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**Comparison Of Cytokines And Chemokines In QFT Positive And Negative
House Hold Contacts Of TB Patients**

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Comparison Of Cytokines And Chemokines In QFT Positive And Negative House Hold Contacts Of TB Patients

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Abstract

Background: Tuberculosis (TB) is a highly contagious bacterial infection caused by *Mycobacterium tuberculosis*(MTB). It is the leading cause of death worldwide resulting in approximately 1.6 million deaths annually, yet only a small fraction of those infected with TB ever develop the illness depending on the host's immune status. Our immune system fight pathogens by secretion of chemicals called cytokines. Cytokines are substances released to help our bodies fight infection and their levels can determine if we are infected or not. These cytokines serve multiple purposes: they stimulate the immune response; inhibit bacterial growth; and induce cell-death. Together these actions protect us from MTB infections by eliminating infected cells and limiting further spread of bacteria.

Objective: Comparison Of Cytokines And Chemokines In QFT Positive (LTBI) And Negative House Hold Contacts Of TB Patients

Methods: The study was conducted inhouse hold contacts of TB patients and Community controls from selected health facilities in Addis Ababa, Ethiopia. A Comparative cross-sectional study was used from April 2022 to September 2023. Study involed three groups; Household contacts of TB patients (QFT Negative and QFT positive group(LTBI)) and Community controls with total sample size of 65 were recruited. Socio demographic data was collected using structured questionnaires and blood sample of participants were drawn and the QuantiFERON assay which contains TB antigen tubes(TB1 and TB2) coated with ESAT-6 and CFP-10 peptides to stimulate IFN- γ production. The QuantiFERON assay was performed on House Hold contacts of TB patients (QFT Negative group 16 and QFT positive group(LTBI) 21) and Community controls (CC of QFT Negative group) 28 participants. Cytokine analysis was performed using Luminex technology. Statistical analysis was done using GraphPad Prism 9.5 and Statistical Package for Social Sciences (SPSS). The unstimulated level (Nil) and the background-corrected TB stimulated level (TB1 and TB2 -Nil) of each marker were included as separate variables in the statistical analyses. The Kruskal-Wallis test (the ANOVA one-way non-parametric multiple comparison test coupled to the Dunn's multiple comparison test was used to detect differences between the study groups. A p-value <0.05 with a 95% confidence interval was considered statistically significant.

Results: When assessing socio-demographic, behavioral, clinical and hematological parameters of all participants, the majority were female including 82% (n=23) of CCs, 76% (n=16) of LTBI,

and 69% (n=11) of . HHC QFT negative Regarding age distribution, the majority of participants across the three groups were in the 26-35 years category, with 29% of CCs, 43% of LTBI, and 38% of HHC QFT negative in this age range. In terms of BCG vaccination scar, 46% of HCCs, 33% of LTBI, and 56% of resistors had a visible scar present. Latently infected participants have shown higher levels of proinflammatory molecules like IFN- γ (p<0.0001), IFN- α (p=0.0074), IL1RA(p<0.0001), IL2(p<0.0001), IL12(p=0.0464), IL17A(p=0.0002), IL17F(p=0.0015) compared to HHC QFT negative and Community Controls. Likewise, the level of chemokines like MCP-1(p<0.0001), MIP-1 β (p<0.0001), IP-10(p<0.0001), and MIG(p<0.0001) were significantly higher in the LTBI group as compared to HHC QFT negative and Community Controls. The growth factors FGF basic(p=0.0175), GCSF(p=0.0009), GMCSF(p<0.0001), VEGF(p=0.0104), as well as immunoregulatory cytokines like IL-2R(p=0.0002), IL-13(p=0.0172), IL-15 (p=0.0248) and IL-9(p=0.0361) and anti-inflammatory cytokine IL-22 were also significantly higher in HHC QFT negative groups compared with the other two groups. The following cytokines have no statistically significant difference between the groups (p> 0.05) TNF- α , IL-1 α , IL-1 β , IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, RANTES, MIP-1 α , Eotaxin, EGF, HGF.

Conclusion: The observed cytokine profile difference indicates that LTBI individuals show a distinct immunological signature that distinguishes them from HHC QFT negative and Community Controls in that they have heightened proinflammatory cytokines, chemokines, growth factors, and immunoregulators and Anti-inflammatory cytokines. This indicates a sustained activation of the immune system in LTBI individuals which controls the infection from activation. Our findings could help in understanding the interaction of the immune system with MTB infection for therapeutics and vaccine delivery and development

Recommendation: Further research should delineate immune cell phenotypes and antimicrobial functions across infection stages to identify correlates of protection.

Keywords: TB, Cytokine and chemokine , QFT Assay, Luminex Assay.

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Acronyms and Abbreviations

AAERC = AHRI-ALERT Ethics Review Committee

AHRI = Armauer Hansen Research Institute

BCG = Bacilli Calmette-Guerin

CC = Community Control

CD = Clusters of differentiation

CFP-10 = Culture filtrate protein 10 kDa

EC = Early Clearance

EGF = Epidermal growth factor

ESAT-6 = 6-kDa early secretory antigenic target

FGF-Basic = Fibroblast growth factor –basic

G-CSF = Granulocyte- colony-stimulating factor

GM-CSF = Granulocyte-macrophage colony-stimulating factor

HGF = Hepatocyte Growth Factor

HHCs = Household Contacts

HIV = human immunodeficiency virus

IFN- γ = Interferon- gamma

IGRA = Interferon- gamma release assay

IL = Interleukin

IP-10= Interferon-gamma inducible protein of 10 kDa

IRTBHHC = Immune responses to mycobacterial antigens in house hold contacts of Tuberculosis patients

LTBI = Latent Tuberculosis Infection

MCP-1 = monocyte chemoattractant protein-1

MIG = Monokine

MIP -1 alpha = Macrophage inflammatory protein1 alpha

MIP -1 batá = Macrophage inflammatory protein-1 batá

Mtb =Mycobacterium Tuberculosis

PPD = Purified protein derivative

QFT –Assay =QuantiFERON –TB Gold

SOP = Standard Operating Procedure

SPHMMC = St. Paul's Hospital Millennium Medical College

SPSS = Statistical Package for the Social Sciences

TB = Tuberculosis

Th -1 = T- helper type 1

TLR s= Toll-like receptors

TNF = Tumor Necrosis Factor

TNF-alpha- Tumor Necrosis Factor alpha

TST= Tuberculin skin test

VEGF =Vascular endothelial growth factor.

WHO = World Health Organization

1. Introduction

1.1 Background

Tuberculosis (TB) is a serious bacterial disease caused by *Mycobacterium tuberculosis* (MTB) that primarily affects the lungs, but it can also affect other organs such as the kidney, spine, and brain (1). Transmission of TB occurs via in air droplets, which are small enough to remain airborne and disperse in the air(2,3). The disease is more common in adults, accounting for 90% of total reported cases, and is reported more frequently in males than females(4). According to the 2023 WHO Report, about 10.6 million people fell ill with TB in 2021, representing a 4.5% increase from 2020. Over one fourth of world's population contracted TB but do not develop TB. Additionally, 1.6 million people died from TB in 2021, including 187,000 among HIV-positive individuals. The burden of drug-resistant TB (DR-TB) also increased by 3% between 2020 and 2021, with 450,000 new cases of rifampicin-resistant TB (RR-TB) in 2021(2,5).

Although approximately one-quarter of the world's population may have been infected with MTB, only 5-10% will ever develop active TB disease. Following MTB aerosol exposure, three potential clinical outcomes are described. The first possible outcome is resistance to infection or early clearance, where adaptive immunity has not yet developed and clearance relies on innate immune functions such as alveolar macrophage activation. Another outcome is asymptomatic or latent tuberculosis infection (LTBI), where adaptive immunity contains but does not eliminate bacteria sequestered within lung granulomas for decades with an approximated 10% lifetime risk of reactivation into active disease when immunity wanes. Conversely, insufficient immune control permits unchecked bacterial replication in macrophages, causing active pulmonary TB disease (6–8).

A significant number of people with high MTB exposure may not have LTBI that can be detected by conventional Tuberculin Skin Test (TST) or Interferon Gamma Release Assay (IGRA) (9). Protection against LTBI is dominated by CD4 and CD8 T cells. T cells immune response restrict the exponential growth of MTB and are responsible for the establishment of a plateau level of bacterial load in infected tissues, which usually develops 3–4 weeks after infection and remains at a constant magnitude for months through cellular immunity mechanisms such as macrophage activation, cytotoxic effects, and cytokine production (9–15).

CD4 T cells are involved in all phases of the acquired immune responses and are pivotal for the control of MTB during the plateau phase. In the absence of CD4 T cells, MTB maintains exponential growth without the formation of a stationary phase. Bacterial replication exacerbates pathology and reduces survival time. The majority of specific CD4 T cells are Th-1 cells, and IFN- γ and TNF- α produced by these cells are considered central for protection. Protective mechanisms mediated by CD8 T cells include cytotoxicity against infected cells as well as the production of Th1 cytokines such as TNF- α and IFN- γ (9–11,13–15)

Cytokines are small proteins that our bodies produce in response to infection by pathogens such as the MTB. These cytokines help stimulate and regulate the immune system's response, including the production of other cytokines needed to fight the infection. The cytokines also act directly on infected cells to inhibit bacterial growth, enhance cell-mediated immunity, and trigger apoptosis (programmed cell death). This helps protect our body from TB infections by clearing out infected cells, preventing further spread of the bacteria. In particular, IFN- γ is essential for sensitizing macrophages to recognize MTB, inhibit its growth and contribute to an overall effective immune response. In addition, both TNF- α and IFN- γ have been shown to not only initiate an inflammatory response but also promote activation of CD4⁺ T lymphocytes(16–18).

Moreover, pro-inflammatory cytokines have been shown to not only initiate an inflammatory response but also promote activation of CD4⁺ T lymphocytes. However, excess production of inflammatory cytokines can potentially cause tissue damage. To counteract this, regulatory cytokines help resolve inflammation and maintain immune homeostasis. Growth factors also play a role in regulating immune cell proliferation and activity. Chemokines help recruit immune cells like lymphocytes and macrophages to sites of infection. The coordinated interplay between pro-inflammatory cytokines, regulatory cytokines, growth factors, and chemokines is important for mounting an effective immune response to antigens like those from MTB, while minimizing negative effects. Moreover, cytokines mediate significant cytotoxicity against MTB by stimulating the proliferation of cells that recognize specific mycobacterial antigens. This proliferative response leads to an increase in cells capable of killing infected macrophages. Although there is a lot of evidence regarding cytokine production in TB as well as in LTBI, there is a gap of knowledge as to the cytokine production in the QFT Negative of house hold contact of TB patients. Hence this study was conducted to evaluate antigen specific cytokine production in household contacts of TB patients (16–19).

1.2 Statement of the Problem

MTB infections is the leading cause of death from infectious diseases in Africa and an estimated one-fourth of the world's population have had MTB infections, causing over 10 million new cases and more than 1 million fatalities annually. Although preventive measures such as vaccination and medication therapy have been implemented, the growing prevalence of anti-TB drug and difficulty in diagnosing and treating make TB a severe global health problem with an economic burden. Moreover, the lack of effective vaccine and inability to identify latently infected individuals who are likely going to progress to active TB hinder understanding of protective mechanisms involved in development of a better vaccine, or finding better biomarkers for early diagnosis or progression from latency to disease (4,20–22).

Despite advancements made regarding the research concerning QFT negative and QFT Positive , very few studies have been conducted to understanding how these groups differ in their immune responses. Analysis of antigen specific cytokine production during TB infection provides insight into resistance against infection (21,23).

1.3 Rationale of the Study

Globally, TB is the leading cause of mortality from an infectious agent where more than 1.6 million people die out of it every year. However, majority of the MTB infected population globally clears the bacteria and only 5-10% develops active TB disease. This indicates that the immune system is able to control the infection in the majority of cases, and only a small proportion of those infected go on to develop the active disease. Understanding this scenario and revealing the mechanism to clearance may contribute to development of better vaccines and prophylaxis.

In this study, we aimed to investigate antigen-specific cytokine production during tuberculosis exposure and analyze the difference in antigen-specific cytokine production between the QFT Negative and QFT Positive house hold contact of TB patients . This information could potentially help elucidate disease mechanisms, support diagnosis, and inform therapeutic approaches for mitigating later stage TB symptoms in those at high risk.

Data gathered from this study would be used to better understand how antigens specific cytokine contribute to host immune response, and how these results can be applied to develop interventions that could prevent or reduce the spread of TB.

2. Literature Review

2.1 Epidemiology of TB

Tuberculosis (TB) remains a significant global health challenge, with nearly 1.6 million lives lost each year and 10 million new cases annually worldwide. According to updated global report 2023, the estimated number of people falling ill with tuberculosis increased by 4.5% in 2021 reached 10.6 million (95% UI: 9.9–11 million). Furthermore, the tuberculosis incidence rate (new cases per 100 000 population per year) has increased by 4.5% between 2020 and 2021, after 2 decades of decrease of around 2% per year. Despite this reversal, the net reduction in cases since 2015 only reached 10%, leaving a 50% reduction to the first milestone of the End Tuberculosis Strategy (2,4,20,22).

Africa carries an exceptionally high burden of MTB infection compared to other regions. Recent surveys estimate the prevalence of MTB in the general population across Africa is between 258 and 402 cases per 100,000 population. This is more than double the global average prevalence of 140 per 100,000. Sub-Saharan Africa accounts for the majority of MTB cases on the continent. High rates of HIV co-infection, which fuels MTB transmission, along with overcrowded living conditions in urban areas contribute to widespread community transmission(2,4,24). Poverty, malnutrition, and limited access to healthcare also drive prevalence upward in parts of Africa. Efforts to control MTB are further challenged by lack of diagnostic facilities in many settings as well as emergence of drug resistant strains. While a few countries such as The Gambia and Ethiopia are seeing declines in MTB prevalence, the disease remains entrenched at the population level across much of Africa. Concerted efforts in active case finding, expanded access to molecular diagnostics, and research on new vaccines are needed to alter the MTB prevalence trajectory(4,24,25).

Ethiopia, is one of the 30 nations with a heavy TB/HIV burden, in 2019, Ethiopia's TB incidence was 157 per 100,000 people, with 23,800 deaths from the disease. The prevalence of MDR-TB was 0.71% in newly diagnosed cases and 12.0% in previously treated patients. A study in Eastern Amhara, Ethiopia, showed a decrease in Mtb prevalence from 13.9% in 2015 to 8.8% in 2017, then a slight increase to 9.5% in 2018 and 9.3% in 2019. Another study in Addis Ababa found that the prevalence of pulmonary and extrapulmonary TB was 46.0% among people with chronic

psychotic disorders. Ethiopia had an estimated TB incidence rate of 140 per 100,000 populations and 21,000 TB deaths in 2018. The age-standardized TB incidence rate decreased from 201.6/100,000 to 88.5/100,000 between 1990 and 2016. Despite the consistent decline in the tTB incidence rate, the decline in TB mortality in Ethiopia has been slower than the decline in incidence. However, Ethiopia is on track to achieve the WHO End TB milestone(26–31).

2.2 Transmission and risk factors of tuberculosis

TB is a highly contagious disease that can be contracted through the inhalation of the bacillus released in the air by coughing, sneezing, or talking of those who have active TB (32). The bacteria enter the lungs and begin to replicate, forming a primary lesion. From there they are able to spread to lymph nodes, as well as other parts of the body such as the meninges, joints and kidneys (20,33). According to the World Health Organization (2004), two weeks of coughing, producing sputum and weight loss are key signs of pulmonary TB. Other symptoms, such as chest pain, hemoptysis, breathlessness, fever, night sweats, fatigue and loss of appetite can also be present in persons infected with this condition (34).

There are several risk factors that might influence the occurrence and spread of TB, according to a number of studies. The most significant is HIV, which has been discovered to be a separate risk factor that has a particularly strong link to active pulmonary cases of TB (35). It has indicated that age can be a contributing risk factor to the development of TB, as well as a higher rate of susceptibility in men than women. A correlation has been found between TB risk factors, such as overcrowding and financial hardship, and incidence rates of the illness in industrialized countries. Furthermore, economic and financial obstacles can impede access to health care services for diagnosis and completion of treatment, leading to catastrophic costs for nearly half of all TB patients and their households(20,36,37) .

2.3 Out come after tuberculosis exposure

After inhaling aerosols containing MTB, individuals can experience three different potential outcomes. These are early elimination of the bacteria, the development of latent (asymptomatic) tuberculosis where the bacteria remain dormant for years, or active disease that can lead to pulmonary problems and the spread of infection (35).

Latent TB infection (LTBI) is an asymptomatic, long-term condition in which a person has been infected with MTB but not yet developed active TB disease. The concept of LTBI has been known for more than 60 years and involves a persistent immune response to antigens of MTB without evidence of clinical manifestation as active tuberculosis disease. Some individuals with LTBI will progress to active disease while others will remain without any symptoms or illness indefinitely. People with LTBI may be at higher risk for progression to active tuberculosis due to co-existing medical conditions such as HIV infection, diabetes mellitus, and malignancies. The highest number of new tuberculosis cases per year can be expected in individuals with latent tuberculosis infection. A systematic review and meta-analysis reported Household contacts (HHCs) of tuberculosis cases are more likely to acquire latent tuberculosis infection (LTBI) than the general population, with prevalence ranging from 34.8% in high-income countries to 52.9% in lower and middle-income countries(7,38–41).

