and GDP per capita. Although spending about 5-6 percent GDP on health care (both public and private expenditure), Vietnam has continued to make impressive progress in reducing maternal mortality and morbidity as well as infant mortality and under-five mortality rates. Vaccine-preventable diseases, such as measles, diphtheria and tetanus; polio were completely eradicated in 1996 (57).

However, Vietnam's health sector has some problems. Government budget for health is low (3$ per capita in 2000, lower than China and the Philippines). The introduction of user fees in health facilities and the emergence of private practitioners and drug sellers lead to a very high private spending on health, (80% of total health spending), which is mainly concentrated on pharmaceuticals. As the social health insurance covers only 21% of population, there is a considerable gap between rich and poor in term of access to services. Moreover, a strong legal framework has not yet been in place for the private sector leading to a very irrational use of resources, particularly in the pharmaceutical sector with a large expenditure on unnecessary and sometimes useless drugs. Finally, there is little coordination between different programs, despite the fact that they often have the same target population (as in the case of tuberculosis and HIV/AIDS) and there is also no mechanism to ensure that these programs are not discontinued before their objectives are achieved.

1.7.3. System of tuberculosis control program in Vietnam.

Established from 1985, National Tuberculosis Program (NTP) in Vietnam is integrated within the structure of primary health care system. There are four levels of TB control activities:

- ***The centre level***: Located in Hanoi, the National Hospital Tuberculosis and Lung Diseases are responsibilities for the direction and management of TB control activities for the whole country. The hospital also has responsible for supporting Ministry of Health (MOH) in developing strategies for TB prevention and control, and in handling management and professional guideline for the system.
- ***The provincial level***: Provincial TB centre has responsibilities for diagnosis, treatment and managing TB patients, implementation of TB policies issued by NTP, supporting the district and commune levels, monitoring and evaluation local program activities.
- ***The district level***: District centre has responsibilities for detecting and treating TB cases, implementing and monitoring the NTP, supervision and management of TB program in commune centre.
- ***The commune level***: Each commune centre has a nurse or assistant physicians who have responsibilities in detecting suspected cases, providing treatment under the control of district level. They also manage the village health workers in identifying suspected patients, conducting counseling for examination and tests as well as paying home visit to patients undergoing treatment.

Concerning to laboratory of diagnosis tuberculosis, there are two reference laboratories (one from Hanoi and one from Ho Chi Minh city) which are responsible for controlling quality for more than 600 district TB laboratories (58).

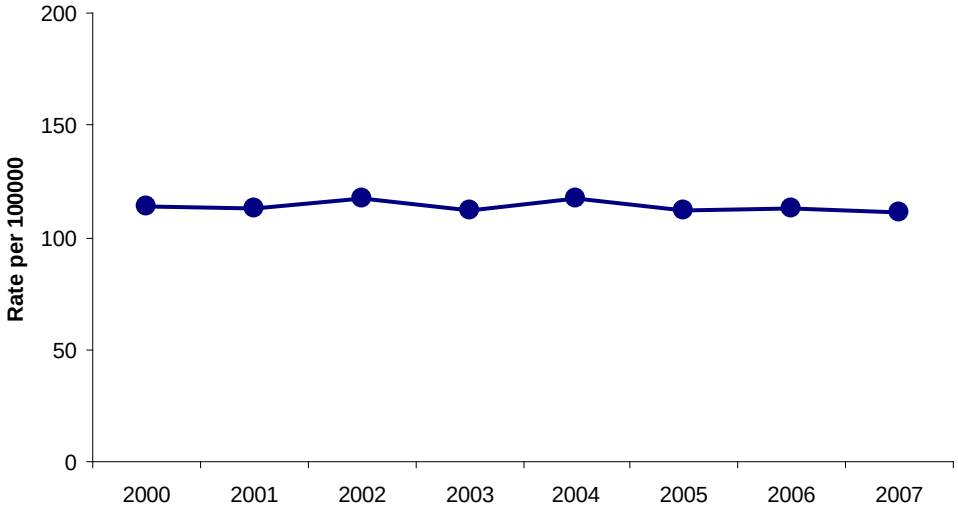
1.7.4. Tuberculosis in Vietnam

The National Tuberculosis Control Program in Vietnam is based on the principles of the DOTs strategy recommended by WHO. According the evaluation of WHO, Vietnam is only among high burden countries has reached and exceeded the targets for 70% case detection and 85% treatment success since 1997 (5;7).

While the TB control program in Vietnam has gained some achievements, it finds itself facing new challenges. Vietnam still ranks 13th on the list of 22 countries with the highest tuberculosis burden in the world (50). Data from the drug resistance survey carried out in 2002 show that the multidrug - resistant tuberculosis (MDR-TB) in Vietnam was slightly increased from 2.3% in 1996 (61) to 3.0% in 2002 (49). The other major challenge is the rise of TB and HIV co-infection. According to surveillance in

2002, the prevalence of HIV infection in TB patients has increased from 0.5% (1994) to 9.3% (2002) and in some provinces, the rate is as high as 14 percent ((54;59). Additionally, there is no evidence of decrease in any TB reports in Vietnam (figure 1.5).

Figure 1.5: TB notification rates (new and relapse cases/100,000 population) in Vietnam, 2000-2007



Source: *WHO Report 2009, Global Tuberculosis Control*.

As seen in fig 1.5, TB notification rate in Vietnam was unchanged since 2000 (114/100,000 population in 2000 and 111 in 2007).

Tuberculosis prevalence surveys in Vietnam also indicated that the tuberculosis incidence rate varies around the country. It is estimated that the level of transmission in the south is twice as high as in the north (58;60).

1.8. JUSTIFICATION OF THE STUDY

The goal of tuberculosis control program is to eliminate the disease by breaking the chain of transmission. Thus to effectively prevent tuberculosis, after detecting a cases, it is imperative that the possible risk factors for tuberculosis infection among contacts should be identified so that the chain of transmission can be broken. In the world, many studies have been conducted towards describing the epidemiology and risk factors associated with tuberculosis infection among children contacts to have an overview of recent ongoing tuberculosis transmission in communities. However, TB prevention program in Vietnam has mostly focused on detecting and treating index cases rather than identifying the risk factors for TB transmission. Additionally, from my knowledge tuberculin skin test is still considered as the standard method for investigation of TB

infection in Vietnam. False positive from TST may lead to unnecessary anxiety among population as well as over estimated annual risk TB infection rates in community.

My study was undertaken with the aim of finding the best toll for diagnosing tuberculosis infection among child contacts by comparing INF gamma assay with tuberculin skin test, and identifying possible risk factors for TB transmission.

CHAPTER 2

RESEARCH QUESTION, HYPOTHESIS AND OBJECTIVES

2.1. RESEARCH QUESTION:

What is the best tool for diagnosis of tuberculous infection in Vietnam?

What are the potential risk factors for tuberculous infection in household contacts among children under 15?

2.2. HYPOTHESIS:

We hypothesize that:

- Host, pathogen, and environment together were potential risk factors for TB infection in this population.
- Interferon gamma assay is more specific than tuberculin skin test in diagnosis tuberculous infection.

2.3. OBJECTIVES OF STUDY:

General objective:

- To evaluate TB transmission in the community contributing for TB control activities in Vietnam.

Specific objectives:

- To identify the better tools for diagnosing tuberculous infection in Vietnam.
- To estimate the proportion of tuberculous infection in child contacts.
- To identify risk factors associated with TB infection among children who has close contact with index cases.

CHAPTER 3

METHODS AND MATERIALS

3.1. STUDY SITE

The current study was carried out in three provinces in the north of Vietnam: Ha Noi, Ha Tay and Thai Binh.

Ha Noi has an area of 922.8 square kilometers and the population of 3,398,889 million people (2007). GDP per capital is approximately 620 USD (2005). Like many areas in northern Vietnam, Ha Noi has a hot and rainy season (from May to September), and a cold season (from October to April). The average temperature is 23^0 C. The average rainfall is 1,500 to 2,000 mm. The humidity ranges around 80%.

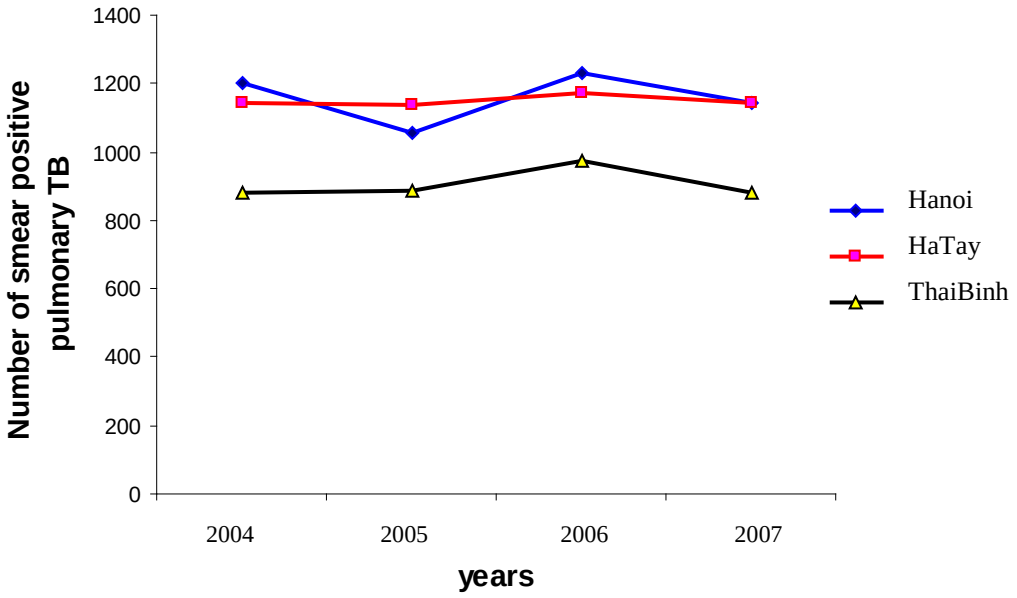
Ha Tay province lies to the southwest of the entrance to the capital city of Ha Noi. It is located in the Red River Delta surrounded by Ha Noi, Hung Yen, Ha Nam, Hoa Binh and Phu Tho provinces. The tropical monsoon climate is divided into three microclimates according to landscape: the plain zone, with a hot and humid climate affected by marine winds; the hilly zone, with a continental climate affected by western winds; and the Ba Vi mountainous zone, with a cool climate and an average temperature of 18°C.

Thai Binh is an eastern coastal province in the Red River Delta region of Vietnam, it is about 110 km from Ha Noi. Thai Binh has the population of 1,827,000 (2002), including 94.2 % countryside and 5.8% city residence. The climate is the same as in Hanoi city. The economic growth is rapid, in 2004 export turnover estimated 78 million USD (increased 22%) while import turnover 57 million USD (increased 20.3%).

From National Tuberculosis Program (NTP), 1057 new sputum smear positive TB cases were reported in 2005 in Hanoi (33.5 per 100,000 population), 38/100,000 population in 2006 and 35.4 in 2007.

Figure 3.1 showed the number of case detection from 2004 to 2007 in Hanoi, Hatay and Thai Binh provinces. This indicated that the incidence of TB was not sufficiently reduced in those provinces despite high DOTS coverage and successful achievement of NTP in Vietnam (61).

Figure 3.1 : Case-detection per province per year



Source: *Vietnam National Tuberculosis Control Programme (NTP), unpublished data*

Ha Noi, Ha Tay and Thai Binh provinces were chosen for this study because they have specific characteristics of an urban as well as rural provinces where existing distribution of population, incidence of TB, health care provider remain problem. In addition, no similar research, identifying the potential risk factors for TB infection among children who has close contact with TB cases, had been carried out in these cities before.