Early Clearance (EC) of MTB has been postulated to occur when the bacteria have been successfully removed before a body's immune system can mount a response. This theory is evidenced by contact studies indicating that individuals in heavy contact with MTB do not always become infected, as well as through genetic expression markers associated with individual reactions to tuberculin. EC largely depends on alveolar macrophages being able to kill the bacteria at an initial stage of infection, and cytokines or chemokines produced by these macrophages activate target cells for further infection. It was reported an effective early clearance process of MTB can be achieved through various approaches such as phagocytes activation, cytokine responses and macrophage functions. A study found that individuals with a T-cell count greater than 1,000 cells per mm³ were substantially less likely to develop active tuberculosis than those with lower numbers (42,43).

2.4 Antigen specific cytokines related to TB exposure

In regard to tuberculosis, antigen-specific cytokines are integral in helping detect the presence of an infecting bacteria (MTB). These cytokines can be detected not only in infected individuals but also in those exposed through close contact to an infected person in a household setting. It has been found that production of specific cytokines for example: IFN- γ , TNF- α , IL-2, and interleukin-17A (IL-17A) are frequently seen among household contact TB patients but not necessarily in symptomatic individuals(44). IL-2 levels were significantly higher in those infected with both

active TB and LTBI than in TB-uninfected controls, though it was not sensitive enough to differentiate between active TB and LTBI(45).

Cross-sectional study done in Ethiopia including newly diagnosed smear-positive and smear-negative PTB patients, non-TB patients, and healthy contacts with or without LTBI, finding that smear-positive PTB had higher MTB-specific TNF α and IFN γ CD4 T cells than smear-negative PTB, albeit not statistically significantly, and both smear-positive and -negative PTB had significantly higher antigen-specific cytokine responses than non-TB patients and LTBI- controls, with smear-negative PTB displaying higher levels of activation and proliferation markers on antigen-specific cytokine-producing CD4 T cells than controls and smear-positive PTB showing even higher cytokine, activation and proliferation marker expression than other groups, while consideration of the fraction of activated/proliferating cells among antigen-specific cytokine producers helped discriminate smear-negative TB from LTBI and non-TB illness(46).

The study investigated the cytokine responses (IFN- γ , TNF- α , IL-10) to the latency antigen in active TB patients, their household contacts, and community controls in Ethiopia. At baseline, specific cytokine responses were highest in controls, intermediate in contacts, and lowest in untreated TB patients, suggesting they may be biomarkers of latent/protected TB status. IFN- γ /IL-10 and TNF- α /IL-10 ratios were lowest in untreated TB patients and highest in controls, suggesting these ratios may indicate infection outcome. Contacts had significantly lower cytokine responses to ESAT-6-CFP-10 at baseline(47).

The Longitudinal study done in Addis Ababa Ethiopia aimed to identify plasma biomarkers to differentiate active TB cases from latently infected contacts and assess treatment responses. 17 cytokines/chemokines were evaluated in untreated TB cases, contacts and post-treatment cases via multiplex analysis. At baseline, TB cases had significantly higher levels of EGF, IFN γ , IL-4, MCP-3 and IP-10 versus contacts. No markers differentiated QFT-positive and -negative contacts, suggesting cytokine levels did not correlate with LTBI. After effective TB treatment, IFN γ , MCP-3, MIP-1 β levels increased while IL-4 and IP-10 decreased significantly. HIV coinfection did not affect cytokine levels in TB cases(48).

The study done in north Shewa Ethiopia a total of 80 study participants comprising of 40 newly diagnosed smear positive TB patients and 40 healthy blood donors were included in the study.

From our study participants 19 (23.75%) were females and 61(76.25%) were males with mean age of 30. Results from the study showed that the median level of IFN- γ (72.8) and IL-6 (13.45) in TB patients was higher when compared with controls IFN- γ (44.8) & IL-6 (4.08) ($P > 0.001$) while on the other hand, the median level of CD4 (565.5) and BMI (18.00) were lower compared to controls CD4 (857) & BMI (20.9) ($P > 0.001$)(49) .

Acquired resistance to mycobacteria involves a complex interplay of T cells, macrophages, cytokines, and Reactive nitrogen intermediates (RNI). IFN- γ , IL-4 and IL-6 trigger macrophage functions that are responsible for inhibiting the growth of mycobacteria. TNF- α stimulates the production of RNI while IL-10 suppresses it. Understanding this process better may lead to improved treatments for tuberculosis ((50)

The results of a study examining the cytokine response to exposure to MTB antigens in healthcare workers with latent infection found that those with a positive latent TB infection (LTB+) exhibited greater levels of proinflammatory cytokines GM-CSF, IFN- γ , IL-1 β , IL-2, IL-6, IL-8, IL-12p70 and TNF- α than those with a negative latent infection (LTB-). Although there were statistically significant differences detected between the two groups in terms of the th2 cytokines IL-5 and IL10, these absolute differences were relatively minor(21).

A study conducted in Uganda reported latently infected health care workers (HCWs) and community control (CCs) had significantly higher IFN- γ , IL-2, IL-5 and IL-8 responses than their non-infected counterparts, whereas high TB exposure in infected HCWs caused production of pro-inflammatory (GM-CSF, IFN- γ , IL-1 β , IL-2, IL-6, IL-8, TNF- α) and anti-inflammatory/ Th2 cytokines (IL-5 and IL-10) (39)

A study conducted in Ghana showed that a combination of different cytokines (IL-6, IL-10, and IP-10) can effectively distinguish between TB patients based on the levels of different cytokines in their plasma, namely IL-6, IL-10, and IP-10, and that IL-6 has potential as an early biomarker for treatment response. Four of the seven cytokines (IL-6, IP-10, IL-10, and IL-22) were detected in the majority of tuberculosis patients (51).

IL-4 expressed within MTB infected human macrophages can subvert the containment of mycobacterial infection by altering host TLR pathways and expression of cytokines(52). Another study reported the role of IL-10 in MTB infection and how it can modulate the processes of

inflammation and immunity. Furthermore, they found that other cytokines, such as TNF- α , IFN- γ , and CXC chemokine receptor 4/CXCR4 play an important regulatory role in MTB infection, acting as both stimulants for immune activation and mediators to inhibit inflammatory responses (53).

A study conducted in China investigates the patterns of cytokines and chemokine combined with IFN- γ production reflecting MTB infection and disease. The results demonstrate that the expression levels of these combinations not only differ between individuals with TB infection or disease, but also exhibit clear temporal dynamics throughout the course of the disease. Moreover, a combination of IFN- γ with IL-2, IL-10, IL-6, IL-9 was identified to be significantly associated with MTB infection or disease; this combination may provide diagnostic applications for TB screening or management (54).

In conclusion, the exact role of antigen-specific cytokines in tuberculosis infection has yet to be fully elucidated. However, there is increasing evidence that suggests the involvement of several cytokines may play a key role in promoting an effective immune response to TB and possibly also modulating the subsequent pathology. Although more research is necessary, some studies suggest that modulating cytokine signaling could be a promising approach toward treating TB infections. Additionally, further research should focus on elucidating how antigen-specific cytokines interact with other components of the innate and adaptive immune system to influence the course of TB infection.

3. Objective of the study

3.1 General Objective

- ✓ Comparison Of Cytokines And Chemokines In QFT Positive And Negative House Hold Contacts Of TB Patients

3.2 Specific Objectives

- ✓ To measure the level of cytokine and chemokin production among: QFT Positive And QFT Negative House Hold Contact Of TB Patients and Community controls.
- ✓ To identify antigen specific cytokines and Chemokin that can differentiate LTBI from HHC QFT Negative

4. Methods and Materials

4.1 Study Setting

The study was conducted among 7 Health Centers (HC) found in Addis Ababa, Ethiopia. Those were Jagemia Kelo HC (Nifasilk Lafto sub city), Alem Bank HC (Kofe- keranio sub city), T/Haimanot HC (Lideta sub city), Kofe HC (Kofe- Keranio sub city), Addis Raey HC (Addis Ketema sub city), Hana Mariam HC (Nifasilk Lafto sub city) and Meshualekia HC (Kirkos sub city). Addis Ababa is a capital city of Ethiopia with a population of over 4 million people, located at 9° 1' 48" north and 38° 44' 24" east. The city spans an area of 540 square Km and has an annual growth rate of 4.4%. It is divided into 10 sub-cities, which are in turn divided into 116 Woredas, making up the smallest government administrative units. Health centers in Addis Ababa are managed by Addis Ababa City Administration Health Bureau (AACAHB) and provide primary health care services(55–57).

4.2 Study Design and Period

A comparative cross-sectional study was conducted from April 2022 to September 2023 to compare Cytokines And Chemokines In QFT Positive And QFT Negative House Hold Contacts Of TB Patients

4.3 Source of Population

The source population was all household contacts of TB patients visiting the selected health facilities and all HIV negative individuals who seek for medical service at the ART department.

4.4 Study Population

The study population was household contacts (HHC) of TB patients who lived with newly diagnosed TB patients for 1 month and above. Based on the results of an interferon-gamma release assay (IGRA), they were categorized into two groups:QFT Positive (LTBI) and QFT Negative HHC. Additionally, Community controls (CC) who had not been directly exposed to TB were recruited from HIV-negative individuals at the ART clinics of the health centers. To ensure their overall health, all participants underwent a physical examination and answered health-related

questions administered by a data collector. HIV counseling and testing were provided to all participants, and HIV-positive individuals were referred to the nearest ART clinic. The HCC group underwent further testing using IGRA, and only those with negative results were included in the group. At the baseline visit, all participants provided blood samples for various assays, and a follow-up visit occurred after two months for retesting with IGRA to check if the “QFT Negative group” continue to remain as IGRA negative.(Figure 1)

4.4.1 Inclusion criteria

The participants in this study were eligible if they were 15 years of age or older and tested negative for HIV. Household contacts consist of those who live in the same house (under the same roof) as newly diagnosed TB patients, without TB signs and symptoms. Community controls were apparently healthy individuals with no chronic illnesses, no history of TB, and no known previous exposure to TB, especially in terms of co-habitation with TB patients.

4.4.2 Exclusion Criteria

Participants who were ill at the recruitment time, those under the age of 15, individuals who had active TB and displaying symptoms such as cough and fever during recruitment, pregnant women, participants with chronic conditions or experiencing severe pain or discomfort, individuals taking medication that modifies their immunity, such as Methotrexate, Chloroquine, and Chemotherapy, those with Helminth infection, participants with severe anemia, individuals on any steroid medication, and participants who do not provide consent were excluded. Additionally, participants aged 15 to 18 and whose parents do not provide consent were also excluded from the study.

4.5 Sample Size

The study initially identified 624 household contacts (HHC) of 266 active pulmonary TB index cases, among which 176 were eligible after excluding those who were pregnant, had traveled extensively, or could not provide consent. Of the eligible HHC, 101 attended visit one and 59 attended visit two. Additionally, 138 and 126 individuals were enrolled at visits one and two respectively among Community Controls (CC) with no known TB exposure. From these enrolled participants, QuantiFERON-TB gold testing identified 16 QFT Negative of House Hold Contac who were exposed but tested Negative, 21 LTBI with who tested QFT Positive, and 28 Community Controls who tested negative. Each of these 65 individuals then had their QuantiFERON

supernatant analyzed using the Cytokine Human 35-Plex Panel for the Luminex platform to measure levels of 35 cytokines and relate cytokine and chemokine profiles to differential outcomes of exposure.

4.6 Sampling Procedures

All eligible household contacts of TB patients and Community Controls were differentiated and shortlisted to be included in the study. A Convenient sampling technique was used to include the study participants.

4.6.1 Dependent variable

The dependent variable in this study is the analysis of mean antigen specific cytokine and chemokine concentration.

4.6.2 Independent variable

The independent variables in this study include QFT Positive and QFT Negative of household contacts of TB patients, socio-demographic factors, physical examination results, and hematological profiles. These variables serve as key components for the analysis and exploration of the study's objectives.

4.6.3 Instrument

During sample collection and laboratory procedures, various instruments and aids were utilized, such as a questionnaire, QFT Gold Plus test tube, QFT PLUS ELISA kit, cytokine Human magnetic 35-plex panel for Luminex, disposable Vacutainer tube with needle, Luminex MAGPIX instrument, ELISA reader, Hematology Analyzer, Hemo Quest, and SPSS software version 26. All laboratory materials and equipment were used from the AHRI.

4.7 Operational Definition

- ❖ **Household contacts of TB (HHC):** are individuals living with an active TB Patients.
- ❖ **Community Controls:** are individuals with **no known contact** history with active TB patients.

- ❖ **HHC QFT positive:** House Hold contacts tested positive with Interferon-Gamma Release Assay (IGRA) from two consecutive visits but has no TB sign and symptoms.
- ❖ **HHC QFT negative:** House Hold contacts tested negative with IGRA from two consecutive visits despite exposure to an active case.

4.8 Data Collection Procedures

4.8.1 Data collection

The health centers were randomly selected and they are expected to have 1-2 TB patients flow per week. The data collectors used standardized questionnaires, consent and assent forms, and eligibility criteria to identify the study groups (household contacts and healthy community controls) at the health centers. Socioeconomic and demographic data about the participants were collected, along with the results of some physical exams. Additionally, a stool test was conducted to check for intestinal helminths. If a parasitic infection was detected, participants were referred to the outpatient department (OPD). Other tests included a chest X-ray, a pregnancy test for women, a hemoglobin test to check for severe anemia, and an HIV test. Within two hours of collection, the blood samples were transported to the AHRI laboratory. IGRA tests were conducted for household contacts and community controls, and household contacts were grouped into two categories (HHC QFT positive, HHC QFT negative) based on their IGRA results. (Figure 2)

4.8.2 Sample collection and laboratory procedure

IRTBHHC (Immune response in house hold contacts of Tuberculosis patients) project used the aseptic technique to collect 40 ml of whole blood from each study participant. From this 40 ml, 4 ml of whole blood was used for QuantiFERON-TB Gold (QFT) testing to differentiate the three groups: HHC QFT positive, HHC QFT negative, and CC QFT negative. QuantiFERON TB Gold plus (QFT-plus) blood collection tube (QFT-Plus Lot-059069551, GmbH, Germany) was used and the ELISA was done using QuantiFERON TB Gold plus (QFT-plus Lot-56904764, GmbH, Germany). Subsequently, human cytokines were quantified using QFT supernatants by the Cytokine Human 35-Plex Panel.(Figure 3)

4.8.3 Laboratory testing

QuantiFERON TB gold assay

The QFT-Gold (QuantiFERON-TB Gold) assay or IGRA is a blood test that helps in the diagnosis of tuberculosis infection when used together with clinical signs and symptoms. It measures the response of T-cells to specific TB antigens to detect tuberculosis infection. The test uses a peptide cocktail simulating ESAT-6 and CFP-10 proteins, which are unique to MTB, and not found in the BCG vaccine or most non-tuberculosis mycobacteria. As a result, the QFT-Gold assay is more specific than the traditional tuberculin skin test. QFT-Plus has two distinct TB antigen tubes: TB Antigen Tube 1 (TB1) and TB Antigen Tube 2 (TB2). Both tubes contain peptide antigens from the MTB-complex-associated antigens, ESAT-6 and CFP-10. Both the TB1 tube and TB2 tubes contain peptides from ESAT-6 and CFP-10 that are designed to elicit CMI responses from CD4+ T-helper lymphocytes; the TB2 tube contains an additional set of peptides targeted to the induction of CMI responses from CD8+ cytotoxic T lymphocytes. The test involves collecting 1ml of whole blood into specialized blood collection tubes containing negative and positive controls. After incubation and plasma removal, the concentration of IFN- γ (IU/ml) is measured, with a positive result indicated by an IFN- γ response in the TB Antigen tube exceeding any background from the Nil IFN- γ IU/ml value. The test can provide quicker results within 24 hours compared to other diagnostic methods, without the risk of false-positive results or side effects from live bacteria injections. However, the QFT-Gold assay does not diagnose TB on its own, but aids in diagnosis along with consideration of clinical signs and symptoms(58).

Antigen specific cytokine analyses by Luminex

The Cytokine Human 35-Plex Panel for the Luminex™ (Invitrogen Lot 310337-004, Austria) was used to quantify human cytokines, chemokines and growth factors in QFT supernatant. By measuring 35 analytes simultaneously, the Luminex assay panel helps provide more data from each sample. The Human Cytokine Magnetic 35-Plex Panel having reagents which include sensitive quantitation of human proteins including: EGF(Epidermal Growth Factor), Eotaxin, FGF-basic (basic fibroblast growth factor), G-CSF (granulocyte colony stimulating factor), GM-CSF (Granulocyte-macrophage colony stimulating factor), HGF (Hepatocyte Growth Factor), IFN-alpha(interferon alpha), IFN- γ (interferon gamma), IL-1 β (Interleukin-1 beta), IL-1 α

(Interleukin-1 alpha), IL-1RA (interleukin 1 receptor antagonist), IL-2 (interleukin 2), IL-2R (interleukin 2 receptor), IL-3 (interleukin 3), IL-4 (interleukin 4), IL-5 (interleukin 5), IL-6 (interleukin 6), IL-7 (interleukin 7), IL-8 (interleukin 8), IL-9 (interleukin 9), IL-10 (Interleukin-10), IL-12 (interleukin 12), IL-13 (interleukin 13), IL-15 (interleukin 15), IL-17A (interleukin 17A), IL-17F (interleukin 17F), IL-22 (interleukin 22), IP-10 (interferon gamma induced protein 10), MCP-1 (monocyte chemoattractant protein-1), MIG (monokine induced by interferon gamma), MIP-1 α (macrophage inflammatory protein-1 alpha), MIP-1 β (macrophage inflammatory protein-1 beta), RANTES (Regulated on Activation, Normal T Cell Expressed and Secreted), TNF- α (Tumor Necrosis Factor-alpha), VEGF (vascular endothelial growth factor). Antigen specific cytokine analyses by MAP Luminex involves collecting the sample, adding a cocktail of anti-cytokines antibodies, incubating the mixture for a period of time at an optimal temperature, binding the antigen to the beads conjugated with respective capture antibody, washing away unbound components, and finally analyzing the sample using a Luminex instrument to detect relative concentrations of various cytokines bound to its respective antibody (59).

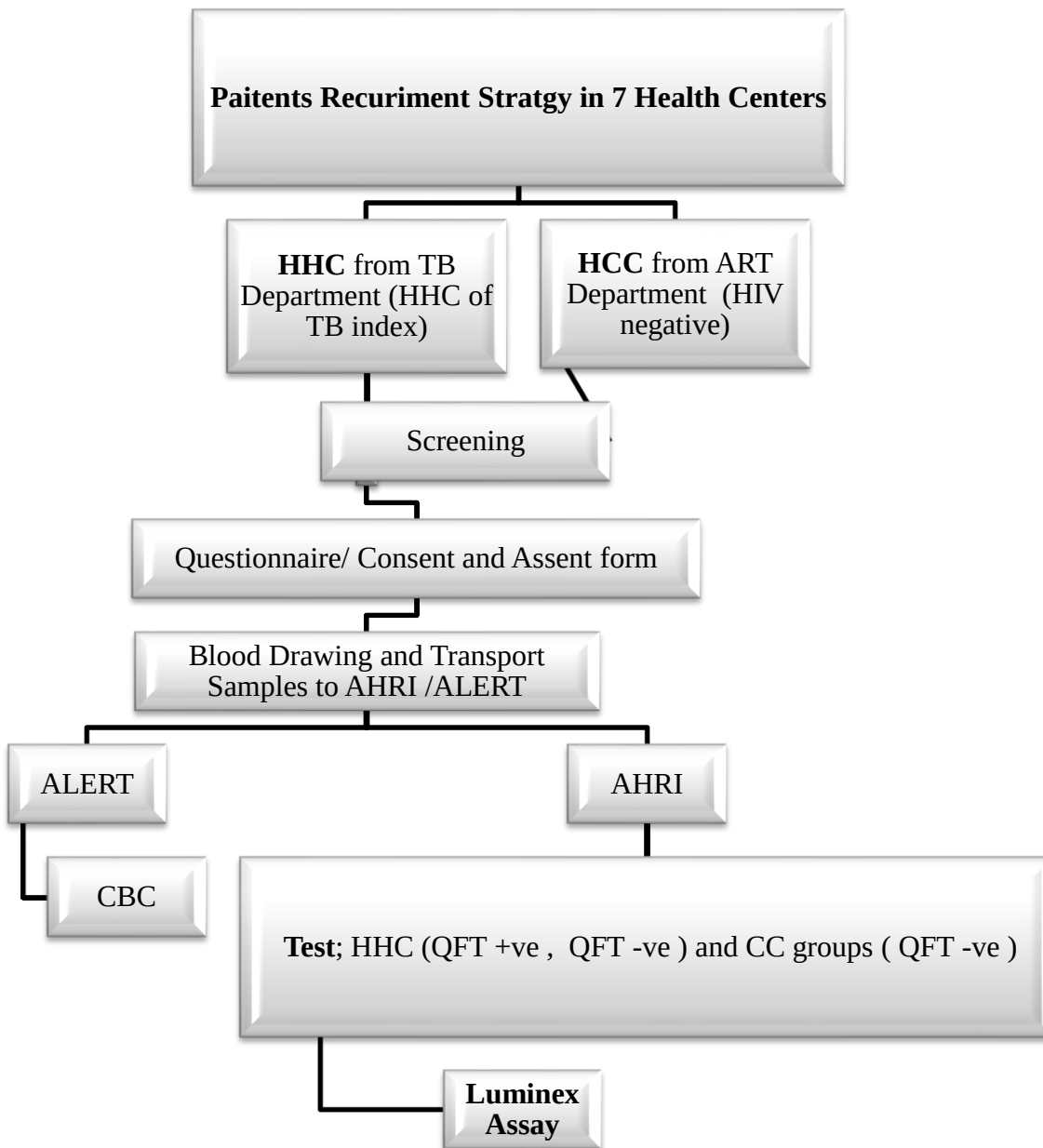


Figure 1. A flow chart that shows the participant recruitment, and the sample collection and processing steps

4.9. Quality Assurance

All the questions in the structured questionnaire were prepared in a valid and specific way. Data collectors were oriented, and the entire questionnaire was assessed for completeness during and after data collection by the data collectors. A pretest assessment was conducted to check for any unexpected limitations. Additionally, all laboratory assays were performed by maintaining quality control procedures. Standard operating procedures (SOPs) were strictly followed, confirming that reagents met expiration dates and quality control parameters. Assay results were cross-checked for completeness and validity before further analysis.

4.10. Statistical Analysis

All statistical analyses were performed using GraphPad Prism 9 and Statistical Package for Social Sciences (SPSS) statistics. The background-corrected mycobacteria-specific antigen-stimulated cytokine level (TB1 and TB2 Ags - Nil) was used in statistical analyses. The Kruskal-Wallis test (the ANOVA one-way non-parametric multiple comparison test coupled to the Dunn's multiple comparison test) was used to detect differences between the study groups. A p-value <0.05 with a 95% confidence interval was considered statistically significant. Statistical analysis with a Kruskal-Wallis test was used to determine differences in cytokine concentrations between test groups, and P-values were corrected for multiple comparisons with GraphPad Prism version 9 at an alpha level of $p < 0.05$.

4.11. Ethical consideration

Ethical approvals were received from the research and ethical review committee of the ALERT/AHRI and SPHMM prior to the study's execution. A formal letter was sent to all the relevant bodies in the study area to seek their assistance in supporting the study. Before collecting data, the data collectors were instructed on the objective, benefits, confidentiality, and risks of the study. During the interview, each participant was also informed of the study's objective and the confidentiality concerns and was asked for consent. For participants who were under the age of 18, parental or guardian consent was requested, and participant assent was also obtained. The use of study codes and restricted access to the data to protect participants and relevant ethical and regulatory bodies ensured the privacy and confidentiality of participants' data.

5. Results

5.1 General information of study participants

5.1.1 Socio-demographic characteristics of participants

The study included a total of 65 participants from three groups; 28 Community controls (CCs), 21 contacts with latent TB infection (LTBI), and 16 HHC QFT negative. Of all participants, the majority were female including 82% (n=23) of CCs, 76% (n=16) of LTBI, and 69% (n=11) HHC QFT negative. Regarding age distribution, the majority of participants across the three groups were in the 26-35 years category, with 29% of CCs, 43% of LTBI, and 38% of HHC QFT negative in this age range. (Table 1)

Table1. Socio-Demographic and behavioural Characteristics House Hold Contacts of Tuberculosis Patients mong Selected Health Facilities of Addis Ababa Ethiopia

Study participant	CC	LTBI	HHC QFT negative
Age category n(%)			
15-25	9(32)	8(38)	7(44)
26-35	8(29)	9(43)	6(38)
36-45	11(39)	2(9.5)	3(19)
>45	0(0)	2(9.5)	0(0)
Gender n (%)			
Male	5(18)	5(24)	5(31)
Female	23(82)	16(76)	11(69)
Marital Status n (%)			
Single	13(46)	11(52)	7(44)
Married	14(50)	9(43)	8(50)
Divorced	1(3.6)	1(4.8)	1(6.3)
Educational Status, n (%)			
No formal education	0(0)	1(4.8)	0(0)
Grade 1-8	4(14)	8(38)	3(19)
High school	12(43)	1(4.8)	2(13)
Diploma	8(29)	2(9.5)	4(25)
Degree and above	4(14)	2(9.5)	4(25)
Occupation, n (%)			
Civil Servant	22(79)	1(4.8)	3(19)
House wife	1(3.6)	3(14)	3(19)
Private Business	3(11)	10(48)	6(38)
Unemployed	2(7.1)	7(33)	4(25)
Monthly Income, n (%)			

Below 1500	1(3.6)	4(19)	3(19)
1500-3000	18(64)	2(9.5)	1(6.3)
3001-5000	4(14)	4(19)	7(44)
Above 5000	1(3.6)	2(9.5)	3(19)
Can't estimate	4(14)	9(43)	2(13)
Physical Exercise frequency			
Never	13(46)	19(90)	12(75)
Once in month or less	3(11)	1(4.8)	1(6.3)
2-4 times a month	5(18)	0(0)	0(0)
2 -3or more a week	6(21)	1(4.8)	3(19)
4 or more a weak	1(3.6)	0(0)	0(0)
Alcohol consumption n (%)			
Never	11(39)	21(100)	11(69)
Once in month or less	13(46)	0(0)	2(13)
2 -4 times a month	2(7.1)	0(0)	1(6.3)
2 -3or more a week	2(7.1)	0(0)	1(6.3)
4 or more a weak	0(0)	0(0)	1(6.3)
Chew chat, n (%)			
Never	28(100)	16(76)	15(94)
1 -2 days per weak	0(0)	5(24)	0(0)
5 -6 days per weak	0(0)	0(0)	1(6.3)
Relation with TB index , n(%)			
Family member	11(68.75)	19(90.48)	-
Relative	5(31.25)	2(9.52)	-
Close Friend	0(0)	0(0)	-
Over Past 3 month ,how often did you come in contact with the index case , n(%)			
1-3 day per week	0(0)	2(9.52)	-
4-6 days per week	1(6.25)	0(0)	-

7 days per week	15(93.75)	19(90.48)	-
On the days you had contact with the index case, how much time did you spend with the index case? , n(%)			
2-6 hrs a day	7(43.75)	5(23.81)	-
7-12 hrs a day	7(43.75)	12(57.14)	-
13-18 hrs a day	1(6.25)	2(9.52)	-
Over 18 hrs a day	1(6.25)	2(9.52)	-
Number of rooms , n(%)			
1 room	10(52.5)	7(33.35)	-
2 rooms	2(112.5)	6(28.57)	-
3 rooms	1(6.25)	6(28.57)	-
4 rooms	3(18.75)	2(9.52)	-

5.1.2 Hematological and Clinical investigation of participants

The median vital signs were generally similar between groups, with HHC QFT negative having slightly lower median systolic and diastolic blood pressures (110 and 74 mmHg) compared to CCs (120 and 80 mmHg) and LTBI (115 and 80 mmHg). In terms of BCG vaccination scar, 46% of CCs, 33% of LTBI, and 56% of QFT Negative of HHC had a visible scar present. For complete blood count measurements, QFT Negative of HHC had lower median hemoglobin (13.65 g/dL), hematocrit (42.8%), and red blood cell count (4.66 million/mcL) compared to CCs (14.8 g/dL, 42.9%, 4.79 million/mcL) and LTBI (14.9 g/dL, 44.8%, 4.99 million/mcL). (Table 2)

Table 2. Hematological and Clinical characteristics of House Hold Contacts of Tuberculosis Patients among Selected Health Facilities of Addis Ababa, Ethiopia

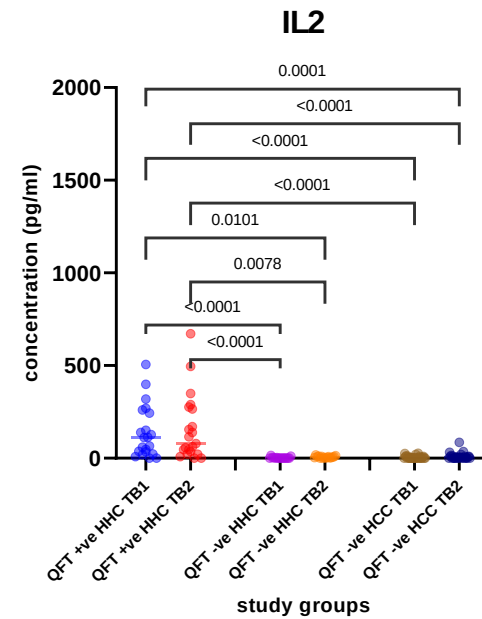
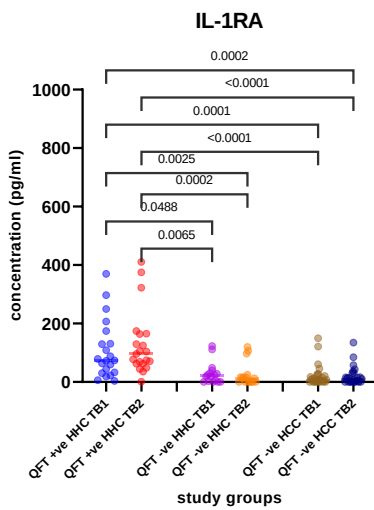
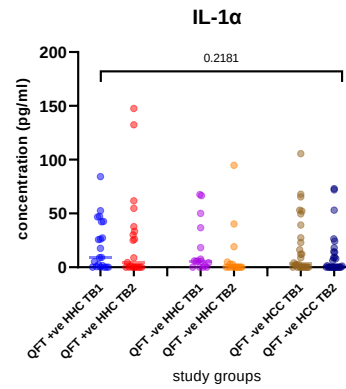
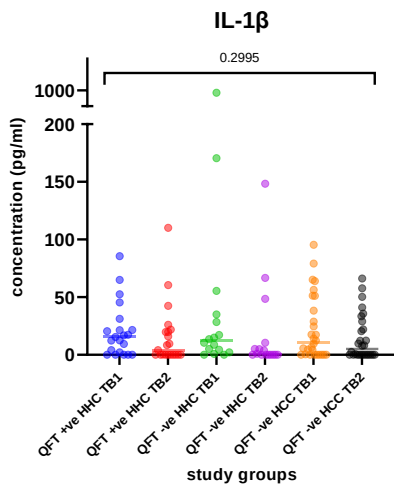
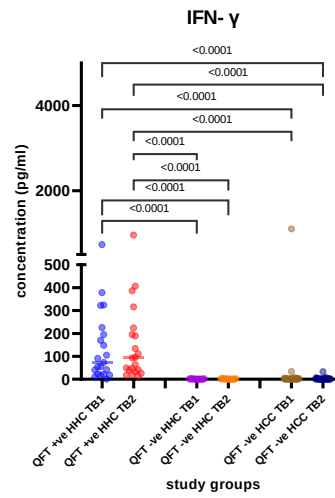
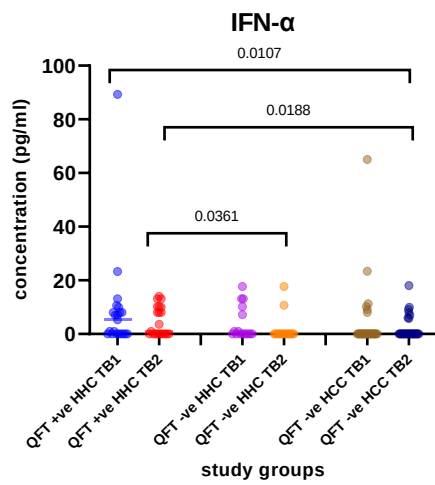
VARIABLE	CC	LTBI	HHC QFT negative
Temperature, median (IQR)	35.90(35.48-36.13)	35.80(35.60-36.30)	35.70(35.20-36.25)
Pulse, median (IQR)	76(72-80)	72(68-75)	73(70-78)
Systolic BP, Median (IQR)	120(110-125)	115(110-120)	110(110-120)
Diastolic BP Median (IQR)	80(70-83)	80(70-80)	74(70-80)
BMI, Median (IQR)	24.1(21.7-26.9)	21.2(18.4-24.2)	21.1(20.1-23.5)
COVID treatment n (%)			
Yes	7(25)	3(14)	3(19)
No	21(75)	18(86)	13(81)
COVID Vaccine n (%)			
Yes	23(82)	8(38)	3(19)
No	5(18)	13(62)	13(81)
BCG Scar n (%)			
Present	13(46)	7(33)	9(56)
Absent	15(54)	13(62)	7(44)

QFT Test n(%)			
QFT Negative	28(43.07)	0(0)	16(24.6)
QFT POSITIVE	0(0)	21(32.3)	0(0)
Hemoglobin, Median (IQR)	14.80(14.08-15.53)	14.90(13.50-16.20)	13.65(13.23-15.03)
HCT, Median (IQR)	42.9(40.8-45.5)	44.8(41.9-48.8)	42.8(42.5-43.7)
RBC Count, Median (IQR)	4.79(4.55-5.12)	4.99(4.69-5.42)	4.66(4.52-4.99)
WBC Count, Median (IQR)	5.65(4.58-6.58)	5.80(4.00-7.30)	5.60(4.08-6.43)
Lymphocyte, Median (IQR)	34(26-39)	35(24-39)	30(26-34)
Neutrophil, Median (IQR)	54(48-45.5)	57(44-69)	61(56-63)
Eosinophil, Median (IQR)	2.7(1.6-4.9)	2.5(0.9-3.7)	2.6(2.0-3.5)
Basophil, Median (IQR)	0.65(0.40-0.93)	0.50(0.30-0.70)	0.45(0.20-0.75)
Monocyte, Median (IQR)	6.70(5.38-8.00)	6.50(5.20-7.30)	5.90(4.95-7.08)
MCV, Median (IQR)	89.0(86.1-91.8)	89.1(87.8-91.8)	92.5(87.7-94.4)
MCH, Median (IQR)	30.45(28.95-31.10)	29.80(29.30-3.60)	3.65(3.28-31.45)
MCHC, Median (IQR)	33.80(33.28-34.30)	33.50(33.30-34.10)	33.75(33.05-14.05)
RDW, Median (IQR)	13.85(13.08-14.23)	13.70(13.20-13.90)	13.65(13.05-14.03)
RDWSD, Median (IQR)	43.1(41.6-44.6)	42.4(41.6-44.6)	43.8(42.2-46.0)
Platelet, Median (IQR)	256(186-321)	242(223-285)	271(247-302)