3.2. STUDY DESIGN*: Cross sectional study*

Epidemiology is concerned with the distributions and determinants of disease frequency in human populations. The basic design strategies used in epidemiological research can be broadly categorized according to whether such investigations focus on describing the distributions of disease or elucidating its determinant (62). In the epidemiological approach to investigate associations between a disease and possible risk factors, cross-sectional, case-control and cohort designs can be employed (62-64).

Cohort study: Cohort study is a study in which a group of individuals who exposed to the agent under investigation (index subjects) are followed over a period of time and compared with another group who not exposed to the agent under investigation (control subjects) . Thus, in cohort study, groups of individuals are defined on the basis of presence or absence of exposure to a suspected risk factor for a disease. The occurrence of disease in exposed group of individuals will be compared with its occurrence in non-exposed group after of a period time of following up. In cohort studies, the measure of

disease is the incidence rate which is the proportion of individuals who develop the disease within the time of following. The measure of association between exposure and disease is the relative rate which is the ratio of the incidence rate of index subjects to that of control subjects. A principle advantage of general cohort study is that it can provide a picture of range of health and disease outcome. Additionally, cohort studies also provide a clear temporal sequence of exposure and disease because participants of this study were free from the disease under investigation at the time that exposure status is identified. Moreover, since exposure and outcomes are being continuously assessed within the period time of following, recall bias is minimized.

Beside the advantages that were mentioned above, cohort studies also have some disadvantages. In cohort studies, since subjects are required to follow up over time, usually for many years, loss of information may be occurred that result in producing bias (62;64). Besides, the expenditure of carrying out cohort studies is high because it requires a large number of subjects with a long time of following up.

Looking at our study, time for developing tuberculosis is flexible depending on immunology status of each individual and range from 2 years to life time, thus with the limited time and funding, cohort study design was not suitable for this study.

Case-control study: In case-control studies, subjects are selected on the basis of whether they do or do not have a particular disease under study. Risk factors of group of exposure or diseases (cases) then are compared with group of non-exposure or without disease (control). The outcome of a case-control study is an estimate of the associated risk factors which is measured by the odds ratio (62-64).

By this design, case-control studies have many advantages for identifying the association between exposure and disease. They allow estimating a wide range of potential exposures. Since case control studies start with individuals known to have outcome rather than starting with population free of disease and having to wait for many years to identify who will develop disease, it is possible to select sufficient number of patient with rare disease in a short time. Moreover, case control studies are often retrospective because it starts with an outcome and then traces back to investigate exposure. Thus, case control studies can be conducted more quickly, less expensive and easier to carry out than cohort studies.

However, case control studies also have some limitations including the uncertainty of the exposure – disease time relationship and the inability to provide a direct calculation of the incidence of disease in exposed and non-exposed groups. The greatest limitation of

case-control studies is that they are more susceptible to bias than other analytic studies. (62). Selection bias occurs if the relationship between the exposure and disease observed among those who participate in the study is different from that for individuals who would have been eligible to participate but was unwilling or not selected by the investigator. Similarly, if alternate controls are selected to replace those who initially chosen but could not be contacted or refused to participate, biased estimates could also result. Another important bias related to case control studies is recall bias which occurs when individuals in case group who have experienced a disease tend to think about the possible causes of their illness, and thus they are likely to remember their exposure histories differently from those who are unaffected by the disease.

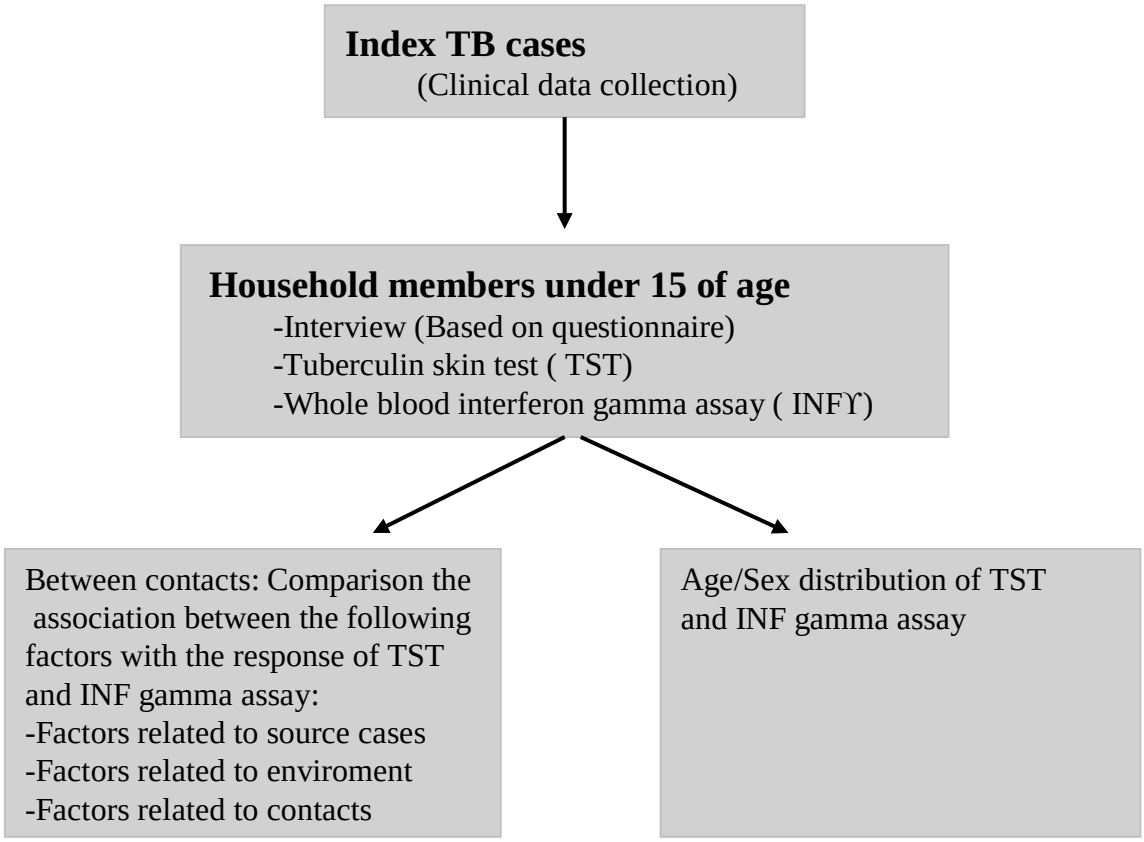
Because of all limitations that mentioned above, case control study was not chosen for presented study.

Cross-sectional study*:* is a type of observational descriptive investigation, in which exposure and disease statuses are assessed simultaneously among individuals in a well-defined population and at one point of time. Thus, cross-sectional studies provide information on the prevalence and characteristics of a disease or other health outcomes of the population at a specified time. Cross-sectional studies establish association, not causality because exposure and disease histories are taken at the same time (62). The most important advantage of cross-sectional studies is that it is inexpensive and easy to conduct.

However, cross-sectional studies have disadvantages. First, since these studies consider prevalent rather than incident cases, it will be difficult to distinguish factors related to risk of disease among factors related survival. The second major limitation applies to the ability to study disease of low rate (62)

Our objectives were to estimate the proportion of latent tuberculosis infection among children contacts and determine the risk factors associated to tuberculosis infection. Thus we decided to adopt a cross sectional study design based on recruitment of tuberculosis infectious cases and their child contacts. The general design of the study is showed as bellow:

Figure 3.2: The general design of the study



3.3. STUDY POPULATION.

Target population included:

- Newly detected positive pulmonary tuberculosis cases that had children less than 15 years of age living with at least three months.
- Children less than 15 years of age who lived in the same house with tuberculosis cases.

Household of each tuberculosis infectious individual were visited from 2 to 15 weeks after diagnosis of index cases to collect all information related to index cases and childhood contacts. Since the children were too young to be interviewed, their mother instead was interviewed to identify risk factors associated with TB infection. Children contacts were invited to Health Centre to take interferon gamma assay and tuberculin skin test.

3.4. SAMPLE SELECTION

3.4.1. Sample size:

The number of children was calculated based on the precision of the proportion with the following formula (65):

SE(p) = $\sqrt{p(1-p)/n}$

n: the required sample size

p: Proportion of TB infection in contacts from previous study

d: The length of 95% confidence interval of the proportion

From a previous study (61), we estimated that the proportion of tuberculous infection in this population is around 25%. The precision of the proportion was estimated as ± 0.06. Then the required sample size (n) is calculated as bellow:

1.96 * SE(p) =1.96 * $\sqrt{p(1-p)/n}$

$d = 1.96 * \sqrt{p(1-p)/n}$

$d^2 = (1.96)^2 * p(1-p)/n$

$n = \frac{(1.96)^2}{d^2} * p(1-p)$

$n = \frac{(1.96)^2 0.25(1-0.25)}{(0.06)^2}$

$n \approx 200$

Based on the number of children contacts needed, we calculated the number of index cases:

$$\text{Number of index case } (N) = \frac{\text{Number of needed children contacts}}{\text{Number of estimated children per each family}}$$

$$N = \frac{200}{2}$$

$$N = 100$$

-> Totally 100 TB cases and 200 their children contacts should be collected.

3.4.2 Sampling technique

Sampling method is classified either probability or non-probability. In non-probability sampling, members are selected in nonrandom manner. These included convenience sampling, judgment sampling, quota sampling and snowball sampling. The most important disadvantage of convenience sampling is they may not representative for the tuberculosis population. But since it is inexpensive and easy to conduct in a limited time, we decided to use convenience sampling method to select tuberculosis cases. By this way, all pulmonary tuberculosis cases who had children under 15 of age admitted Provincial Tuberculosis and Respiratory Diseases Hospital were selected. All identified children contacts under 15 of age were subsequently investigated. The period time for

selecting subjects was done from 15th July to 29th January 2009. With the period of seven months, we recruited 110 index cases and 180 children contacts into the study. Eighteen tuberculosis cases and 28 of their children contacts that were collected at the end of 2007 also were recruited in this study. They were selected because of meeting all criteria of this study.

Selection of index cases:

- An index case was defined as a newly pulmonary tuberculosis case who had at least one contacts less than 15 years of age living with at the same house for >3 months. Pulmonary tuberculosis was confirmed by 3 consecutive sputum smears positive for acid-fast bacilli and/or positive culture. Informed consent was obtained before enrollment.

Selection of contacts:

- Children contacts were defined as a family members or living with the index case in the same house more than 3 months before the starting of tuberculosis treatment of index case. They were recruited into study, and consent was obtained from the parent/representatives to undertake the study.

3.5. DATA COLLECTION

Pre-testing: Pre-testing or pilot study serves as a trial run at the beginning of a research project that allows us to identify potential problems in the proposed study. However, pre-testing is simpler and less time-consuming and costly than conducting an entire pilot study. Therefore pre-testing was used in this study.

The pre-testing was conducted on 5 smear-positive pulmonary TB cases admitted to the hospital and their children contacts. These people were not be recruited into the study after the selection of subjects process. The pre-testing was to check if they understood the questions to avoid information distortion.

After conducting pre-testing questionnaire, some changes were made. Most of the index cases were farmers who are self-reliant in supporting their own demand. Thus they could not calculate exactly their income per month. Therefore we added more question regarding their family facilities for additional analysis. The results then were checked by community leaders to classify poor and non-poor household

To have more clear information about the previous expose to cases in the family, we added one question to find out if there is one more family members had tuberculosis or not. These results then were checked based on the records of NTP.

Research assistant training: Four research assistants were recruited for the study, one from Department of Immunology and Molecular Biology - NIHE, one from Hanoi Tuberculosis and Pulmonary Hospital, one from Hatay tuberculosis controlling program and one from Thai Binh tuberculosis hospital. The questions and their meanings were thoroughly explained to the assistants. They were then instructed how to ask the questions and how to exactly report what the respondent answered. The assistants practiced together to ensure a standardized way of collecting information.