5.3 Cytokine production of the participants

5.3.1 The level of proinflammatory molecules was higher in LTBI group

We measured 35 different cytokines in the three groups HHC QFT negative, LTBI, and CC. The proinflammatory cytokines; IFN- γ , IFN- α , IL-1RA, IL-2, IL-12, IL-17A, and IL-17F were significantly higher ($p < 0.05$) in the LTBI group compared to the HHC QFT negative and CC groups based on Kruskal-Wallis testing. The LTBI group showed increased production of IFN- γ ($p < 0.0001$), IFN- α ($p = 0.0074$), IL-1RA ($p < 0.0001$), IL-2 ($p < 0.0001$), IL-12 ($p = 0.0464$), IL-17A ($p = 0.0002$) and IL-17F ($p = 0.0015$) in response to MTB antigens compared to the other study groups, However, IL-1 α , IL-1 β , IL-6 and TNF- α were not significantly different ($p > 0.05$) among the three study groups.(Figure 2)



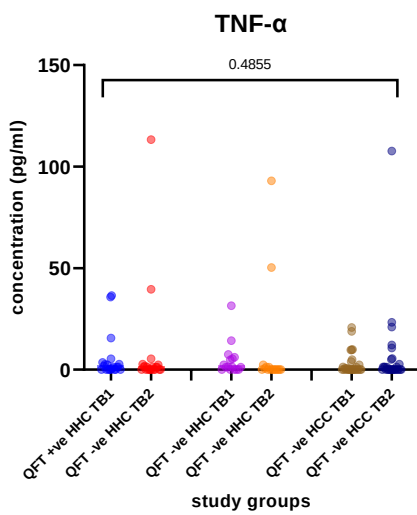
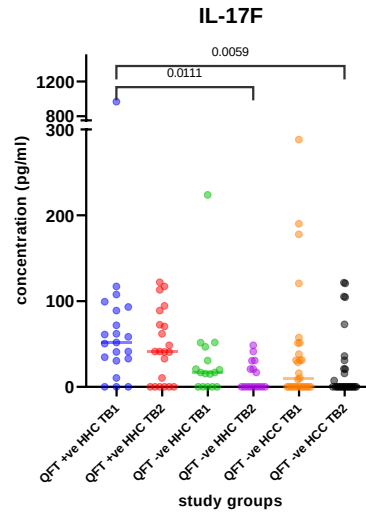
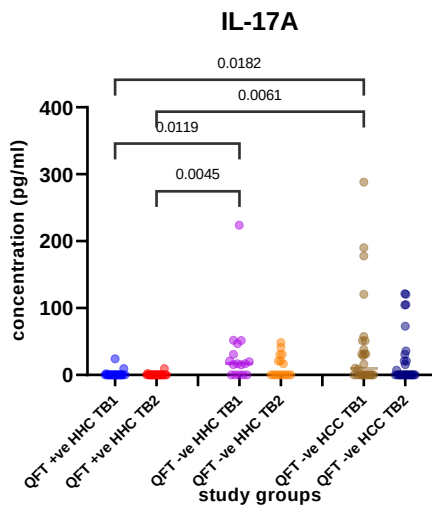
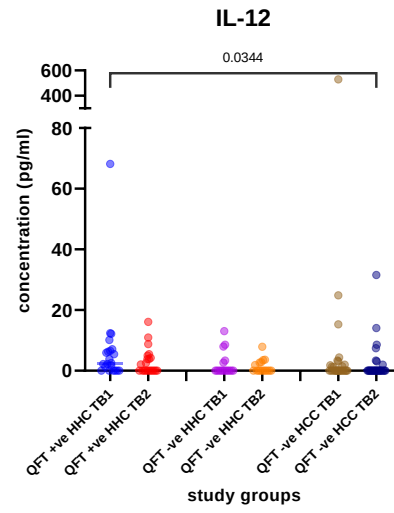
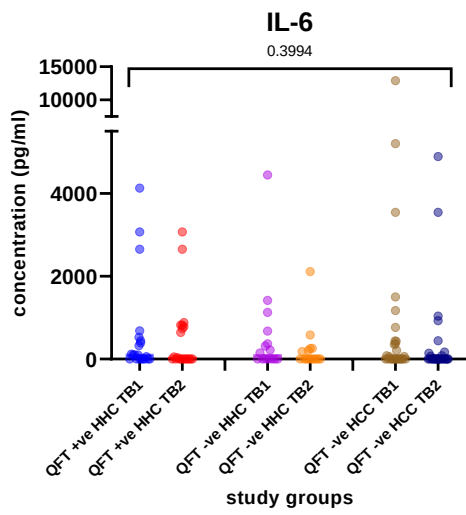
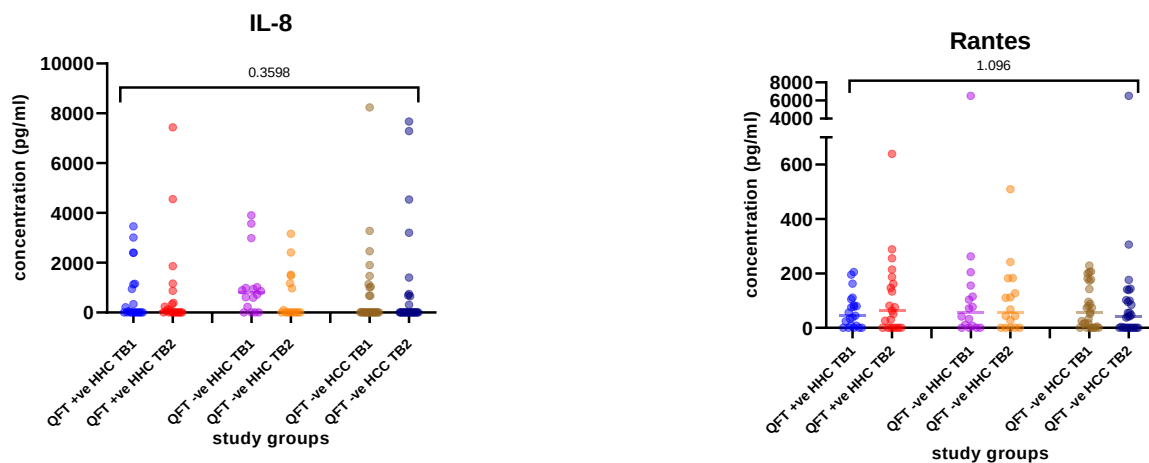


Figure 2. Concentration of the proinflammatory cytokines among study groups; IFN- γ , IFN- α IL1RA, IL2, IL12, IL17A, IL17F, IL-1 α , IL-6, TNF- α and IL-1 β in the supernatant obtained from the QFT® assays of *M. tuberculosis*.

5.3.2 Higher chemokine level was observed among LTBI group

There was no statistically significant difference in IL-8, Rantes, MIP-1 α , and Eotaxin among the groups however concentration of chemokines MCP-1, MIP-1 β , IP-10, and MIG were significantly higher ($p < 0.05$) in the LTBI group compared to HHC QFT negative and CC based on Kruskal - Wallis testing. Levels of MCP-1 ($p < 0.0001$), MIP-1 β ($p < 0.0001$), IP-10 ($p < 0.0001$), MIG ($p < 0.0001$) were significantly higher in the LTBI group compared to the HHC QFT negative and CC. The LTBI group also showed a higher antigen-induced production of MCP-1, MIP-1 β , IP-10, and MIG. (Figure 3)



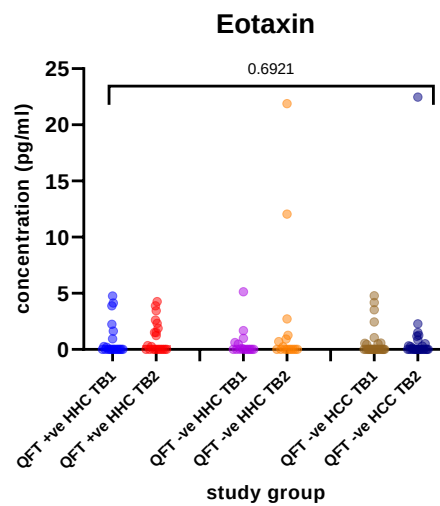
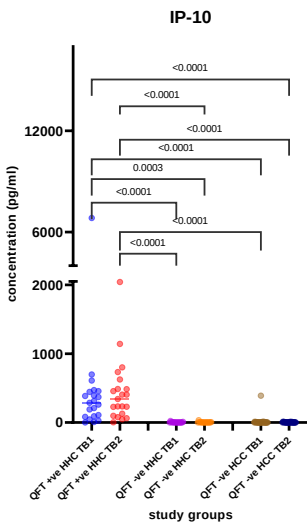
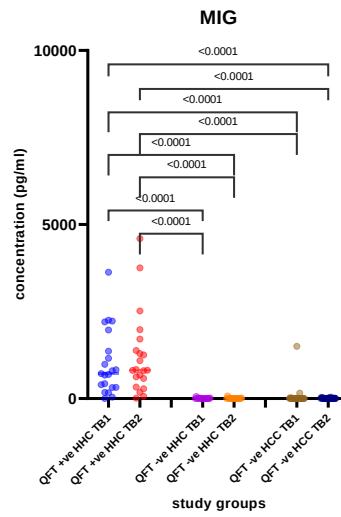
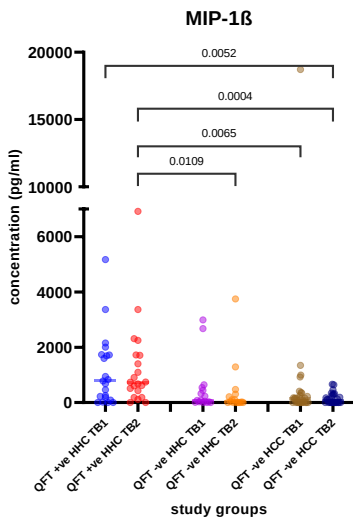
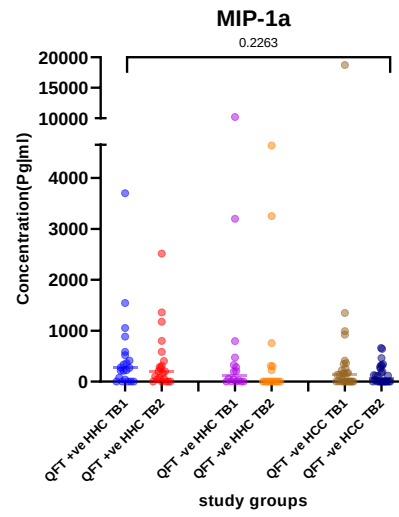
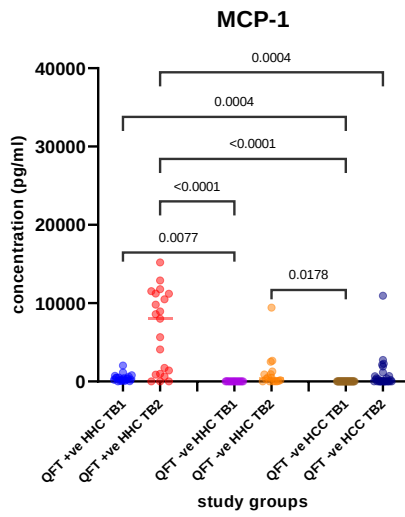
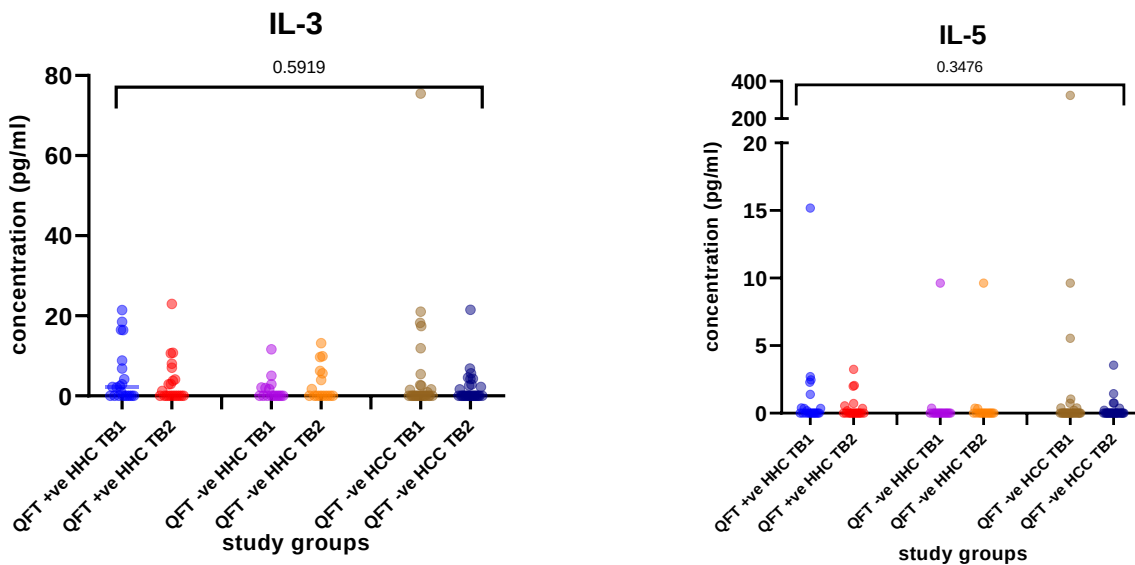


Figure 3. Concentration of the chemokine's family among study groups; MCP1, MIP-1 β , IP-10, MIG, IL-8, Rantes, MIP-1 α and Eotaxin in the supernatant obtained from the QFT® assays.

5.3.3 The level of growth factors molecules was higher in LTBI group

The growth factors FGF basic, GCSF, and GMCSF were significantly elevated ($p < 0.05$) in the LTBI group compared to the HHC QFT negative and CC groups based on Kruskal-Wallis analysis. FGF basic was increased in LTBI with $p = 0.0175$. GCSF was higher in LTBI with $p = 0.0009$. GMCSF was elevated in LTBI with $p < 0.0001$. The angiogenic growth factor VEGF was significantly elevated ($p = 0.0104$) in the LTBI group compared to the HHC QFT negative. There were no significant difference ($p > 0.05$) in IL-3, IL-5, HGF and EGF. (Figure 4)



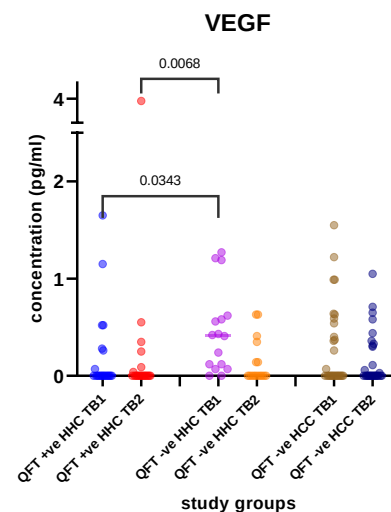
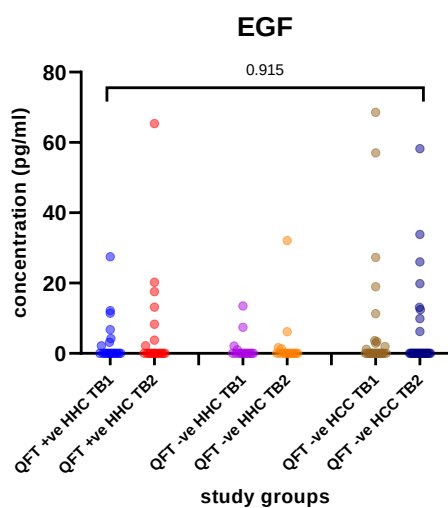
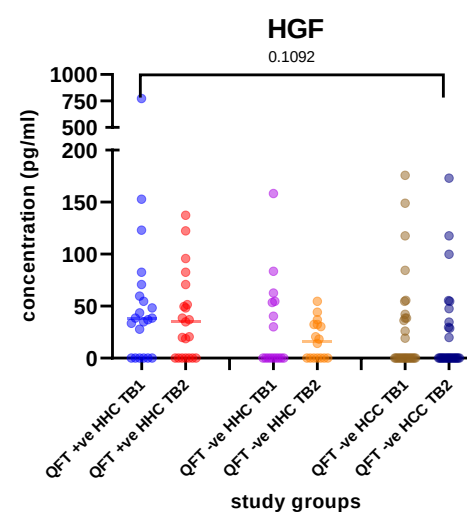
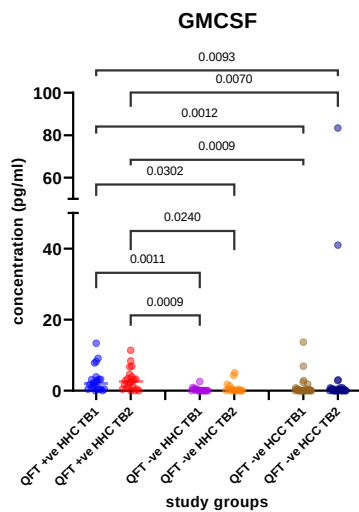
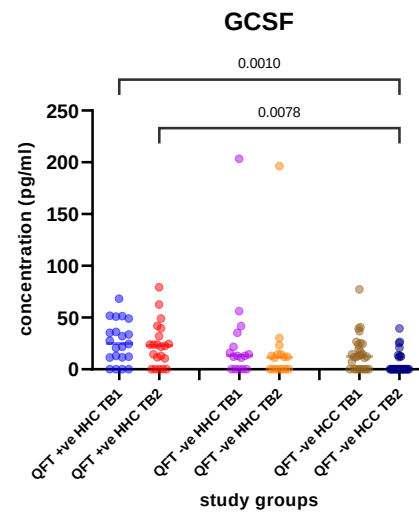
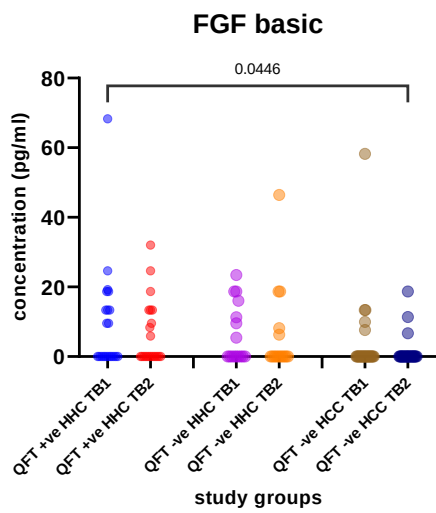


Figure 4. Concentration of the growth factors among study groups; FGF basic, GCSF, GMCSF, VEGF, IL-3, IL-5, EGF, HGF in the supernatant obtained from the QFT® assays.

5.3.4 The level of immunoregulatory molecules was higher in LTBI group

There was no significant difference in IL-7 between any groups. The concentration level of immunoregulatory cytokines IL-2R, IL-9, IL-13, IL-15 were significantly increased ($p < 0.05$) in the LTBI group compared to the HHC QFT negative and CC. IL-2R levels were elevated in the LTBI with $p = 0.0002$ and IL-15 ($p = 0.0248$). IL-9 and IL-13 levels were higher in the LTBI group with $p = 0.0361$ and $p = 0.0172$ respectively. The LTBI group had increased production of IL-2R, IL-9, IL-13 and IL-15 in response to TB antigens compared to the other study groups (Figure 5).

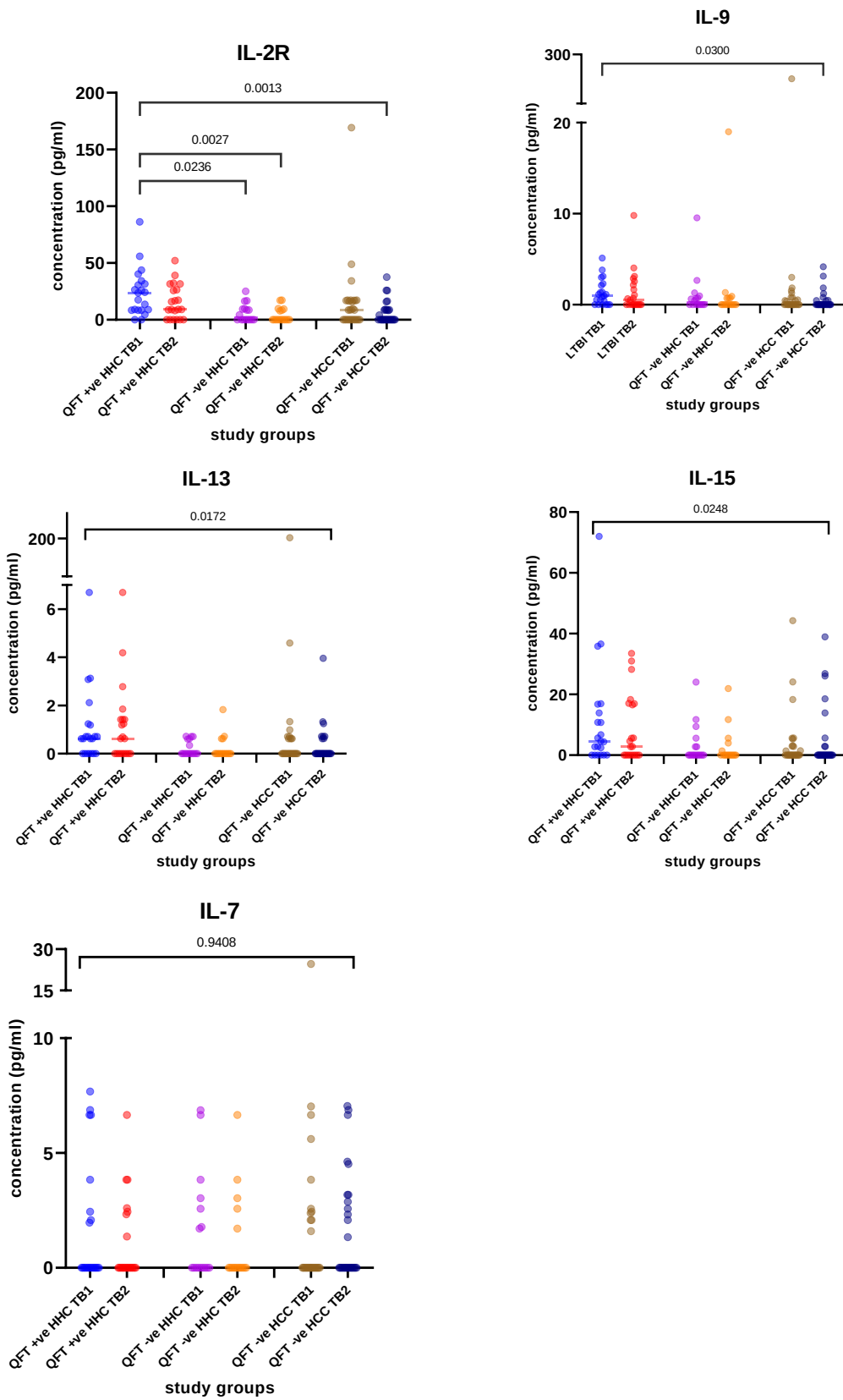


Figure 5. Concentration of the Immunoregulators cytokines among study groups; IL2R, IL-9, IL-13, IL-15 and IL-7 in the supernatant obtained from the QFT® assays.

5.3.5 The level of anti-inflammatory molecules was higher in QFT Negative of HHC group

The anti-inflammatory cytokine IL-22 was significantly increased ($p=0.0008$) in HHC QFT negative compared to the LTBI and Community Control groups based on Kruskal-Wallis testing. IL-22 levels were elevated in the HHC QFT negative with $p=0.0008$. The HHC QFT negative group had elevated IL-22 production in response to TB antigens compared to the other study groups. The heightened IL-22, which can limit inflammation. However, IL-4, IL-10 have no significance difference between the study groups. (Figure 6)

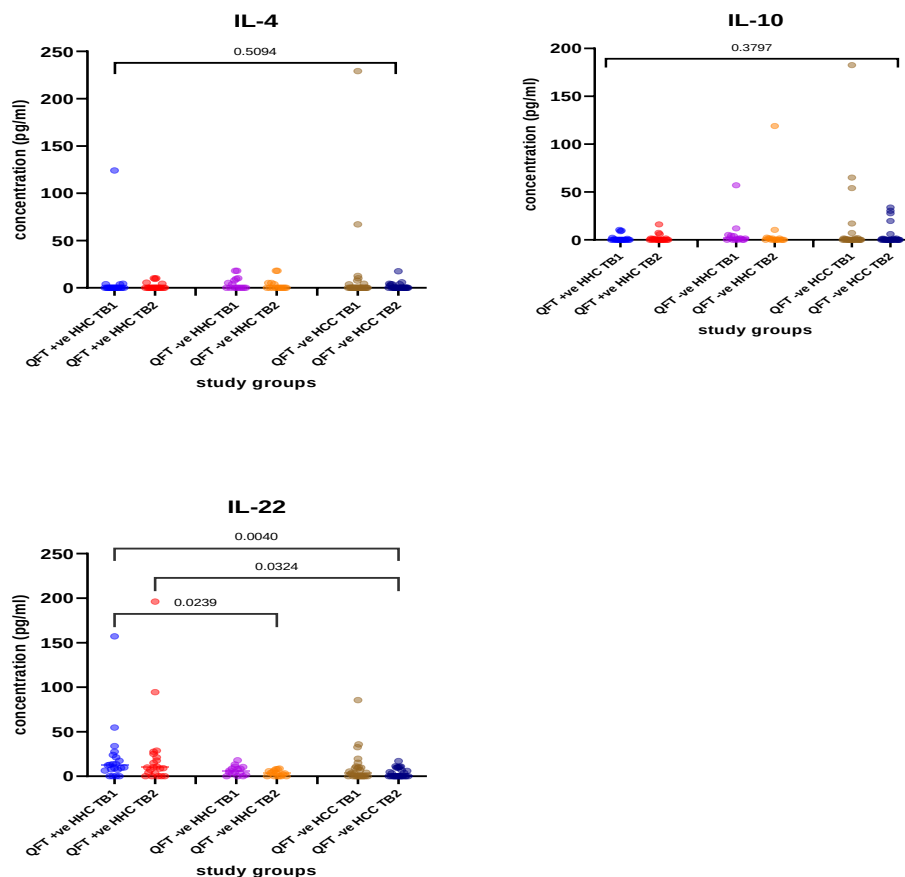


Figure 6. Concentration of the Anti-inflammatory cytokines among study groups; IL22, IL-4 and IL-10 in the supernatant obtained from the QFT® assays between the study groups.

6. Discussion

We found significant differences in cytokine levels between the three groups, suggesting distinct immunological phenotypes that may influence TB susceptibility and disease outcomes.

The significantly higher levels of the proinflammatory cytokines found in LTBI patients, closely matched previous observations across multiple studies showing increased antigen-specific proinflammatory cytokine production in whole blood or PBMCs of latently infected TB contacts relative to HHC QFT negative. Therefore, coordinated elevation of these cytokines indicates functional Th1 responses are actively containing infection in latent TB(60–64). In contrast, the QFT Negative of HHC group showed significantly lower levels for the same spectrum of cytokines compared to both latently infected contacts and Community controls. This implies HHC QFT negative have a muted inflammatory response, despite equivalent intensive exposure histories to index tuberculosis cases within household contacts. Previous studies similarly found HHC QFT negative exhibited reduced IFN- γ responses to MTB antigens compared to person with latent infection (63–67).

The elevated IL-17 levels in latently infected individuals could indicate strategic activation of Th17 responses alongside Th1 immunity to aid infection control. Early production of IL-17A/F helps recruit neutrophils to infected lung sites to form granulomas and contain initial infection (68).

The elevated levels of the chemokines IP-10, MIG, MCP-1, and MIP-1 β in LTBI, reflects ongoing recruitment of immune cells to contain infection. The finding was similar to study done by Yang Y. et al., who showed higher levels of IP-10 in LTBI patients. The substantially elevated circulating levels of this chemokines in LTBI likely reflects persistent infiltration of immune cells into lung granulomas to help contain bacterial growth through sustained formation and remodeling of these organized immunological structures (54,69,70).

Our results showed LTBI individuals had markedly higher concentrations of the hematopoietic growth factors FGF basic, GCSF, and GMCSF as compared to HHC QFT negative. This finding is in line with a study done among health care workers highly exposed to TB, that showed elevated FGF, GCSF, GMCSF in LTBI. The elevated levels likely reflect ongoing hematopoiesis to replenish effector cells at sites of contained infection, maintaining latent immunity through

sustained immune cell turnover. HHC QFT negative conversely showed reduced growth factor levels, reflecting a lack of bone marrow-derived leukocyte expansion and mobilization after initial TB exposure. Without replenishing these cells, HHC QFT negative may fail to establish infection containment through cellular immunity. We observed significantly higher levels of vascular endothelial growth factor (VEGF) in HHC QFT negative compared to latently infected patients. This finding is consistent with the well-established role of VEGF as the major mediator of angiogenesis and permeability. The higher VEGF levels detected in HHC QFT negative could indicate increased angiogenesis and vascular permeability. (64,71–73).

LTBI individuals exhibited markedly higher levels of the immunoregulatory cytokines IL-1RA, IL-9, IL-13, and IL-15 compared to QFT Negative of HHC. This was in line with a study done in India that showed higher levels of IL-9, IL-13, IL-15(74). IL-1RA inhibits the pro-inflammatory actions of IL-1, while IL-9 can suppress IFN- γ production under some conditions(69,70). Meanwhile, IL-13 and IL-15 have varied context-dependent immunomodulatory effects on macrophages, granulocytes, and lymphocyte subsets (75,76). These findings suggest LTBI exhibit coordinated counter-regulation of inflammation alongside Th1 activation, which may help prevent excessive tissue damage from prolonged infection (77).

Our analysis revealed increased levels of the anti-inflammatory cytokine IL-22 in HHC QFT negative compared to latently infected contacts. Different studies have also shown higher levels of IL-22 in QFT Negative of HHC. The selectively elevated IL-22 production in HHC QFT negative may indicate retained potential for deploying such frontline mucosal defenses against initial infection events, averting bacterial dissemination beyond lung niches. In contrast, latently infected contacts showed lower IL-22 levels, potentially signifying loss of mucosal control enabling infection persistence (74,78–81). The key Th1-polarizing cytokine IL-12 is expressed at higher levels in latently infected individuals compared to HHC QFT negative. Reduced IL-12 in HHC QFT negative may impede the development of protective Th1 responses after exposure(81–84).

These differences may also reflect distinct cellular sources. IL-22 is derived from numerous cell types including Th 22, γ δ T cells, innate lymphoid cells and mucosa-associated invariant T (MAIT) cells (80,81,85,86). MAIT cells can mediate early protective surveillance at epithelial barriers by detecting microbial metabolites via MR1 restriction the higher IL-22 in HHC QFT

negative could potentially indicate maintained innate-like defenses from MAIT cells that resist infection establishment but become counter-regulated in infected contacts.(80,87).

Further clarification is still needed to fully understand the influence of IL-22 on ultimate TB outcomes based on co-ordination with additional mediators and pathways. Overall, differential cytokine profiles likely capture heterogeneous immune activation dynamics determining protection vs pathogenesis following TB exposure. Understanding these complex networks that influence the development of medical conditions can help predict and monitor outcomes and guide treatments.

7. Conclusion

This study found significant differences in levels of inflammatory cytokines, chemokines, growth factors, immunoregulators, and angiogenic factors between individuals with LTBI, HHC QFT negative, and Community Controls in response to MTB antigens. Specifically, those with latent TB had increased production of IFN-gamma, IFN- α , IL-1RA, IL-2, IL-12, IL-17A, IL-17F, IL-15, MCP-1, MIP-1 β , IP-10, MIG, FGF basic, GCSF, GMCSF, IL-2R, IL-9, IL-13, IL and VEGF compared to TB “HHC QFT Negative” and controls.

These results suggest latent TB induces a distinct immunological signature characterized by heightened proinflammatory cytokines, chemokines, growth factors, and angiogenic mediators. The antigen-stimulated cytokine profile shows that LTBI is associated with upregulation of inflammatory pathways involved in cell-mediated immunity, leukocyte recruitment, cell growth and proliferation, immunoregulation, and angiogenesis.

Individuals with latent infection had much higher levels of Th1 cytokines like IFN- γ and chemokines like IP-10 compared to uninfected HHC QFT negative, even though both groups have TB exposure. This suggests ongoing cell-mediated immunity that controls dormant infection. In contrast, HHC QFT negative exhibited a muted cytokine response despite high infectious risk, indicating an inability to mount adequate adaptive defenses against TB challenge. But the resulting limited inflammation likely prevents progression to full-blown disease as well. Selectively lower IL-22 in LTBI may represent controlled neutrophil activity while strategic Th1 activation maintains asymptomatic persistence.

In summary, the cytokine patterns observed useful in differentiating individuals with latent TB infection from HHC QFT negative despite similar exposure histories. The findings provide insight into the immune response during latent MTB infection.

8. Limitations

The inability of the assay to evaluate the immune cell types involved in the measured cytokine production

9. Recommendations

The distinct cytokine profiles associated with latent TB infection and HHC QFT Negative group phenotypes in this study provide insight into immune mechanisms influencing differential susceptibility to TB outcomes. Screening high-risk contacts and evaluating cytokine biomarkers could aid early diagnosis and preventative management of those most vulnerable to progression. Modulating key inflammatory pathways may represent a host-directed approach to contain subclinical infection. The cytokine differences also suggest coordinated Th1 cell and innate immunity activation could improve TB vaccines. Further research should delineate immune cell phenotypes and antimicrobial functions across infection stages to identify correlates of protection. Integrated systems biology approaches are needed to unravel heterogeneity in TB susceptibility from demographics, genetics, comorbidities, and pathogen strains. Overall, this work helps elucidate determinants of latent infection persistence versus resistance to infection after exposure to TB for translating into preventative, diagnostic and therapeutic applications.