In the process of collecting data, the principal researcher and the assistants checked data qualify after each field day of data collection. Corrections were made as necessary and possible

Laboratory technicians training: Training of laboratory technicians was held in Hanoi Tuberculosis and Pulmonary Hospital, Thai Binh Tuberculosis and Pulmonary Hospital. This ensured that stool sample collection and storage were complied with a standard protocol which has been applied in Laboratory. At least 6 hours since being collected, blood samples were transported to Bio-molecular and Immunology Department-NIHE for the INF-ϒ test.

Questionnaire: The questionnaire was developed in English and translated into Vietnamese language (See attached file). It was a pre-tested questionnaire and had both closed and open-ended questions. The questionnaire had three sections: a section on general information of households including household size, house structure, hygiene, presence of animals, economic status, a section on clinical data of index cases and a section on demographic information of contacts including duration of residence in the compound, related to index case, exposure to the index case, medical history, BCG scar assessment.

Interview: Face to face interview based on questionnaire was conducted on hospitalized patients. Their households were invited into community health care centre and a face to face interview was taken on mother or caregiver of child to investigate the presence of risk factors and assess the degree of exposure to index case. BCG vaccination was assessed by physical examination for the presence or absence of BCG scar.

Laboratory method:

Acid-fast bacilli microscopy: AFB microscopy was taken in laboratory of Hanoi Tuberculosis and Pulmonary Hospital, Thai Binh Tuberculosis and Pulmonary Hospital, Chuong My (Ha Tay) Community health Centre according to recommendation in the

IUALTD and WHO guides and is used as a standard method of Vietnam National Tuberculosis Program (66).

Principle: Mycobacterium cell walls contain a waxy substance composed of mycolic acids which can present a barrier to dye entry as well as elusion. Thus cells of species of mycobacterium do not stain ready with ordinary dyes. However, cold carbon fuchsin for several hours or at high temperatures for five minutes will dye the cell. After stained, acid fast in mycobacterium cells will hold the stain fast in the presence of the acidic decolorizing agent and have red color.

Steps of protocol:

* *Sputum collection*: 3 sputum smears were obtained early morning after rising in the first 3 day of each adult hospitalized patient.

* *Acid fast bacilli staining*:

- Add Ziehl-Neelsen carbon fuchsin (0.3% fuchsin) to the slide for 10 minutes while applying heat.
- Follow with a gentle wash with water to cool the slide
- Decolorising by 20% sulfuric acid in water and alcohol 70% separately or acid alcohol (3% hydrochloric acid and 95 % ethyl alcohol).
- Wash the slide in water
- Counterstaining by methylene blue 0.25% in 1% acetic acid.
- Acid fast bacteria retain the red color, and therefore look hot pink or red. Non acid-fast bacteria will not be red.

* *Microscopic reading*: Recording and reporting was done by technician, the IUATLD/WHO scale was used as evaluation tool, in case the ordinary microscopy is graded as: negative (0); 1-9 AFB/100 fields (scanty); 10-99 AFB/100 fields (1+); up to 9 AFB/field (2+); 10 AFB/field (3+) (66).

Tuberculin Skin Test (TST): Tuberculin skin test was done by trained health care workers according to International guidelines (67;68). Results were reading after 72 hours of injection in health care centers. Home visit was done for participants who did not return for TST reading

Principle: Purified protein derivative (PPD) from Institute of Paster Nha Trang, Vietnam (Registered number: VNDP - 194-0604), a solution of protein derivative of *M.TB* is injected into inner surface of the forearm. Then the delayed-type hypersensitivity response to the PPD is measured at from 48 to 72 hours after injection.

Steps of protocol:

- 0.1 ml of PPD containing 5 tuberculin units is injected into the inner surface of the forearm, normally in an area free of lesion and away from veins.
- 72 hours after injection, the reaction is read based on the area of duration around the site of injection.
- TB infection is defined as positive skin test: ≥ 10 mm is regarded as positive

Interferon gamma assay (IFN-γ **assay)**: QuantiFERON®-TB Gold In-Tube (Cellestis Limited, Carnegie, Victoria, Australia)

Principle: The test is based on the release of interferon gamma by sensitized lymphocytes when exposed to antigen of mycobacterium tuberculosis (69).

Steps of protocol:

Blood collection:

- Mix QuatiFeron TB gold tubes with 1 ml of blood sample by shaking vigorously 5 seconds
- Incubate tubes at 37 ^{0}C for 16 to 24 hours
- Centrifuge tubes at 2000 to 3000 G for 15 minutes
- Harvest at least 200µl of plasma each tube and store at -20^{0}C within 1 month for continuing step

ELISA assay:

- Add 50 µl of working conjugate to each well, then add 50 µl of plasma or standard.
- Shake covered plate for 1 minute, then incubate plate for 120 minutes at room temperature.
- Wash plate 6 times with washing buffer. Add 100 µl substrate, incubate 30 minutes at room temperature.
- Add 50 µl stop solution. Read absorbance at 450 nm (620 ref)
- IFN-γ values (IU/ml) for TB-specific antigens were corrected for background by subtracting the value obtained for the respective negative control. As recommended by the manufacturer, the cut-off value for a positive test was IFN-γ ≥ 0.35 IU/ml

3.6. VARIABLES AND DEFINITIONS USED IN THIS STUDY

Two type variables are used in this study, namely dependence and independence variables.

3.6.1. Dependence variable:

Dependence variable in this study is tuberculous infection which was defined according to the result of TST and IFN-γ assay.

3.6.2. Independence variables:

The independent variables in the study are regarded as the potential risk factors for latent tuberculosis infection among children contacts based on the literature review, including demographic, socio-economic factors, knowledge of tuberculosis prevention stated by the mothers, degree of exposure, and pathogen characteristic**.**

Demographic and socio-economic factors: included age of both the child and mother, level of mother's education, marital status, total number of children per family, birth order, and occupation of the parents and economic status of the family. Economic status of the family was assessed using the variable income level which was categorized as low-income and medium income. A family was said to be non-poor if average personal income was over 260,000 VND per month for urban area and 200,000 VND per month for rural area. A family that could not satisfy the above condition was considered to be poor *(Based on national baseline of poverty for 2005-2010, decision of Ministry of Labour, Invalids and Social Affairs, Vietnam).*

Degree of exposure: Mothers were asked to identify their children's degree of exposure including frequent level and duration of exposure. Proximity of exposure was identified based on house size, household size, and average number of persons per room.

Questions were asked to find out the number of room in the house, the number of family members in the household, room ventilation (How many window and door in the house, it is opened or closed during the day)

Mothers were also asked if their children have to share bedroom/sleeping with source cases; the relationship with source cases; how they prevent TB transmission among household contacts.

Clinical examination of index cases: Variables related to TB cases including history of fever, chronic cough, sputum grading testing. The interviewers assessed the health status of index cases both by asking the patients and by collecting clinical examination from hospital.

3.7. DATA ANALYSIS.

Data collected was entered into a computer in the SPSS 16.0 software for the analysis.

Numerical variables like age of child and mother, number of siblings of the child, etc, were entered as they are without being recoded. Categorical variables like sex, mother's marital status, parents' occupation, etc, were entered after being recoded.

Economic status of the family was categorized in 2 groups namely non-poor and poor. The criteria to categorize were mentioned above.

Categorization of demographic variable such as house size, household size, duration of exposure, proximity of exposure (social proximity and genetic proximity), medical history, presence of BCG scares, presence of symptoms of tuberculosis are presented in attached files of questionnaire.

The outcome of this study was the difference in the prevalence of tuberculous infection according to interferon gamma assay and TST as indicated by the frequency of positive results for each test. We then assessed the level of concordance between the results of these two tests. Concordance was calculated as the overall percent agreement between the results of the two assays using two-by-two contingency tables. The strength of this agreement was examined using Cohen's kappa (κ) with a κ value of >0.75 representing excellent agreement beyond chance, 0.40 to 0.75 representing fair to good agreement beyond chance, and <0.40 representing poor agreement beyond chance (70).

Univariate analysis was performed to identify risk factors associated with positive tuberculin skin test and interferon gamma assay by calculating the Odds ratios and 95 % CI, with the statistical significance that was set at the level $p < 0.05$.

Multivariate analysis was then used to find out whether (or not) the factors, which were identified in univariate analysis, remain associated with the risk of tuberculous infection. In multivariable analysis, generalized estimating equation (GEE) was used to determine the risk factors for tuberculous infection among child contacts of sputum-positive pulmonary tuberculosis patients. The GEE was optioned because the outcome for different contacts being exposed to the same source cases, thus, tuberculosis infection tends to aggregate within household. In that case, the outcomes within cluster of contacts were not independent. This was taken into account by the GEE procedure. The GEE corrects for correlation and lack of independence of responses for contacts with an index case in common (71). Adjusted odds ratios and their 95% confidence intervals were also estimated. Associations were considered significant at p-value < 0.05.

3.8. RESEARCH TEAM

In the collaboration between Department of Immunology and Molecular-Biology Department - NIHE and Hanoi Pulmonary Tuberculosis Hospital, a research team was

established to conduct the study, including the principal researcher, 2 researchers from Department of Immunology and Molecular-biology - NIHE and 5 staffs from Hanoi Pulmonary Tuberculosis Hospital**.**

3.9. ETHICAL CONSIDERATION.

The researchers explained the purpose and benefits of the study to the subjects and asked them for their permission to interview and collect specimens. Participation in the study was totally voluntary. They are not forced or persuaded to participate in the study and free to withdraw in the course of the study if they did not wish to continue.

Children recruited into study were appointed at provincial Tuberculosis and pulmonary Hospital for blood collecting. Information regarding risk factors for TB infection will be gathered by asking TB patients and mothers or caregivers of children. Thus, the conduct of the study did not pose any health risk to the participants.

The study was approved by the Department of International Health, Faculty of Medicine, University of Oslo-Norway and Vietnam Institute of National Hygiene and Epidemiology. The project was submitted to the two bodies for ethical clearance. Also, permission from Hanoi Tuberculosis and Pulmonary Hospital was obtained before conducting the study.

3.10. TIME TABLE

Month	Works
June 2008	
1 - 17	- Visit and work with provincial tuberculosis Hospital's directorate. - Recruit researcher assistants. - Meet and discuss with all members of the research team to reach a consensus on the study's schedule. - Train interviewers.
20 – 30	- Conduct the pre-testing. - Modify the questionnaire as necessary.
July	- Collect data - Work in laboratory. - Arrange meetings for the research team to discuss and decide solutions to problems occurring in the process.
August	- Collect data - Work in laboratory
September	- Collect data - Work in laboratory
October	- Collect data - Work in laboratory
November	- Collect data - Work in laboratory
December 2008	- Collect data - Work in laboratory - Enter data into the computer - Collect data
January 2009	- Work in laboratory - Data compilation and analysis
February - June 2009	- Write thesis - Defend thesis

CHAPTER 4

RESULTS

4.1 CHARACTERISTICS OF STUDY SAMPLE

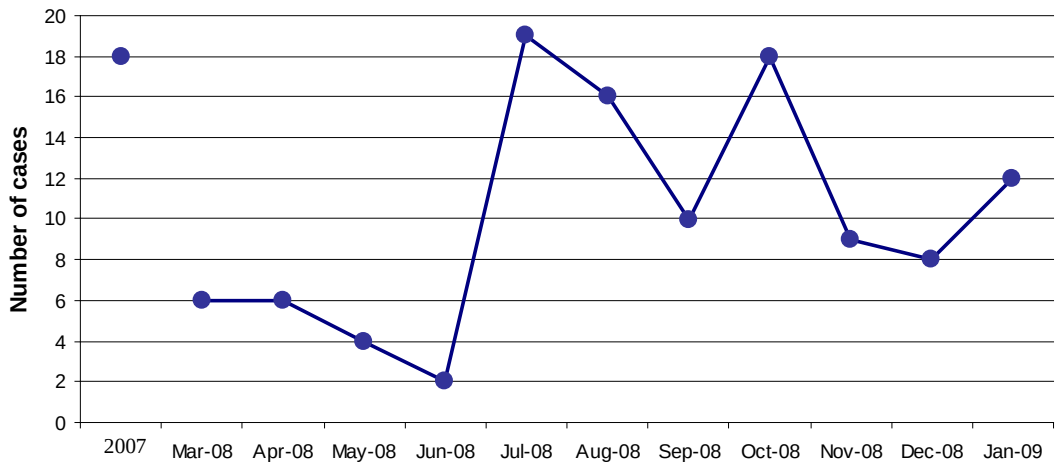
A total of 128 TB cases and 208 their children contacts were recruited into the study after meeting the inclusion criteria. The hospital record of tuberculosis patients were done including information on patient demographic, sputum smear result, chest X radiograph and duration of symptoms. All subjects were required to complete a structured questionnaire. An information regarding children contacts were done by child's parent or care-taker. Children contacts were invited to the Health Care Centre for tuberculin skin test. 208 blood samples were collected and transferred to Immunology Laboratory – NIHE to take IFN-γ assay.