10. Reference

1. Chackerian AA, Alt JM, Perera T V., Dascher CC, Behar SM. Dissemination of *Mycobacterium tuberculosis* is influenced by host factors and precedes the initiation of T-cell immunity. *Infect Immun*. 2002;70(8):4501–9.
2. Global tuberculosis report 2023 . 2023. Available from: <https://iris.who.int/>.
3. for Disease Control C, National Center for HIV P, Hepatitis V, Prevention T. Core Curriculum on Tuberculosis: What the Clinician Should Know.
4. Global Tuberculosis report 2022 . 2022. Available from: <http://apps.who.int/bookorders>.
5. Schildknecht KR, Pratt RH, Feng PJI, Price SF, Self JL. Morbidity and Mortality Weekly Report Tuberculosis-United States, 2022. 2023. Available from: <https://www.census.gov/programs-surveys/popest/data/tables.html>;
6. Chedid C, Kokhraidze E, Tukvadze N, Banu S, Uddin MKM, Biswas S, et al. Relevance of QuantiFERON-TB Gold Plus and Heparin-Binding Hemagglutinin Interferon- γ Release Assays for Monitoring of Pulmonary Tuberculosis Clearance: A Multicentered Study. *Front Immunol*. 2021 Feb 2;11.
7. Simmons JD, Stein CM, Seshadri C, Campo M, Alter G, Fortune S, et al. Immunological mechanisms of human resistance to persistent *Mycobacterium tuberculosis* infection. Vol. 18, *Nature Reviews Immunology*. Nature Publishing Group; 2018. p. 575–89.
8. Shanmugasundaram U, Bucsan AN, Ganatra SR, Ibegbu C, Quezada M, Blair R V., et al. Pulmonary *Mycobacterium tuberculosis* control associates with CXCR3- And CCR6-expressing antigen-specific Th1 and Th17 cell recruitment. *JCI Insight*. 2020 Jun 23;5(14).
9. Mogues T, Goodrich ME, Ryan L, Lacourse R, North RJ. The Relative Importance of T Cell Subsets in Immunity and Immunopathology of Airborne *Mycobacterium tuberculosis* Infection in Mice [Internet]. Vol. 193, *J. Exp. Med*. 2001. Available from: <http://www.jem.org/cgi/content/full/193/3/271>
10. Mittrü HW, Steinhoff U, Kö A, Krause M, Lazar D, Mex P, et al. Poor correlation between BCG vaccination-induced T cell responses and protection against tuberculosis [Internet]. 2007. Available from: www.pnas.org/cgi/doi/10.1073/pnas.0703510104
11. Du Bruyn E, Ruzive S, Lindestam Arlehamn CS, Sette A, Sher A, Barber DL, et al. *Mycobacterium tuberculosis*-specific CD4 T cells expressing CD153 inversely associate with bacterial load and disease severity in human tuberculosis. *Mucosal Immunol*. 2021 Mar 1;14(2):491–9.

12. Woodworth JSM, Behar SM. Mycobacterium tuberculosis specific CD8 + T cells and their role in immunity.
13. Lindestam Arlehamn CS, Lewinsohn D, Sette A, Lewinsohn D. Antigens for CD4 and CD8 T cells in tuberculosis. Cold Spring Harb Perspect Med. 2014;4(7).
14. Lu YJ, Barreira-Silva P, Boyce S, Powers J, Cavallo K, Behar SM. CD4 T cell help prevents CD8 T cell exhaustion and promotes control of Mycobacterium tuberculosis infection. Cell Rep. 2021 Sep 14;36(11).
15. Prezzemolo T, Guggino G, La Manna MP, Di Liberto D Di, Dieli F, Caccamo N. Functional signatures of human CD4 and CD8 T cell responses to Mycobacterium tuberculosis. Vol. 5, Frontiers in Immunology. Frontiers Research Foundation; 2014.
16. Narayana SV PRPH. Role if Interferon Gamma on innate immune Response against Mycobacterium Tuberculosis. Journal of Immunology Research . 2018;1–14.
17. Cox MTD TE and HN. the role of cytokines in infection with Mycobacterium Tuberculosis. Frontiersin Immunology. 2019;10:3226:1–14.
18. Chauhan S PR and GP. The role of TLR2 Ligation Induced Interleukin- 2 in Pathogenesis of Mycobactrium Tuberculosis. Frontiers Immunology . 2021;11:661914:1–15.
19. Banerjee U, Baloni P, Singh A, Chandra N. Immune Subtyping in Latent Tuberculosis. Front Immunol. 2021 Apr 7;12.
20. World Health Organization. Global tuberculosis report 2019. 283 p.
21. Essone PN, Leboueny M, Maloupazoa Siawaya AC, Alame-Emane AK, Aboumegone Biyogo OC, Dapnet Tadatsin PH, et al. M. tuberculosis infection and antigen specific cytokine response in healthcare workers frequently exposed to tuberculosis. Sci Rep. 2019 Dec 1;9(1).
22. GLOBAL TUBERCULOSIS REPORT 2021. 2021. Available from: <http://apps.who.int/bookorders>.
23. Ai JW RQLQZW. Updates on the risk factors for latent tuberculosis reactivation and their managements. Emerg Microbes Infect. 2016;1–10.
24. Jamison DT, Feachem RG, Makgoba MW, Bos ER, Baingana FK, Hofman KJ, et al. Disease and Mortality in Sub-Saharan Africa.
25. Conan N, Simons E, Ohler L, Mbatha M, van Cutsem G, Huerga H. Prevalence of TB and health-seeking behaviour. Vol. 26, International Journal of Tuberculosis and Lung Disease. International Union Against Tuberculosis and Lung Disease; 2022. p. 463–5.

26. Godoy P. Guidelines on controlling latent tuberculosis infection to support tuberculosis elimination. Vol. 23, *Revista espanola de sanidad penitenciaria*. NLM (Medline); 2021. p. 28–36.
27. Kefyalew Addis Alene APDJWAEZAWAKPWGACAC. Mapping tuberculosis prevalence in Ethiopia using geospatial meta-analysis. *International Journal of Epidemiology* . 2023 Aug;52(4):1124–36.
28. Deribew A, Deribe K, Dejene T, Tessema GA, Melaku YA, Lakew Y, et al. Tuberculosis Burden in Ethiopia from 1990 to 2016: Evidence from the Global Burden of Diseases 2016 Study. *Ethiop J Health Sci*. 2018 Sep 1;28(5):519–28.
29. Haileamlak A. Ethiopia is on Track of Achieving the WHO End Tuberculosis Milestone. Vol. 31, *Ethiopian journal of health sciences*. NLM (Medline); 2021. p. 1–2.
30. Temesgen E, Belete Y, Haile K, Ali S. Prevalence of active tuberculosis and associated factors among people with chronic psychotic disorders at St. Amanuel Mental Specialized Hospital and Gergesenon Mental Rehabilitation center, Addis Ababa, Ethiopia. *BMC Infect Dis*. 2021 Dec 1;21(1).
31. Arja A, Tadesse S, Agachew M, Getnet F, Beksisa J, Mohammed S, et al. The Burden of Tuberculosis across Regions in Ethiopia: A Systematic Subnational Analysis for the Global Burden of Disease Study 2019 . Available from: <http://www.healthdata.org>
32. diagnostic-standards-and-classification-of-tuberculosis-1990.
33. Festenstein F, Grange JM. Tuberculosis and the acquired immune deficiency syndrome. Vol. 71, *Journal of Applied Bacteriology*. 1991. p. 19–30.
34. Sculier D, Hāylayasus Gétāhun, World Health Organization. WHO policy on collaborative TB/HIV activities : guidelines for national programmes and other stakeholders. 34 p.
35. Reid A, Scano F, Getahun H, Williams B, Dye C, Nunn P, et al. Review Towards universal access to HIV prevention, treatment, care, and support: the role of tuberculosis/HIV collaboration [Internet]. 2006. Available from: <http://infection.thelancet.com>
36. Cailhol J, Decludt B, Che D. Sociodemographic factors that contribute to the development of extrapulmonary tuberculosis were identified. *J Clin Epidemiol*. 2005 Oct;58(10):1066–71.
37. Dye C, Lönnroth K, Jaramillo E, Williams BG, Raviglione M. Trends in tuberculosis incidence and their determinants in 134 countries. *Bull World Health Organ*. 2009 Sep;87(9):683–91.

38. Vernon A. Treatment of latent tuberculosis infection. *Semin Respir Crit Care Med*. 2013;34(1):67–86.
39. Chandrasekaran P, Mave V, Thiruvengadam K, Gupte N, Yogendra Shivakumar SVB, Hanna LE, et al. Tuberculin skin test and QuantiFERON-Gold In Tube assay for diagnosis of latent TB infection among household contacts of pulmonary TB patients in high TB burden setting. *PLoS One*. 2018 Aug 1;13(8).
40. Mena JA KR. Latent Tuberculosis Diagnosis Revisited Response Therapy . 2017;(38(1)1082-1087):38(1).
41. Fox GJ, Barry SE, Britton WJ, Marks GB. Contact investigation for tuberculosis: A systematic review and meta-analysis. Vol. 41, *European Respiratory Journal*. 2013. p. 140–56.
42. Verrall AJ, G. Netea M, Alisjahbana B, Hill PC, van Crevel R. Early clearance of *Mycobacterium tuberculosis*: A new frontier in prevention. Vol. 141, *Immunology*. Blackwell Publishing Ltd; 2014. p. 506–13.
43. Choi J SSHJLWKCLJKS. Household contacts of *Mycobacterium tuberculosis* patients may develop resistance to infection by maintaining a higher T-cell count : A cross-sectional study in South Korea. *J . Infect Public Health*. 2018;11(12):1410–5.
44. Wakeetip P, NS, KS and BP. Detection of Antigen-Specific Cytokines in *Mycobacterium Tuberculosis* Infection. *A Review International Journal of Molecular sciences*. 2020;
45. Jafrasteh A, Karimi A, Hoseinialfatemi SM, Azimi L, Tabarsi P, Nasehi M, et al. Evaluation of interleukin-2 to detect active and latent tuberculosis among household contacts of pulmonary tuberculosis cases. *Arch Pediatr Infect Dis*. 2021 Apr 1;9(2).
46. Esmael A, Abebe T, Mihret A, Mussa D, Neway S, Ernst J, et al. *Mycobacterium tuberculosis* antigen-specific T-cell responses in smear-negative pulmonary tuberculosis patients. *Clin Exp Immunol*. 2022 Jul 1;209(1):99–108.
47. Belay M, Legesse M, Mihret A, Bekele Y, Ottenhoff THM, Franken KLMC, et al. Pro- and anti-inflammatory cytokines against Rv2031 are elevated during latent tuberculosis: A study in cohorts of tuberculosis patients, household contacts and community controls in an endemic setting. *PLoS One*. 2015 Apr 21;10(4).
48. Demissie A, Abebe M, Aseffa A, Rook G, Fletcher H, Zumla A, et al. Healthy Individuals That Control a Latent Infection with *Mycobacterium tuberculosis* Express High Levels of Th1 Cytokines and the IL-4 Antagonist IL-4δ2. *The Journal of Immunology [Internet]*. 2004 Jun 1;172(11):6938–43. Available from:

<https://journals.aai.org/jimmunol/article/172/11/6938/80968/Healthy-Individuals-That-Control-a-Latent>

49. Lemma M, wondmagegn T, Mengesha B, Yehuala S, Workineh M, Abate E. Level of Interferon - Gamma, Interlukin – 6, Frequency of Monocyte and Lymphocyte Subsets among Newly Diagnosed Tuberculosis Patients in Northwest Ethiopia. *J Adv Microbiol*. 2019 Jan 15;14(1):1–8.
50. Flesch IEA, Kaufmann SHE. Role of Cytokines in Tuberculosis. *Immunobiology*. 1993;189(3–4):316–39.
51. Vivekanandan MM, Adankwah E, Aniagyei W, Acheampong I, Yeboah A, Arthur JF, et al. Plasma cytokine levels characterize disease pathogenesis and treatment response in tuberculosis patients. *Infection*. 2022 Feb 1;
52. Ronacher K, Sinha R, Cestari M. IL-22: An underestimated player in natural resistance to tuberculosis? Vol. 9, *Frontiers in Immunology*. Frontiers Media S.A.; 2018.
53. Domingo-Gonzalez R, Prince O, Cooper A, Khader SA. Cytokines and Chemokines in *Mycobacterium tuberculosis* Infection . *Microbiol Spectr*. 2016 Oct 14;4(5).
54. Yu Y, Zhang Y, Hu S, Jin D, Chen X, Jin Q, et al. Different Patterns of Cytokines and Chemokines Combined with IFN- γ Production Reflect *Mycobacterium tuberculosis* Infection and Disease. *PLoS One*. 2012 Sep 13;7(9).
55. Ethiopia Addis Ababa Health Facility Location Map as of 10 April 2013.
56. Gezahegn Berhe A, Erena B, Hassen M, Mamaru TL, Soressa YA. CITY PROFILE ADDIS ABABA SES Social Inclusion and Energy Managment for Informal Urban Settlements [Internet]. Available from: www.eiabc.edu.et
57. United Nations Human Settlements Programme. Regional and Technical Cooperation Division. Senegal : profil urbain de Dakar. ONU-HABITAT; 2008. 32 p.
58. English_QFT_ELISA_R04_082016.
59. User Guide MILLIPLEX ® Human Cytokine/Chemokine/Growth Factor Panel A Magnetic Bead Panel 96-Well Plate Assay HCYTA-60K, HCYTA-60K-PX38 HCYTA-60K-PXBK38, HCYTA-60K-PX48 HCYTA-60K-PXBK48.
60. Masood KI, Irfan M, Masood Q, Yameen M, Jamil B, Ram N, et al. Latent M. tuberculosis infection is associated with increased inflammatory cytokine and decreased suppressor of cytokine signalling (SOCS)-3 in the diabetic host. *Scand J Immunol*. 2022 Apr 1;95(4).

61. Sasindran SJ, Torrelles JB. Mycobacterium tuberculosis infection and inflammation: What is beneficial for the host and for the bacterium? *Front Microbiol.* 2011;2(JAN).
62. Kak G, Raza M, Tiwari BK. Interferon-gamma (IFN- γ): Exploring its implications in infectious diseases. Vol. 9, *Biomolecular Concepts*. Walter de Gruyter GmbH; 2018. p. 64–79.
63. Herbst S, Schaible UE, Schneider BE. Interferon gamma activated macrophages kill mycobacteria by nitric oxide induced apoptosis. *PLoS One.* 2011;6(5).
64. Essone PN, Leboueny M, Maloupazoa Siawaya AC, Alame-Emane AK, Aboumegone Biyogo OC, Dapnet Tadatsin PH, et al. M. tuberculosis infection and antigen specific cytokine response in healthcare workers frequently exposed to tuberculosis. *Sci Rep.* 2019 Dec 1;9(1).
65. Chandra P, Grigsby SJ, Philips JA. Immune evasion and provocation by Mycobacterium tuberculosis. Vol. 20, *Nature Reviews Microbiology*. Nature Research; 2022. p. 750–66.
66. Baliashvili D, Gandhi NR, Kim S, Hughes M, Mave V, Mendoza-Ticon A, et al. Resistance to Mycobacterium tuberculosis Infection Among Household Contacts: A Multinational Study. *Clinical Infectious Diseases.* 2021 Sep 15;73(6):1037–45.
67. Caccamo N, Guggino G, Joosten SA, Gelsomino G, Di Carlo P, Titone L, et al. Multifunctional CD4⁺ T cells correlate with active Mycobacterium tuberculosis infection. *Eur J Immunol.* 2010 Aug;40(8):2211–20.
68. Khader SA, Bell GK, Pearl JE, Fountain JJ, Rangel-Moreno J, Cilley GE, et al. IL-23 and IL-17 in the establishment of protective pulmonary CD4⁺ T cell responses after vaccination and during Mycobacterium tuberculosis challenge. *Nat Immunol.* 2007 Apr;8(4):369–77.
69. Shey MS, Balfour A, Masina N, Bekiswa A, Schutz C, Goliath R, et al. Mycobacterial-specific secretion of cytokines and chemokines in healthcare workers with apparent resistance to infection with Mycobacterium tuberculosis. *Front Immunol.* 2023;14.
70. Yu Y, Zhang Y, Hu S, Jin D, Chen X, Jin Q, et al. Different Patterns of Cytokines and Chemokines Combined with IFN- γ Production Reflect Mycobacterium tuberculosis Infection and Disease. *PLoS One.* 2012 Sep 13;7(9).
71. Harding JS, Herbath M, Chen Y, Rayasam A, Ritter A, Csoka B, et al. VEGF-A from Granuloma Macrophages Regulates Granulomatous Inflammation by a Non-angiogenic Pathway during Mycobacterial Infection. *Cell Rep.* 2019 May 14;27(7):2119–2131.e6.
72. Abdullah SE, Perez-Soler R. Mechanisms of resistance to vascular endothelial growth factor blockade. Vol. 118, *Cancer*. 2012. p. 3455–67.

73. Duffy AM BHDHJH. Vascular Endothelial Growth Factor (VEGF) and Its Role in Non-Endothelial Cells: Autocrine Signalling by VEGF. 2023.
74. Bapat PR, Husain AA, Dagainawala HF, Agrawal NP, Panchbhai MS, Satav AR, et al. The assessment of cytokines in Quantiferon supernatants for the diagnosis of latent TB infection in a tribal population of Melghat, India. *J Infect Public Health*. 2015 Jul 1;8(4):329–40.
75. Sia JK, Rengarajan J. Immunology of Mycobacterium tuberculosis Infections . *Microbiol Spectr*. 2019 Jul 19;7(4).
76. Monin L, Khader SA. Chemokines in tuberculosis: The good, the bad and the ugly. Vol. 26, *Seminars in Immunology*. Academic Press; 2014. p. 552–8.
77. Chandra P, Grigsby SJ, Philips JA. Immune evasion and provocation by Mycobacterium tuberculosis. Vol. 20, *Nature Reviews Microbiology*. Nature Research; 2022. p. 750–66.
78. Wilson MS, Feng CG, Barber DL, Yarovinsky F, Cheever AW, Sher A, et al. Redundant and Pathogenic Roles for IL-22 in Mycobacterial, Protozoan, and Helminth Infections. *The Journal of Immunology*. 2010 Apr 15;184(8):4378–90.
79. Wergeland I, Assmus J, Dyrhol-Riise AM. Cytokine patterns in tuberculosis infection; IL-1ra, IL-2 and IP-10 differentiate borderline QuantiFERON-TB samples from uninfected controls. *PLoS One*. 2016 Sep 1;11(9).
80. Arshad T, Mansur F, Palek R, Manzoor S, Liska V. A Double Edged Sword Role of Interleukin-22 in Wound Healing and Tissue Regeneration. Vol. 11, *Frontiers in Immunology*. Frontiers Media S.A.; 2020.
81. Dudakov JA, Hanash AM, Van Den Brink MRM. Interleukin-22: Immunobiology and pathology. Vol. 33, *Annual Review of Immunology*. Annual Reviews Inc.; 2015. p. 747–85.
82. Li Q, Ren W, Yuan J, Guo H, Shang Y, Wang W, et al. Significant difference in Th1/Th2 paradigm induced by tuberculosis-specific antigens between IGRA-positive and IGRA-negative patients. *Front Immunol*. 2022 Aug 31;13.
83. Cooper AM, Magram J, Ferrante J, Orme IM. Interleukin 12 (IL-12) Is Crucial to the Development of Protective Immunity in Mice Intravenously Infected with Mycobacterium tuberculosis. Vol. 186, *J. Exp. Med*. 1997.
84. Gourgouillon N, De Lauzanne A, Cottart CH, Curis E, Debord C, Guérin-El Khourouj V, et al. TNF- α /IL-2 ratio discriminates latent from active tuberculosis in immunocompetent children: A pilot study. *Pediatr Res*. 2012 Oct;72(4):370–4.

85. Mills KHG. IL-17 and IL-17-producing cells in protection versus pathology. Vol. 23, Nature Reviews Immunology. Nature Research; 2023. p. 38–54.
86. Huangfu L, Li R, Huang Y, Wang S. The IL-17 family in diseases: from bench to bedside. Vol. 8, Signal Transduction and Targeted Therapy. Springer Nature; 2023.
87. Legoux F, Salou M, Lantz O. MAIT Cell Development and Functions: the Microbial Connection. Vol. 53, Immunity. Cell Press; 2020. p. 710–23.

Annexes

Annex 1 - Participants Information Sheet and consent form (English version)

Part I: Information Sheet

Introduction:

The study team is currently working for Armauer Hansen Research Institute (AHRI), one of the agencies under the Ethiopian Ministry of Health, on research on the disease Tuberculosis. The title of our research project is " Comparison Of Cytokines And Chemokines In QFT Positive And QFT Negative House Hold Contacts Of TB Patients ". The Investigator is Ziyada Rahemato. I am going to give you information and invite you to be part of this research. You do not have to decide immediately whether or not you will participate in the research. You can take time to reflect and to talk to anyone you feel comfortable with about the research. If you don't understand any part of what I am explaining to you, feel free to stop me at any time and ask questions. If you have questions later, you can ask me, the primary investigator of the study, or the ethics committee that has reviewed the protocol; their contact information is written at the end of this informed consent document.

Purpose of the research

Tuberculosis is an infectious disease affecting millions of people worldwide. Ethiopia is among one of the highly affected countries. It is transmitted from person-to-person during coughing. Therefore, people who live with TB patients are at greater risk of acquiring the infection and disease. However, not all contacts develop disease; some remain without any signs of infection while living with a TB patient. It is important to do research to understand the disease in order to be able to develop better diagnostic tools, treatment, and vaccines. The reason we are doing this research is understand the disease and identify factors that contribute to protection in our population.

Type of Research Intervention To understand the disease, we will conduct a clinical research that involves investigation of components of the immune system of contacts of TB patients and unexposed members of the community.

Participant selection

You are asked to participate in this study because you are a healthy member of this community with no history of living with a TB patient OR you are currently living with a recently diagnosed TB patient.

Voluntary Participation

Your participation in this research is entirely voluntary. It is your choice whether to participate or not. You may change your mind later and stop participating even if you agree to participate now.

Procedures and Protocol

If you agree to participate in this study, 4 ml (2 teaspoons) of blood will be collected from your arm at visit 1 (now) and 4ml (2 teaspoon) at visit 2 (in two months). Since blood haemoglobin level is an indicator of anaemia, a haemoglobin test will be done. If you have anaemia, no blood will be collected. You will also be asked to give a stool sample at each visit to test for the presence of intestinal parasites. Chest X-ray will be taken one time to examine for the presence of signs of TB infection. A pregnancy test will be done for women on urine sample at both visits. We will also test your blood for HIV at both visits. The results of the HIV test will be communicated to you by the health care professional and if the result is positive, you will be linked to the nearest ART clinic.

The blood will be collected by a health care professional ensuring sterile condition. The blood will be used for this research project to understand the role of immune cells and selected modifications in some of the immune cells. All study participants will be asked questions relevant to their health and the research at both visits and the information will be recorded on questionnaires designed for the study. This will take about 20 minutes. At the end of this research, leftover specimen will be stored at AHRI for future research. For this, we will ask you for an additional separate consent.

Risks

By participating in this research, you will experience minor pain and discomfort from needle prick during blood collection. The nurse will take the necessary precautions to minimize this risk.

Benefits

There may not be any direct benefit for you to participate in this study. However, you will be checked by a health care worker for any signs of TB. As well, one of the tests that will be performed in this project will identify TB infection you may have acquired now or in the past. This test is helpful to diagnosing TB disease together with clinical signs and symptoms. Therefore, you may benefit from this checkup. The findings of this study will also be beneficial to the community because it will support the development of new and more effective vaccines for TB.

Reimbursements

You will not be paid to participate in this study. However, you will be compensated with 300 ETB at each visit to compensate for your lost time and to cover the cost of transportation to the Health Centre. You will also be given additional 300 ETB for extra days you are requested to come to the Health Centre to drop off Chest X-ray results.

Confidentiality

The information that we collect from this research project will be kept confidential. Personal identifier information will be put away and no-one but the researchers will be able to see it. We will use study code/number rather than your name and only the researchers will know what your number is. This document will also be protected with password and lock-and-key where access is given only to researchers. Access will not be shared with or given to anyone except ethics and regulatory authorities as needed.

Sharing the Results, the knowledge that we get from doing this research will be shared broadly to the local and international community through publications and conferences. Confidential information will not be shared.

Right to Refuse or Withdraw

You do not have to take part in this research if you do not wish to do so. You may also stop participating in the research at any time you choose. Refusing to participate or choosing to withdraw from this study will not affect any treatment you may have at this health center now or

in the future. You will still have all the benefits that you would otherwise have at this Health Center.

Who to Contact

This proposal has been reviewed and approved by AHRI-ALERT Ethics Review Committee, which is a committee whose task it is to make sure that research participants are protected from harm. If you have any questions you may ask the principal investigator of this study or the AHRI-ALERT Ethics Review Committee at any time, even after the study has started. You can contact them using the following information:

Principal Investigator =Dr..Martha Zewdie (this project was done under the IRTBHHC study lead by Dr Martha Zewdie Gebeyehu.) =email; martha.zewdie@ahri.gov.et

AHRI-ALERT Ethics Review Committee (AAERC); Tel- 011 3 48 12 89

email -ahri.alerterc@ahri.gov.et

You can ask me any more questions about any part of the research study, if you wish to. Do you have any questions?

PART II: Consent Form

I have read the above-mentioned information, or it has been read to me. I have had the opportunity to ask questions about it and the questions that I have asked have been answered to my satisfaction. I have been told that I will be asked questions related to my health and general status and that blood will be collected from my arm at two visits; 4 ml (2 teaspoons) now and 4ml (2 teaspoons) at two months. I will also be tested for HIV at both visits. I will also give stool sample at each visit, and I understand a chest x-ray will be taken. I consent voluntarily to participate as a study participant in this research.

Name of Participant _____ **Signature of Participant** _____
Date _____ (Day/month/year)

If illiterate

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness _____ AND Thumb print of participant
Signature of witness _____
Date _____ Day/month/year

Name of Study Nurse: _____ **Signature:** _____ **Date** _____
_____ (Day/month/year)

PART III: Consent for Storage and Future Use of Your Samples

After this study is completed, there may be some leftover specimen. We would like to ask your permission to store your leftover specimens for useful studies proposed in the future. We are asking you this because the consent you gave us allows us to use your specimen only for this study. If you do not want your leftover samples to be used in future research, you may refuse storage for future use but still be in this study.

At present, it is possible to trace back the person to whom the specimen belongs to. For storage and future usage, you may choose to keep it this way or you may choose to remove all personal identifiers and link to the individual. If the specimen is unidentified, it may not be possible to trace back to you, and you will not receive the results of any tests conducted in the future that are not part of this research study. If your identity remains linked, it will be possible to inform you any results that have clinical relevance to your health.

Your leftover specimen will not be sold or shared to anyone for profit. However, in some research, using human specimen may help researchers to make medical tests or treatments or vaccines that may eventually generate money. In such instances, you will not receive any money, but you and other members of the community who have similar illnesses may benefit from using them. Research studies using your leftover specimens will be reviewed by the Ethiopian ethics committees before conducted.

There may not be benefit to you from agreeing to let your leftover specimen be used in future research. There are no risks to you also if you agree to store your leftover specimens for future research studies. Confidentiality of your information will be maintained by using study codes rather than your name or other personal identifiers in data storage and communication.

If you decide that you do not want your leftover specimens stored and used for future studies, we will destroy your samples at the end of the current study. If you choose now that your leftover specimens can be stored for future studies, the leftover specimens will be kept at AHRI laboratory for at least 10 years and only certain institutionally approved people will be able to access the samples or your linked biological data.

PLEASE INDICATE YOUR CHOICE BY INITIALING OR MARKING ONE OF THE FOLLOWING OPTIONS:

1. I choose to let my leftover coded specimens be used for ANY type of future research studies.
_____ Yes. _____ No
2. I will let the researchers contact me in the future if they need more information about me for a future research study. _____ Yes _____ No

Name (participant) _____ Signature _____ Date _____

Name (Witness) _____ Signature _____ Date _____

Name of study nurse _____ Signature _____ Date _____

You can ask me any more questions about any part of the research study, if you wish to. Do you have any questions?

Part IV Information sheet and assent form for Minors (Age 15 to 17 years) in English

PART I: Information Sheet

Introduction

The study team is currently working for Armauer Hansen Research Institute (AHRI), one of the agencies under the Ethiopian Ministry of Health, on research on the disease Tuberculosis. The title of our research project is " Comparison Of Cytokines And Chemokines In QFT Positive And QFT Negative House Hold Contacts Of TB Patients ". The investigator is Ziyada Rahimeto. I am going to give you information and invite you to be part of this research. You do not have to decide immediately whether or not you will participate in the research. You can take time to reflect and to talk to anyone you feel comfortable with about the research. If you don't understand any part of what I am explaining to you, feel free to stop me at any time and ask questions. If you have questions later, you can ask me, the primary investigator of the study, or the ethics committee that has reviewed the protocol; their contact information is written at the end of this informed consent document.

Purpose of the research

Tuberculosis is an infectious disease affecting millions of people worldwide. The disease also affects many people in Ethiopia. It is transmitted from person-to-person during coughing. Therefore, people who live with TB patients are at greater risk of becoming sick. However, not all people who contact patients become sick; some remain without any signs of disease. It is important to do research to understand the disease in order to be able to develop ways to control the disease; for example, to develop better treatment and vaccines. The reason we are doing this research is to understand the disease and identify factors that contribute to protection in our population.

Type of Research

To understand the disease, we will conduct a clinical research that involves investigation of cells in our blood that are components of the immune system. The study participants will include healthy people who live with a TB patient and people who have never lived with a TB patient.

Participant selection

You are asked to participate in this study because you are a healthy member of this community with no history of living with a TB patient OR you are currently living with a recently diagnosed TB patient.

Voluntary Participation

Your participation in this research is entirely voluntary. It is your choice whether to participate or not. You may change your mind later and stop participating even if you agree to participate now.

Procedures and Protocol

If you agree to participate in this study, 4 ml (2 teaspoons) of blood will be collected from your arm now, and 4ml (2 teaspoons) at two months. Since blood haemoglobin level is an indicator of anaemia, a haemoglobin test will be done. If you have anaemia, no blood will be collected. You will also be asked to give a stool sample at each visit to test for the presence of intestinal parasites. Chest X-ray will be taken once to examine for the presence of signs of TB infection. Pregnancy test will be done for women on urine sample at both visits. We will also test your blood for HIV at both visits. The results of the HIV test will be communicated to you by the health care professional and if the result is positive, you will be linked to the nearest ART clinic.

The blood will be collected by a health care professional in a clean and safe manner. The blood will be used for this research project to understand the role of immune cells and selected modifications in some of the immune cells.

You will be asked questions relevant to your health and the research at both visits and the information will be recorded questionnaire designed for this study. This will take about 20 minutes. At the end of this research, leftover specimen will be stored at AHRI for future research. For this, we will ask you for an additional separate assent. For you to participate in this study, we will also require the consent of your parent/guardian.

Risks

By participating in this research, you will experience minor pain and discomfort from needle prick during blood collection. The nurse will take the necessary precautions to minimize this risk.

Benefits

There may not be any direct benefit for you to participate in this study. However, you will be checked by a health care worker for any signs of TB. As well, one of the tests that will be performed in this project will identify TB infection you may have acquired now or in the past. This test is helpful to diagnosing TB disease together with clinical signs and symptoms. Therefore, you may benefit from this checkup. The findings of this study will also be beneficial to the community because it will support the development of new and more effective vaccines for TB.

Reimbursements

You will not be paid to participate in this study. However, you will be compensated with 300 ETB at each visit to compensate for your lost time and to cover the cost of transportation to the Health Centre. You will also be given additional 300 ETB for extra days you are requested to come to the Health Centre to drop off Chest X-ray results.

Confidentiality

The information that we collect from this research project will be kept confidential. Information that can identify you, such as your name will be put away and no-one but the researchers will be able to see it. We will also use study code/number rather than your name and only the researchers will know what your number is. This document will also be protected with password and lock-and-key where access is given only to researchers. Access will not be shared with or given to anyone except ethics and regulatory authorities as needed.

Sharing the Results

the knowledge that we get from doing this research will be shared broadly to the local and international community through publications and conferences. Confidential or personal identifier information will not be shared.

Right to Refuse or Withdraw

You do not have to take part in this research if you do not wish to do so. You may also stop participating in the research at any time you choose. Refusing to participate or choosing to withdraw from this study will not affect any treatment you may have at this health center now or in the future. You will still have all the benefits that you would otherwise have at this Health Center.

Who to Contact

This proposal has been reviewed and approved by AHRI-ALERT Ethics Review Committee, which is a committee whose task it is to make sure that research participants are protected from harm. If you have any questions you may ask the principal investigator of this study or the AHRI-ALERT Ethics Review Committee at any time, even after the study has started. You can contact them using the following information:

Principal Investigator =Dr..Martha Zewdie (this project was done under the IRTBHHC study lead by Dr Martha Zewdie Gebeyehu.) =email; martha.zewdie@ahri.gov.et

AHRI-ALERT Ethics Review Committee (AAERC); Tel- 011 3 48 12 89

email -ahri.alerterc@ahri.gov.et

You can ask me any more questions about any part of the research study, if you wish to. Do you have any questions?

PART II: Assent Form

I have read the above-mentioned information, or it has been read to me. I have given the chance to ask questions about it and I am happy with the answers given to me. I have been told that I and my parent/guardian will be asked questions related to my health and general status and that blood will be collected from my arm at two visits; 4ml (2 teaspoons) now and 4ml (2 teaspoons) after two months. I will also be tested for HIV at both visits. I will give stool samples at all visits, and I understand a chest X-ray will be taken. I understand that my parent/guardian has given consent for me to participate in this study. I assent voluntarily to participate as a study participant in this research.