4.1.1 CHARACTERISTICS OF SOURCE CASES

4.1.1.1 Distribution of cases by month:

Among 128 source cases, 18 cases were recruited from July to December 2007 and 110 cases were collected in 11 months (From March of 2008 to January of 2009). Data on distribution of cases by month are shown in figure 4.1.

Figure 4.1: Distribution of source cases by month



4.1.1.2 Distribution by geography:

Regarding to geographic distribution of index cases, of the total 3 provinces of Ha Noi, Thai Binh, Ha Tay, 13 Community Health Centres were chosen for this study. Detailed number and percentage of cases occurring in the above-mentioned Health Centres are shown in table 4.1.

Table 4.1 Geographic distribution of index cases by Community Health Centre

Community Health Centre	Frequency
1. Thai Binh:	***66***
- Thai Thuy	24
- Hung Ha	6
- Quynh Phu	17
- Dong Hung	19
2. Hanoi:	***41***
- Hoang Mai	11
- Hai Ba Trung	14
- Thanh Xuan	4
- Ba Dinh	12
3. Ha Tay:	***21***
- Tot Dong	5
- Quang Bi	6
- Xuan Mai	2
- Van Vo	3
- Phu Xuyen	5
Total	**128**

4.1.1.3. Social-Demographic and clinical characteristics.

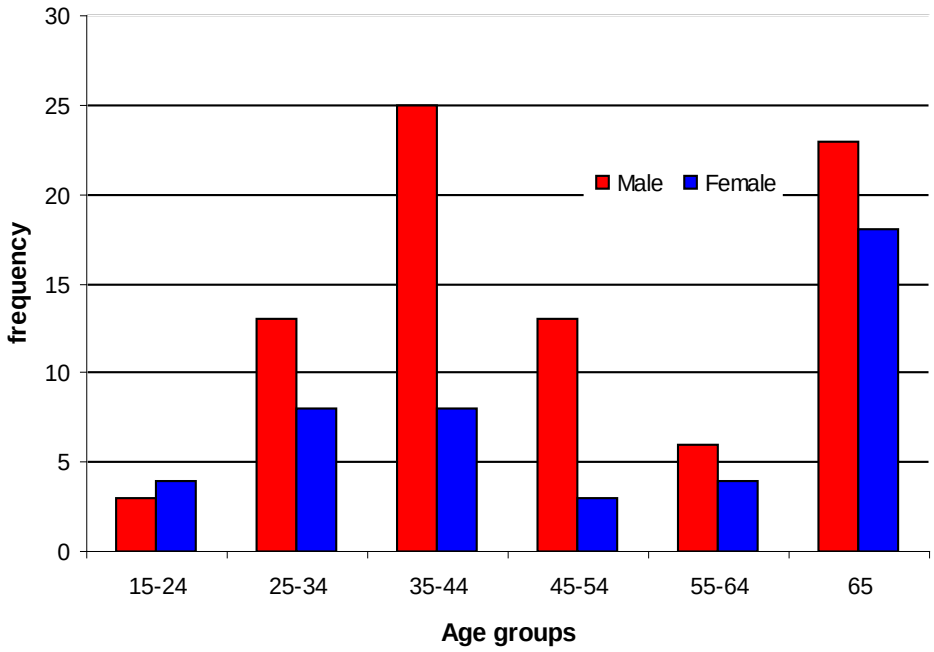
As seen in table 4.2 and figure 4.2 below, of the total 128 cases recruited into the study, there were 83 males and 45 females. The number of males was higher than females in almost all age groups especially groups of 35-54 and over 65 year old**.**

Table 4.2: Distribution of cases by sex and age group.

Age group	Frequency		
	Male	Female	Total
15-24 year old	3	4	7
25-34 year old	13	8	21
35-44 year old	25	8	33
45-54 year old	13	3	16
55-64 year old	6	4	10
≥ 65 of year old	23	18	41
Total	83	45	128

The minimum age of cases was 16 years old; the maximum age was 78 years old. The median age was 46. Most of cases were ranged from 25 to 64 years of age making up 62.5 % of the total. The number of cases more than 65 years of age was 41 (32 %).

Figure 4.2: Distribution of cases by sex and age group.



Along with matching variables (sex and age group), some other demographic and social characteristics of source cases, such as mean age, occupation, family size, relationship to the child, are shown in table 4.3

Table 4.3: Social-demographic and clinical characteristics of source cases

Characteristics		**Source cases (n=128)**
Sex	Male	83 (64.8%)
	Female	45 (35.2%)
Age groups	Under 25 year old	7 (5.5%)
	From 25 to 64 year old	80 (62.5%)
	Over 64 year old	41 (32.0%)
Median age (year)		46
Family size (number of family members)	≤ 4 people	35 (27.3%)
	Over 4 people	93 (72.7%)
Education	Primary school	87 (68 %)
	Secondary school	37 (29%)
	Bachelor	4 (3.1%)
Occupation	Peasant	67 (52.3%)
	Employed	61 (47.7%)
Economic status	Poor	28 (21.9%)
	Non poor	100(78.1%)
Relationship to child	Parents	65 (50.8%)
	Grandparents	54 (42.2%)
	Others	9 (7%)
Duration of cough (Before treatment)	Under 4 weeks	66 (51.6%)
	Over 4 weeks	62 (48.4%)
Sputum microscope	3+	25 (19.5%)
	2+	44 (34.4%)
	1+	35 (27.3%)
	Scanty	24 (18.8%)

4.1.2. Characteristic of child contacts:

4.1.2.1. Social-demographic characteristics of child contacts

In this study, 208 children under 15 years old were fully assessed. Age was ranged from 1 to 15 years old. The mean age of the contacts was 9. There were 96 girls (46.2%) and 112 boys (53.8%). Of 208 children: 44 (21.2%); 69 (33.2%); 57 (27.4%); 38(18.3%) were exposed to index cases whose sputum smear 3+; 2+; 1+; and scanty for AFB

respectively. The number of child contacts per source case varied between 1 to 8 individuals. The source cases were female for 82 children and male for 126. The commonest relationships of source cases to exposed children were parent (47.6%) and grandparent (46.6%). Table 4.4 showed Social-demographic characteristics of children contacts:

Table 4.4: Social-demographic characteristics of children contacts

Characteristics		Number of contacts (n=208)
Sex	Girls	96 (46.2%)
	Boys	112 (53.8 %)
Age groups	Under 5 year old	39 (18.8%)
	From 5 to 10 year old	82 (39.4%)
	Over 10 year old	87 (41.8%)
Mean age (year)		9
Geography:	Ha Noi	57 (27.4%)
	Ha Tay	41 (19.7%)
	Thai Binh	110 (52.9%)
BCG scar	Have BCG scar	205 (98.6%)
	No BCG scar	3 (1.4%)
Relationship to source cases	Parents	99 (47.6%)
	Grandparents	97 (46.6%)
	Others	12 (5.8%)
Exposed to sputum grade	3+	44 (21.2%)
	2+	69 (33.2%)
	1+	57 (27.4%)
	Scanty	38 (18.3%)

4.2. TUBERCULIN SKIN TEST (TST) AND INTERFERON GAMMA TEST (IFN-γ) RESULTS

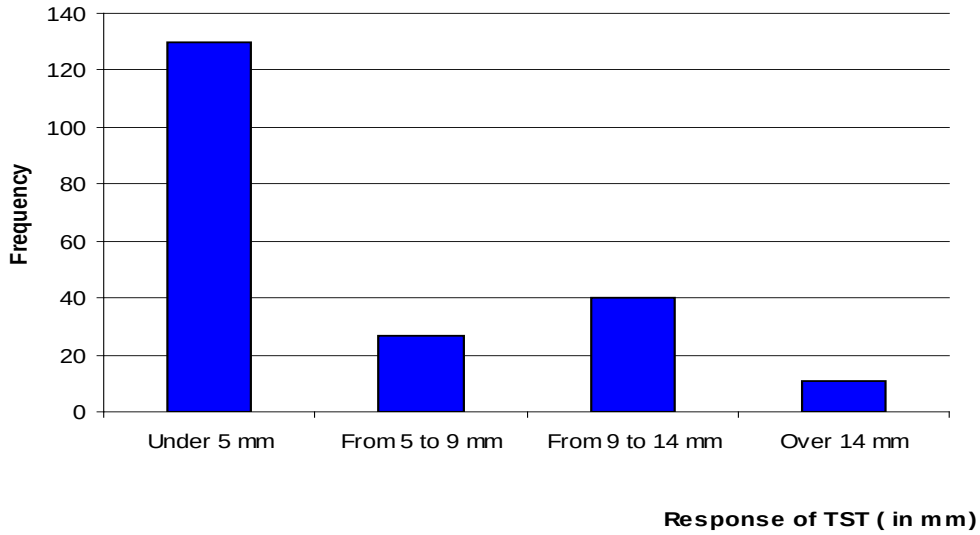
4.2.1. Distribution of TST responses in children contacts (in mm)

As can be seen in table 4.5 and finger 4.3 below, of total 208 children contacts recruited into the study there were 130 children (62.5%) had a TST response < 5 *mm*; 27 (13%) had TST response from 5 to 9 *mm*; 40 (19.2%) had a TST response from 10 to 14 *mm* and 11 (5.3%) $\geq 14mm$

Table 4.5: Distribution of TST responses in children contacts (in mm)

In duration	TST response	
	Frequency	Percent
≤ 4 mm	130	62.5
From 5 to 9 mm	27	13
From 10 to 14 mm	40	19.2
Over 14 mm	11	5.3

Figure 4.3: Distribution of TST responses in child contacts (in mm)



4.2.3. Response of INF gamma assay and TST by duration of exposure

Figure 4.4 showed the response of IFN-γ by duration of exposure. Overall, IFN-γ level in response to ESAT-6 and CFP-10 was increased in group of those who had duration of exposure from 3 to 12 weeks and slightly decreased in group of those who had duration of exposure over 12 weeks.

Figure 4.5 showed the response of TST by duration of exposure to index cases. Overall, duration of TST was not difference by the time of exposure.

Figure 4.4: Response of INF gamma assay by duration of exposure

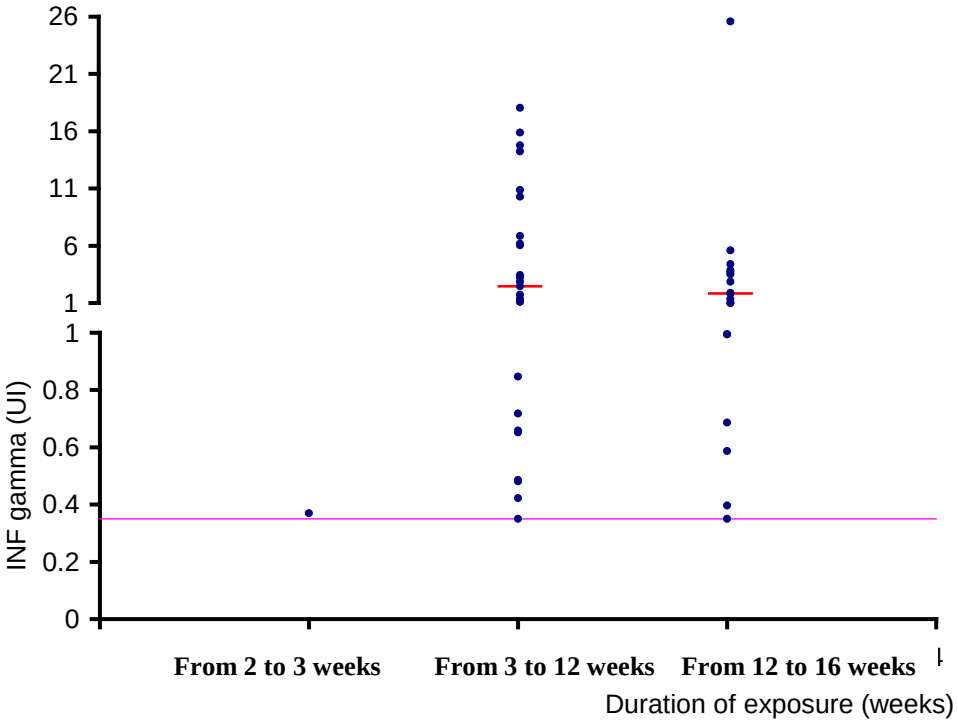
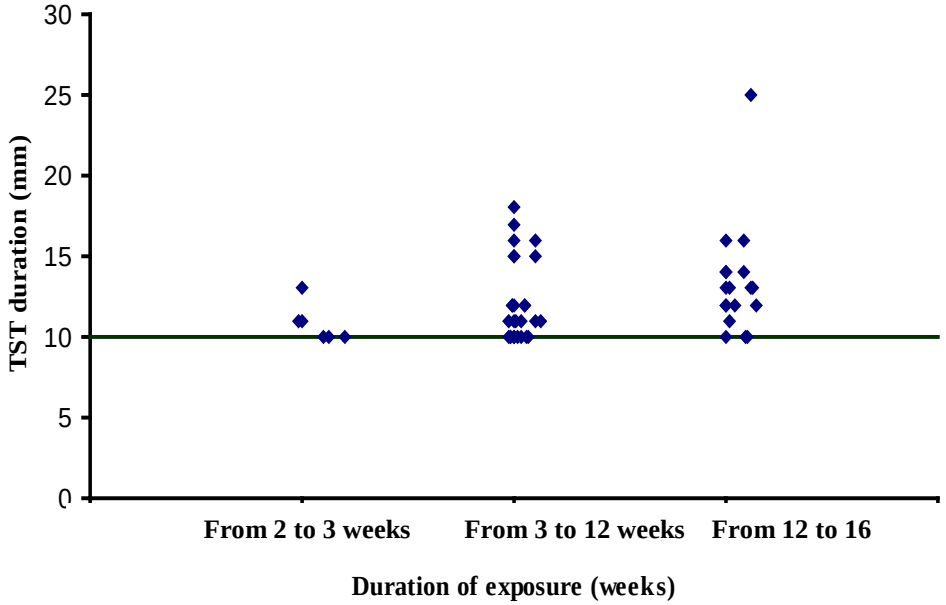


Figure 4.5: Response of TST by duration of exposure



4.2.3. Proportion of latent tuberculosis infection (LTBI) among children contacts

Both tuberculin skin test (TST) and interferon gamma test are used for diagnosis of tuberculous infection among children contacts. For TST there were 78 cases (37.5%) positive with 5 mm cut off point and 51 cases (24.5%) positive with 10mm cutoff point.

For interferon gamma test, there were 42 cases (20.2%) positive. Data was showed in table 4.6.

Table 4.6: Proportion of TST and INF gamma assay positive among contacts

Results	Tuberculin skin test		Interferon gamma assay
	Cut off point of 5 mm	*Cut off point of 10 mm*	
Negative (%)	130 (62.5)	157 (75.5)	166 (79.8)
Positive (%)	78 (37.5)	51 (24.5)	42 (20.2)
Total			

4.2.3. Distribution of LTBI by sex and age groups:

As can be seen in table 4.7 below, for group of under 5 years of age, the proportion of tuberculous infection seems to be higher in boys than in girls but overall it was the same in two groups.

Table 4.7: Distribution of tuberculous infection by sex and age groups

Age groups	Number of tuberculos infection			
	Interferon gamma assay		***TST - Cut off point of 10 mm***	
	(No. positive/ total no. tested)		***(No. Positive/ total no. tested)***	
	Boy	***Girl***	***Boy***	***Girl***
Under 5 of age	6/17	3/24	8/17	1/24
Over 5 of age	17/95	16/72	22/95	20/72
Total	23/102	19/96	30/102	21/96

4.3. AGREEMENT BETWEEN TST AND IFN-γ ASSAY

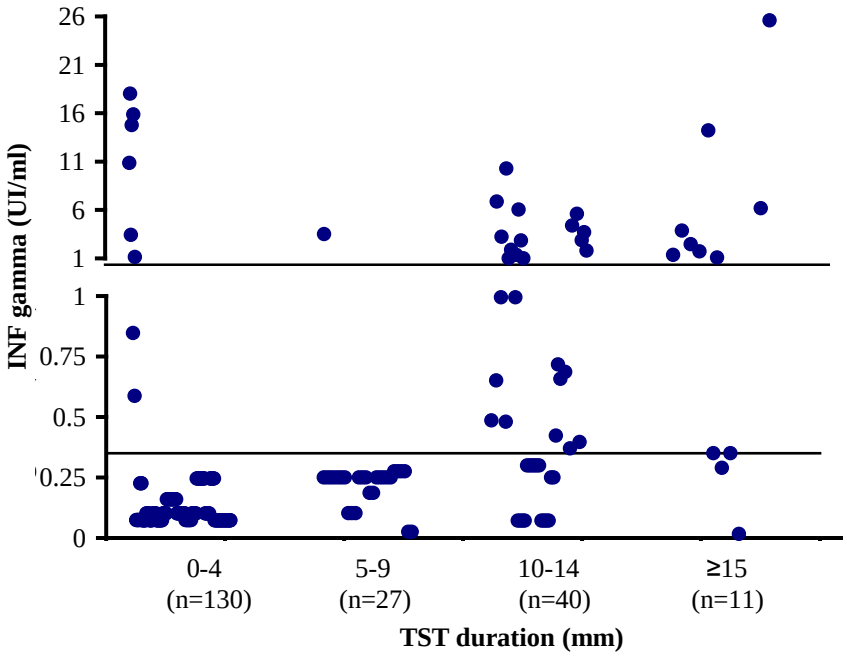
Table 4.8 shows that the overall agreement between TST and IFN-γ assay was moderate with the cut off of 5 mm for TST (Kappa: 0.41, 95% CI: 0.29–0.54), with concordant results in 130 (102 negative and 28 positive) of 208 contacts (62.5%). Agreement between the two tests was substantial with the cut off of 10 mm for TST (Kappa: 0.62;

95% CI: 0.45-0.75). Concordance results was found in 181 (148 negative and 33 positive) of 208 contacts (87%).

Table 4.8 Agreement between TST and IFN-γ assay results *(n=208)*

TST		Interferon gamma assay		*Total*	Kappa
		Negative	*Positive*		**(95% confidence interval)**
5 mm	*Negative*	102	2	130	Kappa : 0.41
Cutoff	*Positive*	40	28	78	95% CI (0.29-0.54)
point	*Total*	166	42	208	*Observed agreement: 75%*
10 mm	*Negative*	148	9	157	Kappa: 0.62
Cutoff	*Positive*	18	33	51	95% CI (0.45-0.75)
point	*Total*	166	42	208	*Observed agreement: 87%*

Figure 4.6: Agreement between TST and INF -ϒ assay.



A following positive tuberculin skin test indicating tuberculous infection was defined as 10 mm cutoff point for this study.

The distribution of discordant and concordant results with respect to sex, age and relationship to source cases were shown in table 4.9 below:

Table 4.9: Distribution of concordant and discordant results for the TST (*10mm cut-off*) and the IFN-γ *(n = 208).*

Variables	No. discordant/ total no. Tested	Distribution (%)			
		+TST/+INF_γ	*-TST/- INF_γ*	*+TST/- INF_γ*	*-TST/+ INF_γ*
Sex:					
- Male	15/112	19 (17)	78 (69.6)	11 (9.8)	4 (3.6)
- Female	12/96	14 (14.6)	70 (73)	7 (7.3)	5 (5.2)
Age group:					
- Under 5	6/41	6 (14.6)	29 (70.7)	3(7.3)	3(7.3)
- Over 5	21/167	27 (16.2)	119 (71.3)	15 (9)	6 (3.6)
Relationship to index cases:					
- Parent	21/99	27 (27.3)	51 (51.5)	13 (13.1)	8 (8)
- Grandparent	6/97	2 (2.1)	89 (91.8)	5 (5.2)	1 (1)
- Others	0/12	4 (33.3)	8 (66.7)	0 (0)	0 (0)

As can be seen in table 4.9, subjects were more likely to be TST positive, INF_γ negative than TST negative, INF_γ positive. Children exposed to grandparent with tuberculosis seem to be more likely to have discordant TST positive than those who exposed to parent (5/7 and 13/40 respectively) but not significant (p =0.48).

4.4. UNIVARIABLE ANALYSIS OF POTENTIAL RISK FACTORS FOR TST POSITIVE OR INF GAMMA ASSAY POSITIVE.

In univariate analysis, based on findings that were presented with odds ratio (OR), 95 % confidence interval (CI) and p value, we found 8 factors significantly associated with two positive tests. Detailed results of univariable analysis are shown in table 4.15

4.4.1. Factors related to source cases:

Among 99 children having their parent with tuberculosis and 109 having relatives with tuberculosis, there were 35 and 7 children positive with the INF gamma assay respectively. We have OR = 7.766; 95% CI (3.206-18.807); p value < 0.001. Similarly

with TST test, there were 40 and 11 children positive respectively, OR = 5.988; 95% CI (2.675-13.403); p value < 0.001. We say that the risk of being positive with INF gamma assay and TST test in children whose parent had tuberculosis is 7.7 times and 6 times higher compared to those whose relatives had tuberculosis respectively.

Comparing with those who were exposed to mother with tuberculosis, children who exposed to other family members with tuberculosis, such as father and other relatives, are associated with increased risk of tuberculous infection. When using GEE model for this variable, considering exposed to relatives with tuberculosis as reference category, the results was shown in Table 4.10 and 4.11. The risk of positive INF gamma assay or TST test in children whose mothers had tuberculosis was 20.4 times (95% CI 5.89-70.776; p < 0.001) and 14 times higher (95% CI 4.577-47.5; p<0.001) compared to those with other relatives as index cases. The risk of being positive with INF gamma assay in children whose father had tuberculosis is higher 5.5 times (95% CI 2.151-14.290; p < 0.001) compared to those whose other relatives had tuberculosis. The results were similar to respond to TST test: The risk of being positive with TST test in children whose father had tuberculosis is 4.5 times higher (95% CI 1.847-10.775; p=0.001) compared to those whose other relatives had tuberculosis.

Table 4.10: Results of GEE model on relationship to source cases - TST

Variables	Sig	Exp (B)	95.0% CI for EXP (B)	
			Lower	Upper
Mother with tuberculosis	<0.001	14.747	4.577	47.514
Father with tuberculosis	0.001	4.462	1.847	10.775
Other relatives with tuberculosis		1		

Table 4.11: Results of GEE model on relationship to source cases – INF-ϒ assay

Variables	Sig	Exp (B)	95.0% CI for EXP (B)	
			Lower	Upper
Mother with tuberculosis	<0.001	20.424	5.893	70.776
Father with tuberculosis	<0.001	5.544	2.151	14.290
Other relatives with tuberculosis		1		

The variable of sputum smear grade were categorized into 3 level: 3+;2+ and 1+ (scanty were included in 1+), considering exposed to a 1+ smear grade in source cases as

reference category, the risk of being positive with INF gamma assay and TST test was increased with increasing intensity of sputum smear grade of the source cases. The risk was highest for those who were exposed to 3+ smear positive cases (OR=4.518; 95% CI 1.799-11.345; p=0.001 and OR=9.047; 95% CI 2.948-27.765; p <0.001 for TST test and INF gamma assay respectively). Results are shown in table 4.12 and 4.13 below:

Table 4.12: results of GEE model on sputum smear of index cases (TST)

Variables	Sig	Exp (B)	95.0% CI for EXP (B)	
			Lower	Upper
Sputum smear grade : 3+	0.001	4.518	1.799	11.345
Sputum smear grade : 2+	0.580	1.312	0.502	3.492
Sputum smear grade : 1+		1		

Table 4.13: Results of GEE model on sputum smear grade of index cases (IFN-γ assay)

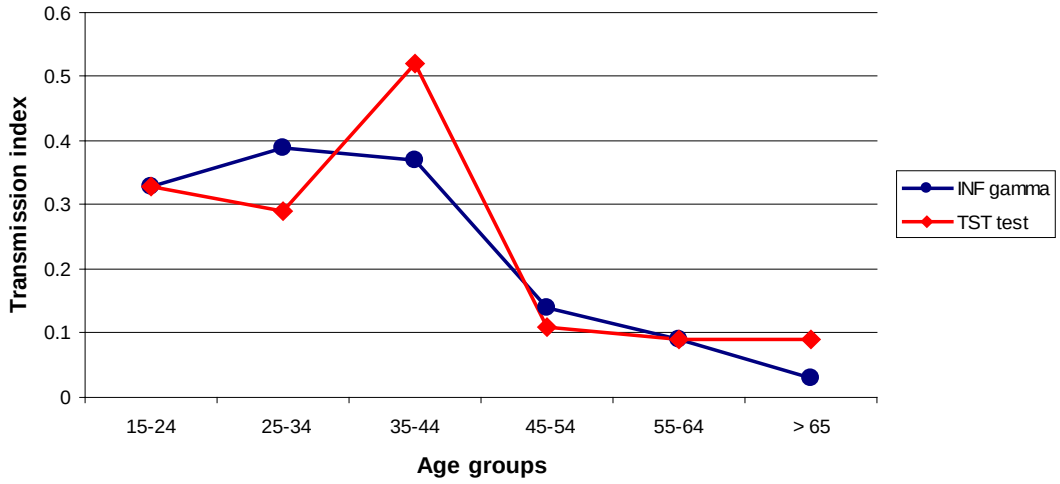
Variables	Sig	Exp (B)	95.0% CI for EXP (B)	
			Lower	Upper
Sputum smear grade : 3+	<0.001	9.047	2.948	27.765
Sputum smear grade : 2+	0.428	1.610	0.496	5.228
Sputum smear grade : 1+		1		

Concerning the results from TST, the risk of being positive in those who were exposed to source cases with a duration of cough before TB treatment ≥4 weeks was 2.9 times higher compared to those who exposed to source cases had duration of cough less than 4 weeks (OR=2.63; 95%CI 1.398-5.862 p = 0.004)

Similarly with the results from INF gamma assay showed that the risk of being positive was increased with the duration of cough before treatment (OR=7.533; 95% CI 2.965-19.240; p < 0.001)

Overall, the number of TST and INF gamma assay positive among children contacts generated per source cases decreased with increasing age of source cases. Finger 4.7 shows that for both TST test and INF gamma assay, the transmission index case tended to decline with increasing with age of source cases.

Figure 4.7: Transmission index of tuberculosis by age group



When we used median age of source cases as a cut-off point to categorize contacts of the source cases into 2 groups: a group of those who exposed to source cases under 46 years of age, and another group of those who exposed to source cases from 46 years of age upwards, we observed significant association between proportion of tuberculous infection and source cases age (OR=6.605; 95% CI 3.010-14.494; p<0.001 and OR=7.784; 95% CI 2.911-20.819, p<0.001 based on the proportion of positive with TST and INF gamma respectively).

Regarding to economic status, based on the poverty line standard issued by Prime Minister (Signed 8 July 2005) and applied for period of 2006-2010, 48 (23.1%), children in 28 families (21.9%) were defined as having low income. Statistic results showed that low income status is associated with having tuberculous infection among contacts (OR=4.888; 95% CI 2.207-10.828 p <0.001 and OR = 6.996; 95% CI 3.089-15.795; p<0.001 for TST and INF gamma assay respectively).

Of 208 children contacts recruited into this study, there were 126 (60.6%) exposed to male and 82 (39.4%) exposed to female. This variable then was analyzed separately by geography, we found that for those who come from rural area, exposed to male and female were significant association with TST and INF gamma assay positive respectively (OR= 3.329; 95% CI 1.349-8.218; p = 0.009 and OR = 3.708; 95% CI 1.354-10.158; p = 0.011)

But overall we found that gender of source cases was not significant association with and interferon gamma assay positive and TST positive (OR = 1.853, 95% CI (0.833-4.120), p =0.13 and OR = 2.046, 95% CI (0.976 – 4.288), p = 0.058 respectively).

Our finding showed that the proportion of positive TST and INF gamma assay was not significant association with education of the source cases (OR= 1.094; 95% CI 0.463-2.585; p=0.838, OR=0.891; 95% CI 0.376-2.184; p=0.794 for TST and INF gamma assay respectively.

Occupation of source cases were also found not significant association with TST positive or INF gamma assay positive (OR=1.193; 95% CI 0.574-2.480; p=0.636 and OR= 1.632; 95% CI 0.739-3.603; p=0.226.

4.4.2. Factors related to contacts

Among 208 children less than 15 years of age recruited into this study, there were 96 (53.8%) girls and 112 (46.2%) boys. Of which 19 and 21 girls, 23 and 30 boys were found positive with INF gamma assay and TST test respectively, showing no association between being infected with *M.TB* and gender of contacts.

It was also found that there was no significant association between being infected and age of contacts.

Regarding geography, contacts were divided into two groups: a group of those who were living in rural provinces (Ha Tay and Thai Binh) and a group of those who were living in an urban province (Ha Noi). Statistic results showed that there was no significant association between being infected and living in urban or rural areas.

When being asked about the duration of sharing activities with source cases, 19 (9.1%) children sleep in the same bed with index cases and 189 (90.9%) sleep in the different bed . Ten and 32 contacts were positive with INF gamma assay respectively indicating a significant association between sleeping in the same bed as source cases and being INF gamma positive (OR=5.490; 95% CI 2.089-14.427; p=0.001) and TST positive (OR=2.627; 95% CI 1.001-6.898; p=0.05).

4.4.3. Factors related to environment.

Regarding household size, contacts were divided into two groups: one group of those who had from 1 to 4 persons living in the same household and a group of those who had more than 4 persons. We found that the number of persons in each families was associated with increasing risk of being INF gamma assay positive (OR = 3.323; 95% CI 1.082-10.204; p=0.036) but not significant associated with TST positive (OR=1.420 95% CI 0.583-3.461; p=0.440).

The number of rooms per family was categorized into 2 groups: one group of those who were living in a house with only one room; one group of those who were living in a house with more than one room. We found that the number of room was significant

association with TST and INF gamma assay positive (OR= 4.612; 95% CI 1.163-18.283; p=0.03 and OR=17.685; 95% CI 3.451-90.627; p=0.001 for TST and INF result respectively)

Among 208 contacts, there were 75 and 133 children who lived in families with only one child and more than one respectively. Statistics showed that being infected was not significant in association with having one sibling or more.

Table 4.14: Univariate analysis of some risk factors associated with LTBI among contacts

Variables	*TST test*		*OR*	*95% CI*	*P value*	*INF-γ assay*		*OR*	*95% CI*	*P value*
	Pos/total	*%*				*Pos/total*	*%*			
Related to source factors:										
Gender of cases										
- Male	25/126	19.8	Ref			21/126	16.7	Ref		
- Female	26/82	31.7	2.046	0.976-4.288	0.058	21/82	25.6	1.853	0.833-4.120	0.130
Education										
- Primary education	36/154	23.4	Ref			31/154	20.1	Ref		
- Higher education	15/54	27.8	1.094	0.463-2.585	0.838	11/54	20.4	0.891	0.376-2.184	0.794
Occupation										
- Farmer	26/120	21.7	Ref			20/120	16.7	Ref		
- Employed	25/88	28.4	1.193	0.574-2.480	0.636	22/88	25	1.632	0.739-3.603	0.226
Economic status										
- Poor	**25/48**	**52.1**	**4.888**	**2.207-10.828**	**<0.001**	**24/48**	**50.0**	**6.996**	**3.089-15.79**	**<0.001**
- Non poor	**26/160**	**16.2**	**Ref**			**18/160**	**11.25**	**Ref**		
Age										
- Under 46 year old	**43/104**	**41.3**	**6.605**	**3.010-14.494**	**<0.001**	**37/104**	**35.6**	**7.784**	**2.911-**	
- Over 46 year old	**8/104**	**7.7**	**Ref**			**5/104**	**4.8**	**Ref**	**20.819**	**<0.001**

Duration of cough before treatment										
- Under 4 weeks	**18/106**	**17.0**	**Ref**			**8/106**	**7.5**	**Ref**		
- Over 4 weeks	**33/102**	**32.4**	**2.863**	**1.398-5.862**	**0.004**	**34/102**	**33.3**	**7.553**	**3.046-19.24**	**<0.001**
Relationship to child:										
- Mother	**15/24**	**62.5**	**14.714**	**4.577-47.5**	**<0.001**	**14/24**	**58.3**	**20.424**	**5.89-70.776**	**<0.001**
- Father	**25/75**	**33.3**	**4.462**	**1.847-10.775**	**0.001**	**21/75**	**28.0**	**5.544**	**2.151-14.29**	**<0.001**
- Relatives	**11/109**	**10.0**	**Ref**			**7/109**	**6.4**	**Ref**		
Sputum smear grade:										
- 3+	**21/45**	**46.7**	**4.518**	**1.799-11.345**	**0.001**	**22/45**	**48.9**	**9.047**	**2.948-27.76**	**<0.001**
- 2+	**15/69**	**21.7**	**1.312**	**0.502-3.429**	**0.58**	**12/69**	**17.4**	**1.610**	**0.496-5.23**	**0.428**
- 1+	**15/94**	**15.9**	**Ref**			**8/94**	**8.5**	**Ref**		
Contact factors										
Gender of contacts:										
- Male	30/112	26.8	Ref			23/112	20.5	Ref		
- Female	21/96	21.9	0.762	0.427-1.357	0.355	19/96	19.8	0.882	0.471-1.652	0.696
Age of contacts:										
- ≤ 5 year old	9/41	22	Ref			9/41	22	Ref		
- Over 5 year old	42/167	25.1	1.148	0.526-2.502	0.729	33/167	19.8	0.935	0.422-2.068	0.867
Sharing activities with cases:										
- Sleeping in the same bed	**8/19**	**42.1**	**2.627**	**1.001-6.898**	**0.05**	**10/19**	**52.6**	**5.490**	**2.089-14.43**	**0.001**
- Sleeping in different bed	**43/189**	**22.8**	**Ref**			**32/189**	**16.9**	**Ref**		

Provincial geography:										
- Rural province	36/151	23.8	0.869	0.391-1.932	0.731	29/151	19.2	0.742	0.319-1.725	0.488
- Urban province	15/57	26.3	Ref			13/57	22.8	Ref		
Environmental factors										
Household size:										
- Under 4 persons	9/49	18.4	Ref			**4/49**	**8.2**	**Ref**		
- Over 4 persons	42/159	26.4	1.420	0.583-3.461	0.440	**38/159**	**23.7**	**3.323**	**1.082-10.20**	**0.036**
House size:										
- Have one room	**6/10**	**60.0**	**4.612**	**1.163-18.28**	**0.03**	**8/10**	**80**	**17.685**	**3.45-90.63**	**0.001**
- Have more than one room	**45/198**	**22.7**	**Ref**			**34/198**	**17.2**	**Ref**		
Sibling										
No siblings	17/75	22.7	1.355	0.627-2.927	0.44	17/75	22.7	0.852	0.392-1.849	0.685
Has siblings	34/133	25.6	Ref			25/133	18.9	Ref		

4.5. MULTIVARIATE ANALYSIS

To identify risk factors that remained significant association after controlling confounders, we put potential factors that had p value ≤ 0.25 in univariate analysis into generalized estimating equation (GEE).

Because of strong correlation between variation of age of source cases and variation of relationship with children, variation of age were taken out from multivariable model.

Multivariable analysis are illustrated in table 4.17 and 4.18

Table 4.15: Multivariate analyses of potential risk factors among 208 child contacts of 128 index cases (Based on IFN-γ assay)

Risk factors	Sig	Exp (B)	95% CI for Exp	
			Lower	Upper
Duration of cough before treatment				
≤ 4 weeks		**Ref**		
> 4 weeks	**0.003**	**6.9**	**1.9**	**25.07**
Sleeping with source cases				
Same bed	0.072	4.635	0.874	24.580
Different bed		Ref		
Economic status				
Poor	**0.001**	**6.142**	**2.055**	**18.358**
Not poor		**Ref**		
Relationship to child				
Parent	**0.044**	**4.458**	**1.041**	**19.093**
Other relatives		**Ref**		
Sputum smear grade of index cases				
3+	**0.006**	**11.313**	**1.972**	**64.903**
2+	**0.143**	**3.325**	**0.664**	**16.739**
1+		**Ref**		
House size				
Have one room	0.310	2.641	0.406	17.199
Have more than one room		Ref		
Household size				
≤ 4 persons	0.103	0.233	0.041	1.342
> 4 persons		Ref		
Gender of TB cases				
Male	0.281	0.514	0.154	1.723
Female		Ref		
Occupation of TB cases				
Farmer	0.655	0.749	0.211	2.656
Employed		Ref		

Table 4.16: Multivariate analyses of potential risk factors among 208 child contacts of 128 index cases (Based on TST test)

Risk factors	Sig	Exp (B)	95% CI for Exp	
			Lower	Upper
Duration of cough before treatment				
≤ 4 weeks		Ref		
> 4 weeks	0.194	1.768	0.748	4.177
Sleeping with source cases				
Same bed	0.695	0.770	0.208	2.843
Different bed		Ref		
Economic status				
Poor	**0.002**	**3.526**	**1.598**	**7.779**
Not poor		**Ref**		
Relationship to child				
Parent	**<0.001**	**7.913**	**2.886**	**21.696**
Other relatives		**Ref**		
Sputum smear grade of index cases				
3+	**0.027**	**3.098**	**1.134**	**8.461**
2+	**0.420**	**1.508**	**0.556**	**4.085**
1+		**Ref**		
House size				
Have one room	0.850	0.875	0.219	3.495
Have more than one room		Ref		
Gender of TB cases				
Male	**0.01**	**0.309**	**0.126**	**0.758**
Female	**Ref**			

In multivariable analysis, the results which were shown in table 4.16 and 1.17, we found that some factors remained significantly associated to a positive INF gamma assay, including: Duration of cough before treatment (OR=6.9; 95% CI 1.9-25.07; p =0.003), having low income (OR=6.142; 95% CI 2.055-18.358; p = 0.001); having parent with tuberculosis (OR= 4.458 95% CI 1.041-19.093; p=0.044) and exposed to sputum smear grade of 3+ (OR=11.313; 95%CI 1.972-64.903); p=0.006).

We also found some factors that remained significantly associated to a positive TST including: Having low income (OR=3.526; 95% CI 1.598-7.779; p=0.002); having a parent with tuberculosis (OR= 7.913; 95% CI 2.886-21.696; p<0.001); being exposed to sputum smear grade of 3+ (OR=3.098; 95% CI 1.134-8.461; p=0.027); being exposed to a female index cases (OR= 0.309; 95% CI 0.126-0.758; p=0.01).

CHAPTER 5
DISCUSSION

Tuberculosis remains a global health problem (50). Mathematical models predict that if at least 70% incidence cases of smear positive TB are detected and treated, and 85% of them are cured, TB transmission will be decline by 7% to 11 % per years(72).

The National TB Program of Vietnam is the only TB control program in a high burden country that have meet the target of 70% case detection and 85% treatment success rate (5;50). But one investigation on TB transmission carried out in six provinces in Vietnam indicated the limited result of TB control in Vietnam despite high case detection and cure rate (60).

Close contact with smear positive TB is considered as an important risk of being infected. Investigation and identification of risk factors for TB transmission as well as the proportion of tuberculous infection among family contacts are important to evaluate ongoing TB transmission. Our study is the initial study on risk factors for TB transmission among children contacts that conducted in Vietnam.

5.1 Strengths of the study:

By convenience sampling technique, it was easily to select consecutive subjects who fulfilled the inclusion criteria in a limited time. In the context of limited time and money, a convenience sample study was relatively easy and inexpensive to conduct.

Trainings for research assistants and pre-testing conducted before data collection ensured a standardized way of collecting information. Among research assistants working in Ha Noi, Ha Tay and Thai Binh Pulmonary Hospitals, there were three medical doctors. They had experience in doing research. Additionally, all of them belonged to the NTP which have the same guideline for reading the TST test. That also ensured quality of collected data as well as recruitment of subjects conforming to the study criteria. Additionally, interferon gamma assay were conducted in Immunology laboratory – standardized laboratory of Vietnam, so the quality of outcome was ensured.

Frequent communication between the principal researcher and the assistants helped to deal with some problems that arose in the process of collecting data.

Vietnamese language, which is the sole language for communication in the area, was used for the interviews. That reduced misunderstanding between interviewers and participants.

5.2 Limitations of the study

There are several important limitations in this study including the cross-sectional study design, the use of convenience sampling techniques and the relative small sample size.

Convenience sampling technique based on hospitalized selection led to the limitation that the source cases are not fully representative for the tuberculosis population of the country.

In a cross-sectional study, we did not have the possibilities to follow individuals at risk for developing tuberculosis. Thus, we were left with the option of comparing the new assay (IFN-γ assay) to TST in order to determine which assay agrees most with the risks of tuberculous infection based on epidemiological characteristics of individuals. Additionally, lacking a true "gold standard" test for diagnosis of tuberculous infection, we are not able to make unequivocal assessments of the sensitivity and specificity of the two assay compared.

The limited sample size reduces the number of potential risk factors for TB infection that we could address in this study.

We could not visit the subjects' house to carry out observations on housing status, ventilation condition of source cases' room due to limited time and manpower. Moreover, the question regarding economic status may have been felt sensitive for some interviewees thus not producing honest answers. The above mentioned limitations may lead to information bias.

Recall bias occurred when interviewees who themselves are source cases fear that they are the cause of TB transmission for the whole family. They might not give true answer about their contact with children on the household. In contrast, when parent were asked about their children's duration of exposure to source cases in their family, they are likely to remember all possible exposure history of their children to the source cases. Thus, it is somehow difficult to interpret and might increase "false positive" as well as false negative findings.

The ability of TST to detect delayed type hypersensitivity reaction is on average optional from 4-12 weeks after exposure to *M. TB*.(73) Therefore, some infected household contacts may have been incubating *M. TB* but were not detected and were wrongly labeled as non-infected.

Finally, as all index cases in this study were smear positive sputum, we could not draw any inference regarding the risk of *M. Tuberculosis* transmission in household of AFB sputum smear negative.

5.3 Results of the study.

5.3.1 Sex distribution of source cases:

Among 128 source cases, the rate ratio between male and female was observed as 1.89 (table 4.2). The prominence of male TB patients by passive case finding was also reported in the annual report of Ha Noi National TB Program as well as in many investigations on TB prevalence (60).

The different ratio between men and women can be explained by social-culture factors. Vietnamese women, by nature, have the tendency to sacrifice for others in their family. If a family's economic status is low, women usually give their husbands and children priorities in food, health care, clothing, etc., resulting in limited assess to health care services. They often choose less qualified provider (self medication, traditional medication, pharmacist) as the first health care action (74-77). Moreover, traditionally, coughing, hawking and spitting sputum in general are less acceptable for women than men. Therefore, women may not report these symptoms to the doctors, leading the doctor's delay among women. One study on TB prevalence in Vietnam indicated that even with advanced pulmonary TB, female may hesitate to seek medical health (60;61). This is one of the reasons leading the longer delays in diagnosis and lower TB detected cases among female compared to male in Vietnam. This could be considered as a potential risk factor for TB transmission that will be discussed later in section 5.3.4

5.3.2. Proportion of positive TST and INF gamma assay among contacts

In this study, the proportion of positive tuberculin skin test and INF gamma assay among 208 contacts was 24.5% (51 contacts) and 20.2% (42 contacts) respectively, lower than in previous report from 43.8% to 48% of positive TST results among close contacts (14;47;78). The lower positive rate in this study might be explained by a number of factors. Firstly, the participants in this study were children less than 15, much more younger than those in the previous study (47). It was reported that approximately 10% of normal children with culture proven TB do not react to tuberculin skin test initially (79-81). Secondly, our study was carried out in Vietnam where TB prevalence is lower than in the previous studies. Finally, the results might concern to false-negative results.

However, in an other study conducted in Ho Chi Minh City, Vietnam, where the ARTI was reported as three times higher than in Ha Noi (60), the proportion of TST positive

among children less than 15 of age reached 21.45% (data not published) indicating that a the higher proportion of TB infections among household contacts that was a likely finding in this study.

5.3.3. Agreement between TST and INF gamma assay.

Increase in prevalence of tuberculosis and emergence of multi-drug resistant strains illustrated the urgent need for early diagnosis tuberculosis infected individuals. Screening for tuberculous infection with TST which was developed in the late 1800s is used as standard method until now. TST has many drawbacks including false-positive results due to exposure to NTM and BCG vaccination strain, and errors related to technical difficulties in administering the test and interpreting the results (82;83).

A new whole - blood interferon gamma (IFN-γ) assay has been reported as more specific than TST and can be used to diagnose tuberculous infection (44;47;84-88).

In the present study, we found that the overall agreement between IFN-γ assay and TST was moderate to substantial depending on whether the cutoff point was 5 mm or 10 mm (table 4.8). The lower concordance at 5 mm cut-off point than 10 mm cutoff point might be explained by the influence of BCG vaccination on tuberculin reactivity. It is well known that BCG induces TST reactivity, especially in the short term. There is an evidence that BCG vaccination affects tuberculin reactivity with in duration $\geq$ 5 but not with in duration $\geq$ 10 mm (89;90). Overall, with the cutoff point 10 mm, the risk factors associated with being infected were similar for both tests. Thus, our findings suggested that IFN-γ assay and TST with the cutoff point of 10 mm are more concordance to identify true infection than with the cut-off point of 5 mm.

The agreement between TST and IFN-γ assay have been showed in many previous studies (88;91-94). Our study showed a strong agreement between two tests (Kappa = 0.62; 95% CI 0.45-0.75), similar to studies that was conducted in rural India (88;92), where the BCG vaccination is given at birth, and prevalence of TB is high. But differently from other studies conducted in Japan and South Korea (47;95;96) where the BCG vaccination is widely used but often boosted. This discrepancy can be explained that previous BCG vaccination may only have minimal effect on TST results several years after the vaccination (97) .

The probable explanation for the higher number of TST positive with the cutoff point of 10 mm (51 contacts) compared to IFN-γ assay positive (42 contacts) is the probably higher specificity of IFN-γ assay, as evidenced by the finding that 18 of the 27 discordant (66.7%) between two tests was TST positive/ IFN-γ negative (Table 4.9) and

the positive IFN-γ assay's stronger association with potential risk factors for tuberculous infection (Table 4.16).

However, positive TST/negative IFN-γ gamma assay also might be explained by the differences in immunological mechanisms that determine the responses in TST and INF gamma assay. Positive TST results are primary mediated by the action of memory T cells (47). Memory T cells can live for a long time in the body and become activated again upon re-encountering antigen stimulation by the pathogen. Positive INF gamma assay are mainly due to the presence of effector T cells (known as T helper cells) (43;47). When being exposed to tuberculosis, effector T cells will be informed to activate macrophages. If this process is successful, effector T cells will be cleared from the circulation and only small number of them will be differentiated into memory T cells. Thus, in some circumstances a positive TST may indicate a remote infection whereas positive INF gamma assay seem to reflect a recent or ongoing infection. A child with a positive TST but negative INF gamma assay might have been infected before, from community contact not from index case in their family.

5.3.4. Risk factors associated with LTBI among household contacts

Multivariate analysis is considered as a standard method to identify risk factors associated with disease while adjusting for potential confounders (98). In this study, using generalized estimating equation (GEE) model, 5 factors were found to remain significant association with risk of TST and IFN-γ positively including exposure to parent with tuberculosis, gender of source cases, duration of cough before treatment, sputum smear grade and economic status (See table 4.17, 4.18)

Gender of source cases and relationship to contacts

Lockman et all (97) found that tuberculosis transmission was associated with increased proximity, close female relative and higher sputum smear grade. One study that conducted in Bangkok, Thailand (99) revealed that children exposure to mothers with TB were 15 times more likely to have tuberculous infection (OR=15; 95% CI 7.9-30.8) compared to exposure to other relatives.

In Vietnam, mothers spend much more time raising their children and doing housework while fathers are responsible for making money outside. Thus, by nature mothers were close to their children than the other relatives. Additionally, in Vietnam there were un equalities between female and male in accessing health care services because of their lower social-economic status, lower education and less information about TB than men. This is more prominent in rural areas, where the husband is seen as the head of the

family, playing the active role in receiving gests, paying visits to others whereas women are restricted to household chores, child rearing and preparing for daily meals. Traditionally, Vietnamese women themselves accepted their lower position in families as a Vietnamese proverb states: "*A boat follows its steering wheel. A woman follows her husband*" (75). This makes an important contribution to gender differences in accessing social benefits in general and health care services in particular. One study from Vietnam National TB Program reported the male/female ratio of TB cases detected passively is 2.98 (60;61) and this predominance of male was not observed among TB cases detected actively (61). The longer delay in diagnosis in women is also reported in other studies that conducted in Vietnam (100).

Thus, spending more time with the child, delaying in TB diagnosis in women can explain our findings that risk of TB infection for those who had mothers with tuberculosis was 20 times and 14 times higher compared to those who had other relatives with tuberculosis based on INF gamma assay and TST respectively (Table 4.10 and 4.11)

Regarding variables of exposure to male and female, overall, we could not find an association between exposure to male and female with being infected. But when we analyze the data separately by geography as rural and urban area, we found a significant association between exposure to male and female with being infected in the group of those who come from rural areas (OR= 3.708; 95% CI 1.354-10.158; p = 0.011 and OR = 3.329; 95% CI 1.349-8.218; p = 0.009) based on INF gamma assay and TST respectively) This finding might be explained by the effect of confounding variables such as: the differences in living standard, access to health care services, women's position between urban and rural areas.

Age of source case

In Vietnam, especially in rural areas, people usually live in the extended families including three or four generations, sometimes living under in the same roof. But by nature, children tend to be close to their parents than grandparents explaining for the finding that age of source cases is associated with tuberculous infection among contacts (Figure 4.4). Our finding agree with the finding from Borg dorff et all that the transmission from index cases tends to decline with increasing age of tuberculosis cases(101).

Duration of cough and Sputum smear grade

Basically, tuberculosis can spread from person to person though the air by drop nuclei. Drop nuclei are produced when a person with pulmonary tuberculosis coughs or sneezes. If someone inhales bacilli with tuberculosis patients, they may be infected. Thus, household contacts exposed to tuberculosis patients with positive smear sputum were more likely to have tuberculous infection. Many studies on contacts indicate that the risk of being infected increase with the density of bacilli on sputum of index cases (14;24;79;99). Our study also confirmed that those who were exposed to tuberculosis patients with 3+ positive smear grades were 4.5 times and 9 times more likely having TST and INF gamma assay positive, compared to those who were exposed to tuberculosis with 1+ positive smear grade (Table 4.12-4.13).

Delay diagnosis of tuberculosis due to low quality of health care, irrational use of drugs, promoted by pharmacist or private doctors is reported in several studies from Vietnam (100;102). It was also revealed that 70% of symptomatic men and women did not seek hospital care for their cough in the first three weeks (100). Longer delay in TB diagnosis was leading to increased risk of transmission (103). Our study also found the risk of being positive INF gamma assay in children exposed to index cases had duration of cough before TB treatment over 4 weeks was 7.5 times and 2.8 times more likely to have INF gamma assay and TST positive compared to those exposed to index case had duration of cough less than 4 weeks (OR=7.553; 95% CI 2.965-19.24; $p < 0.001$ and OR= 2.863; 95% CI 1.398-5.862; p=0.004) (See table 4.16)

Economic Status

The association between tuberculosis and poor socioeconomic factors has been reported by WHO(50). People with low socioeconomic status tend to live in crowded conditions, inadequate sanitations that lead to the increasing of TB transmission. Moreover, poor economic status may also be a barrier in accessing health care services, and this prolongs the period of infectiousness of tuberculosis patients, further increasing the risk of being infected among their contacts. This inter-relationship may explain our finding that poor income was found to be significantly associated with TST and INF gamma positive response among contacts (See table 4.16).

CHAPTER 6

CONCLUSION AND RECOMMENDATION

6.1. Conclusion:

This study has made an effort to investigate risk factors for tuberculosis transmission among household contacts and to compare two methods of diagnosing tuberculous infection. The survey with 128 tuberculosis families has reached some following conclusions:

- In child contacts of infective TB patients, TST results (with 10 mm induration as cut-off) seems equally good as IFN gamma assay to identify infected individuals. The risk factor profiles for both tests were similar and agreed with the known risk factors for developing active TB in this group. However TST identified a slightly larger group of infected children than INF gamma assay, probably due to its lower specificity. TST still was found to be a good tool to identify infected children in households with infectious TB.
- Findings from our study revealed the higher proportion of tuberculous infection among children contacts compared to general population. Analysis of risk factors for having a positive TST or INF gamma assay also showed that the most important index cases to be used as entry point for contact investigations were highly smear positive, mother with tuberculosis and poor income. Thus, the analysis can be used to prioritize a stepwise implementation of contact investigation to prevent childhood TB

6.2. Recommendation:

Given the affordable price and good technical skills among health personal in doing TST, National TB program should consider implementation of contact investigation as part of routine program. A stepwise implementation could start with smear positive mothers caring for children at home. An awareness program to prevent TB infection among children contacts from tuberculosis cases in family should be considered. This could contribute significantly to reduction of TB among children in Vietnam.

Annex 1

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CONSENT FORM

I am …….. from a research team established by Department of International Health, University of Oslo-Norway, Department of Immunology and Molecular Biology - National Institute of Hygiene and Epidemiology, and Hanoi Pulmonary Hospital. I am here to conduct a study Tuberculosis infection among children contacts of tuberculosis patient admitted to Hanoi Pulmonary hospital. The study is trying to find out the factors associated with tuberculosis infection among children contacts and the most effective method for diagnosis latent tuberculosis infection that we could apply in TB prevention program. Since the child is too young to decide on his/her own, If you agree to participant into study, I would like to interview you, and ask you for your permission to collect blood sample and take tuberculin skin test from your child. The amount of blood sample is 2.5 ml, and it will be taken in provincial hospital under your permission.

I also have few questions about tuberculosis and related issues. Your answers will be written and then used for analysis. All information you provide will be handled as confidential and your individual answers will not be known, excepting the interviewer and the coordinator of this study. Your contact details are held on an electronic database for study management purposes only, you could inspect this information. The results will be used only to improve strategies for prevention of tuberculosis, one of the most burden diseases leading to mortality and mobility in Vietnam.

We will need at least 30 minutes to discuss and record the information. You can withdraw from the study at any stage without any consequence for treatment if you do not wish to continue.

Will you participate in this study? Yes □

Do you have any question?

Thank you.

Date: …/…/200...

Interviewee s signature:

Interviewer s signature:

QUESTIONNAIRE

The personal information provided will be used only to make the results more meaningful. It will not be used to identify you and your children contacts in any way.

I. INFORMATION OF TUBERCULOSIS CASES.

1. Participated code: ……..

2. Date of birth ……

3. Sex (Put √ in the applicable box) Male ☐ Female ☐

4. Education: ☐
   1. Primary school
   2. Secondary school
   3. Bachelor

5. Maried status ☐
   1. Single
   2. Married
   3. Divorced

6. Occupation ☐
   1. Peasant
   2. Employed

7. Do you have any children under 15 of age living with? ☐
   1. Yes
   2. No

   If yes, how many?..... ☐

8. Do you have any tuberculosis treatment before?
   1. Yes
   2. No

   If yes, when? Where? How long and result?

   …….

   …….

9. How long (Weeks) do you have symptom of cough before having Tb treatment ☐
   1. From 1 to 4 weeks
   2. Over 4 weeks

10. Do you spit sputum? If yes, how long ? ☐
    1. From 1 to 4 weeks
    2. From 5 to 10 weeks
    3. More than 10 weeks

11. X-ray examination ☐
    (Size of pulmonary lesion is categorized according National Tuberculosis Program, Vietnam)
    1. Small
    2. Medium
    3. Large

12. Sputum examination: ☐
    1. scanty

* Microscop result: 2. +

Categorized according National Tuberculosis Program, Vietnam 3. ++

4. +++

* Cultured result ☐ 1. Negative

2. Positive

13. Do you have other family members with tuberculosis ?

If yes, who, when, where and how long?

……..

……..

II. INFORMATION OF CONTACTS

1.Participated code: ……..

2. Child's age: …… Date of birth…….

3. The child's sex (Put √ in the applicable box) ☐ Male☐ Female☐

4. How does your child have relationship with TB patient? ☐ 1. Mother

2. Father

3. Grandfather

4. Grandmother

5. Others

5. Does your child have BCG scare? ☐ 1. Yes

2. No

6. Does your child have any tuberculosis treatment before? 1. Yes

If yes, when? Where? How long and result? ☐ 2. No

…….

…….

7. Your child's weight and height? (If under 5 of age)

……..

8. Your family's income per month:

(Total family's income devides for family members)

9. How many children are there in your house ? ☐

10. How many people are living in this house? ☐ 1. From 1 to 4 persons

2. More than 4 persons

11. How many rooms are there in your house? ☐ 1. Have one room

2. Have more than one room

12. Room of index case ventilation status ☐ 1. Good (have more than one opened window)

2. Bad (no window)

13. How often your child keep contact with TB patient? ☐ 1. Sleep in different room and bed

(for the last two months from now)

2.Same room, different bed

3. Same bed

14. Activities share with TB case? ☐ 1. Part of day

(For the last two months from now) 2. Most part of day

15. Does any your family members smoke? ☐ 1. Yes

If yes, do they often smoke at home? 2. No

……..

……..