Name of Participant _____ Signature of Participant _____ Date _____
(Day/month/year)

If illiterate I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness _____ AND Thumb print of participant

Signature of witness _____

Date _____ Day/month/year



Name of Study Nurse: _____ Signature: _____ Date _____ (Day/month/year)
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Annex 2: የጥናቱ መረጃ እና የተሳታፊዎች መግለጫ ቅጽ (በአማርኛ የተዘጋጀ)

መግቢያ

የዚህ ጥናት ቡድን በአሁኑ ሰአት በኢትዮጵያ የጤና ጥበቃ ሚኒስቴር ስር በሚገኘው በአርማወር ሃንሰን የምርምር ማዕከል (አህሪ) በሳንባ በሽታ ላይ ምርምር እያካሄደ ነው። የምርምር ጥናቱ ርዕስ " Comparison Of Cytokines And Chemokines In QFT Positive And QFT Negative House Hold Contact Of TB Patients "ሲሆን የጥናቱ ተመራማሪ ዚያዳ ራህመዱ ትባላለች።

ስለዚህ ጥናት መረጃ እንግድትና በጥናቱ ተሳታፊ እንዲሆኑ እጠይቅዎታለሁ። በጥናቱ ተሳታፊ ለመሆንም ሆነ ላለመሆን በችኮላ መወሰን አይጠበቅብዎትም። በቂ ጊዜ ወስደው ፣ ከምትመርጡዋቸው ሰዎች ጋር ስለጥናቱ ተመካከረው መወሰን ይችላሉ። የማስረዳዎት መረጃ ግልጽ ካልሆነ በማንኛውም ሰአት አቁመውኝ ጥያቄ ሊጠይቁኝ ይችላሉ። በታላ ለሚኖርዎ ጥያቄዎች እኔን፣ የጥናቱን ዋና ተመራማሪ ፣ ወይም ይህን ጥናት ገምግሞ ለፈቀደው የምርምር ስነ ምግባር ኮሚቴ መጠየቅ ይችላሉ። የስልክ ቁጥራቸው በዚህ ሰነድ መጨረሻ ላይ ይገኛል፤፤

የምርምሩ አላማ

የሳንባ በሽታ በአለም ላይ በሚሊዮን የሚቆጠሩ ሰዎችን እያጠቃ ይገኛል፤፤ ኢትዮጵያም በከፍተኛ እየተጠቁ ካሉት ሀገሮች መካከል ትገኛለች። በሽታው ከሰው ወደ ሌላ ሰው በሳል የሚተላፍ ስለሆነ ከበሽተኛ ጋር የሚኖሩ ሰዎች በይበልጥ ለመያዝ ስጋት ላይ ናቸው፤፤ ይሁን እንጂ ሁሉም ከበሽተኛ ጋር የሚኖሩ ሰዎች አይታመሙም፤ አንድአንድ ግለሰቦች አብረው እየኖሩ ምንም የበሽታ ምልክት ሳያሳዩ ይቆያሉ፤፤ ለዚህ በሽታ የተሻለ የምርምር ዘዴ፤ ህክምናም ሆነ ክትባት ለማግኘት እና ለዚህም በሽታውን በይበልጥ ለመረዳት ምርምር ማካሄድ ወሳኝ ነው፤፤ ይህን ምርምር የምናካሂደው በሽታውን ለመረዳት እና ከህብረተሰባችን ውስጥ ሊኖሩ የሚችሉ ለመከላከያ የሚጠቅሙ ሁኔታዎችን ለመለየት ነው።

የምርምሩ አይነት

በሽታውን ለመረዳት የምናካሂደው ምርምር ሰውነታችንን ከበሽታ የሚከላከሉ ህዋሳትን በተለያዩ የህብረተሰባችን አባላት ላይ ማየትን ያካትታል። በጥናቱ የሚካተቱ የህብረተሰብ አካላት ከሳንባ በሽተኛ ጋር አብረው የሚኖሩ ወይም ከሳንባ በሽተኛ ጋር ኖረው የማያውቁ ጤነኛ የህብረተሰባችን አባላት ናቸው።

የተሳታፊ አመራረጥ እርስዎ በዚህ ጥናት እንዲሳተፉ የሚጠየቁት ጤነኛ ሆነው ከሳንባ በሽተኛ ጋር ኖረው ስለማያውቁ ወይም አሁን ከሳንባ በሽተኛ ጋር አብረው እየኖሩ ስለሚገኙ ነው።

የተሳታፊ አመራረጥ

እርስዎ በዚህ ጥናት እንዲሳተፉ የሚጠየቁት ጤነኛ ሆነው ከሳንባ በሽተኛ ጋር ኖረው ስለማያውቁ ወይም አሁን ከሳንባ በሽተኛ ጋር አብረው እየኖሩ ስለሚገኙ ነው።

በፈቃደኝነት ስለመስማማት

የዚህ ጥናት ተሳትፎ ሙሉ በሙሉ በእርስዎ ፈቃደኝነት የተመሰረተ ነው። ለመሳተፍም ሁነ ላለመሳተፍ መምረጥ ይችላሉ። አሁን ለመሳተፍ ፈቃደኛ ቢሆኑ እንኳ ወደፊት ሀሳብዎትን መቀየር እና ተሳትፎዎትን ማቋረጥ ይችላሉ።

የጥናቱ ሂደት

የዚህ ጥናት ተሳታፊ ከሆኑ በሁለት ቀጠሮ ቀኖች የደም ናሙና ከክንዶ ላይ እንዲሰጡ ይጠየቃሉ። አሁን አርባ 4 ሚ.ሊ (2 የሻይ ማንኪያ የሚሆን)፣ እና ከሁለት ወር በኋላ 4 ሚ.ሊ (2 የሻይ ማንኪያ የሚሆን) የደም ናሙና እንዲሰጡ ይጠየቃሉ። አነስተኛ ሂሞግሎቢን የደም ማነስ (አኒሚያ) ስለሚያሳይ ይህን ለማወቅ የሂሞግሎቢን ምርመራ ይደረግልዎታል ። በጣም አነስተኛ ሂሞግሎቢን ያለዎት ከሆነ ምንም ዓይነት ደም ከእርስዎ አንወስድም። በተጨማሪም ሆድ ውስጥ የሚገኙ ትላትሎችን ለመመርመር የሰገራ ናሙና በሁለቱም የቀጠሮ ቀኖች እንዲሰጡ ይጠየቃሉ። የሳንባ በሽታ ምልክቶችን ለማየት የሳንባ ራጅም አንድ ጊዜ ይወሰድልዎታል። ለሴቶች የእርግዝና ምርመራ ከሽንት ናሙና በሁለቱም የቀጠሮ ቀኖች ይደረጋል ። ለኤች.አይ.ቪ ምርመራም በሁለቱም የቀጠሮ ቀኖች ይደረግሎታል። የምርመራ ውጤትዎም በጤና ባለሙያ ይነገርዎታል። ውጤቱ ፖዘቲቭ ከሆነ በአቅርባቢያዎ ከሚገኝ የህክምና እና ክትትል ማድረጊያ (ART Clinic) ማዕከል ጋር እንዲገናኙ ይደረጋል።

ደሙ ንፅህና በተሞላበት ጥንቃቄ በጤና ባለሙያ ይወሰዳል። ደምዎ በዚህ ጥናት የሰውነታችንን ከበሽታ የመከላከያ ህዋሳት እና ውስን በነዚህ ህዋሳት ላይ የሚከሰቱ ለውጦችን ለማጥናት ጥቅም ላይ ይውላል። ሁሉም የጥናት ተሳታፊዎች በሚሳተፉባቸው ቀናቶች በጥቅሉ ስለጤንነታቸው የሚገልጽ ጥያቄዎችን ተጠይቀው መልሶቹ (መረጃዎቹ) ለጥናቱ በተዘጋጀ መጠይቅ ይሰፍራሉ። ይህም ወደ 20 ደቂቃ ይፈጃል።

በዚህ ጥናት መጨረሻ የተረፉ ናሙናዎች ወደፊት ላይ ለሚደረጉ ጥናቶች እንዲውሉ በአህሪ ይቀመጣሉ። ለዚህም ተጨማሪ የተለየ የስምምነት መጠይቅ እናደጋለን።

ስጋት

በዚህ ጥናት በመሳተፍዎ ደም በሚወሰድበት ጊዜ ከሌላ ጊዜ ተጨማሪ እና መለስተኛ የሆኑ የምችት መጓደል እና ህመም ሊገጥምዎት ይችላል። ይህን ስጋት ለመቀነስ አስፈላጊ ቅድመ ጥንቃቄ በጤና ባለሙያ ይደረግልዎታል።

ጥቅማጥቅሞች

በዚህ ጥናት መሳተፍዎ እርስዎን በቀጥታ ተጠቃሚ አያደርግዎትም። ሆኖም ግን የሳንባ በሽታ ምልክት ካለብዎት ለማወቅ በጤና ባለሙያ ይታያሉ፤ በተጨማሪም አንዱ የዚህ ጥናት ምርመራ አሁን ወይም ከዚህ በፊት በሳንባ በሽታ ተይዘው ከሆነ ለማወቅ ይረዳል፤ ይህ ምርመራ ከሌሎች ምርመራዎች ጋር ተጣምሮ የሳንባ በሽታን ለመለየት ይጠቅማል፤ ስለዚህ ከዚህ ምርመራ ሊጠቀሙ ይችላሉ፤ በተጨማሪም የዚህ ጥናት ውጤት ወደፊት ለሳንባ በሽታ የተሻለ ክትባት ለማግኘት ያግዛል።

ወጪ ስለመመለስ

በዚህ ጥናት ለመሳተፍ ክፍያ አይሰጥዎትም። ሆኖም ግን ለጥናቱ ሲባል ለሚያጠፉት ጊዜ እና ወደ ጤና ማዕከሉ ሲመጡ ያወጡትን የመጓጓዣ ወጪ ለመሸፈን የሚሆን 300 ብር በሁለቱም የተሳትፎ ቀጠሮዎች ይሰጥዎታል። ተጠርተው የራጅ ውጤት ለማድረስ ሲመጡ በተጨማሪ 300 ብር ይሰጥዎታል።

ሚስጥራዊነት

ለዚህ ጥናት የሚሰበሰበው መረጃ ሚስጥራዊነቱ የተጠበቀ ይሆናል። ማንነትዎን የሚገልፅ መረጃ ከተመራማሪዎቹ በስተቀር ማንም አያየውም። በስምዎ ፋንታ የጥናት ኮድ/ቁጥር እንጠቀማለን እና ይህንን ቁጥር ተመራማሪዎቹ ብቻ እንዲያውቁት ይደረጋል። መረጃዎች የያዙ ሰነዶችም በሚስጥር ቁጥር እና በቁልፍ ይጠበቃሉ እና ፈቃድ ለተመራማሪዎች ብቻ ይሰጣል። እንደአስፈላጊነቱ ከስነምግባር ኮሚቴው እና ተቆጣጣሪ ባለስልጣን መስሪያቤቶች በስተቀር ፈቃድ ለሌላ ሰው አይሰጥም።

ውጤትን ማሳወቅ

በዚህ ጥናት የሚገኙ እውቀቶች/መረጃዎች በአካባቢያችን እና በአለም አቀፍ ደረጃ በሰፊው ለህብረተሰቡ በህትመት መልክ እና በኮንፈረንስ ላይ በማቅረብ ይተላለፋሉ። ሚስጥራዊ መረጃዎች አይተላለፉም።

ያለመሳተፍ ወይም ከተሳትፎ የመውጣት

መብት እርስዎ ካልፈለጉ በዚህ ጥናት አለመሳተፍ ይችላሉ። ተሳትፎዎንም በሚፈልጉበት ጊዜ ማቋረጥ ይችላሉ። አለመሳተፍ ወይም ተሳትፎዎን ማቋረጥ በዚህ የጤና ማዕከል ሊያገኙ የሚችሉትን ማንኛውም አገልግሎት አይለወጥም። በዚህ የጤና ማዕከል የሚያገኙት ጥቅማጥቅሞች እንደተጠበቁ ይቆያሉ።

ቅጽ 2 የስምምነቱ ማረጋገጫ ቅጽ

ከላይ የተገለፀውን መረጃ አንብቤአለሁ፡ ወይም ተነበልኛል። ጥያቄ ለመጠየቅ ዕድል ተሰጥቶኛል፡ እና ለጠየቅኳቸው ጥያቄዎችም አጥጋቢ መልስ ተሰጥቶኛል። ስለጤንነቴ እና አጠቃላይ ሁኔታዬ ጥያቄ እንደምጠየቅ፣ እና የደም ናሙና ከክንዴ ላይ በሁለት የቀጠሮ ቀኖች ፤ 4 ሚ.ሊ (2 የሻይ ማንኪያ የሚሆን) ፡ እና ከሁለት ወር በኋላ 4ሚ.ሊ (የ2 ሻይ ማንኪያ የሚሆን) እንደሚወሰድ ተነግሮኛል። የኤች.አይ.ቪ ምርራም በሁለቱም የቀጠሮ ቀኖች ይደረግልኛል። የሰገራ ናሙናም በሁለቱም የቀጠሮ ቀኖች እሰጣለሁ። የሰገራ ናሙናም በሁለቱም የቀጠሮ ቀኖች እሰጣለሁ። የሳንባ ራጅ ይወሰድልኛል ። በዚህ ጥናት ለመሳተፍ በፈቃደኝነቴ እስማማለሁ።

የተሳታፊ የሚስጥር ቁጥር _____ የተሳታፊ ፊርማ _____ ቀን/ወር/ዓ.ም ____/____/____

ማንበብ ለማይችሉ

ከላይ የተገለፀው መረጃ ለተሳታፊው በትክክል እንደተነበበ እና ተሳታፊው ጥያቄ የመጠየቅ እድል ተሰጥቶት በአግባቡ እንደተመለሰለት እመሰክራለሁ። ተሳታፊው ስምምነቱን በነፃነት እደሰጠ አረጋግጣለሁ።

የእማኝ ስም: _____ እና የተሳታፊ የጣት  አሻራ

የእማኝ ፊርማ: _____

ቀን: _____ (ቀን/ወር/አ.ም.)

የጥናቱ የጤና ባለሙያ ስም: _____ ፊርማ: _____

ቀን: _____ (ቀን/ወር/አ.ም.)

ቅጽ 3፤ የተሳታፊዎች መረጃ እና የስምምነት ቅጽ (ለዕድሜ 15-17 ልጆች)

ክፍል 1፤ የተሳታፊዎች መረጃ

መግቢያ

የዚህ ጥናት ቡድን በአሁን ሰአት በኢትዮጵያ የጤና ጥበቃ ሚኒስቴር ስር በሚገኘው በአርማወር ሃንሰን የምርምር ማዕከል (አህሪ) በሳንባ በሽታ ላይ ምርምር እያካሄደ ነው። የምርምር ጥናቱ ርዕስ " Comparism Of Cytokines And Chemokine In QFT Positive And QFT Negative House Hold Contact Of TB Patients "ሲሆን የጥናቱ ተመራማሪ ዚያዳ ራህመዱ ትባላለች።

ስለዚህ ጥናት መረጃ እንግድትና በጥናቱ ተሳታፊ እንዲሆኑ እጠይቅዎታለሁ። በጥናቱ ተሳታፊ ለመሆንም ሆነ ላለመሆን በችኮላ መወሰን አይጠበቅብዎትም። በቂ ጊዜ ወስደው ፣ ከምትመርጡዋቸው ሰዎች ጋር ስለጥናቱ ተመካክረው መወሰን ይችላሉ። የማስረዳዎት መረጃ ግልጽ ካልሆነ በማንኛውም ሰአት አቁመውኝ ጥያቄ ሊጠይቁኝ ይችላሉ። በኋላ ለሚኖርዎ ጥያቄዎች እኔን፣ የጥናቱን ዋና ተመራማሪ ፣ ወይም ይህን ጥናት ገምግሞ ለፈቀደው የምርምር ስነ ምግባር ኮሚቴ መጠየቅ ይችላሉ። የስልክ ቁጥራቸው በዚህ ሰነድ መጨረሻ ላይ ይገኛል፤፤

የምርምሩ አላማ

የሳንባ በሽታ በአለም ላይ በሚሊዮን የሚቆጠሩ ሰዎችን እያጠቃ ይገኛል፤፤ ኢትዮጵያም በከፍተኛ እየተጠቁ ካሉት ሀገሮች መካከል ትገኛለች። በሽታው ከሰው ወደ ሌላ ሰው በሳል የሚተላፍ ስለሆነ ከበሽተኛ ጋር የሚኖሩ ሰዎች በይበለጥ ለመያዝ ስጋት ላይ ናቸው፤፤ ይሁን እንጂ ሁሉም ከበሽተኛ ጋር የሚኖሩ ሰዎች አይታመሙም፤ አንድአንድ ግለሰቦች አብረው እየኖሩ ምንም የበሽታ ምልክት ሳያሳዩ ይቆያሉ፤፤ ለዚህ በሽታ የተሻለ የምርምር ዘዴ፤ ህክምናም ሆነ ክትባት ለማግኘት እና ለዚህም በሽታውን በይበለጥ ለመረዳት ምርምር ማካሄድ ወሳኝ ነው፤፤ ይህን ምርምር የምናካሂደው በሽታውን ለመረዳት እና ከህብረተሰባችን ውስጥ ሊኖሩ የሚችሉ ለመከላከያ የሚጠቅሙ ሁኔታዎችን ለመለየት ነው።

የምርምሩ አይነት

በሽታውን ለመረዳት የምናካሂደው ምርምር ሰውነታችንን ከበሽታ የሚከላከሉ ህዋሳትን በተለያዩ የህብረተሰባችን አባላት ላይ ማየትን ያካትታል። በጥናቱ የሚካተቱ የህብረተሰብ አካላት ከሰንባ በሽተኛ ጋር አብረው የሚኖሩ ወይም ከሰንባ በሽተኛ ጋር ኖረው የማያውቁ ጤነኛ የህብረተሰባችን አባላት ናቸው።

የተሳታፊ አመራረጥ

እርስዎ በዚህ ጥናት እንዲሳተፉ የሚጠየቁት ጤነኛ ሆነው ከሰንባ በሽተኛ ጋር ኖረው ስለማያውቁ ወይም አሁን ከሰንባ በሽተኛ ጋር አብረው እየኖሩ ስለሚገኙ ነው።

በፈቃደኝነት ስለመስማማት

የዚህ ጥናት ተሳትፎ ሙሉ በሙሉ በእርስዎ ፈቃደኝነት የተመሰረተ ነው። ለመሳተፍም ሁነ ላለመሳተፍ መምረጥ ይችላሉ። አሁን ለመሳተፍ ፈቃደኛ ቢሆኑ እንኳ ወደፊት ሀሳብዎትን መቀየር እና ተሳትፎዎትን ማቋረጥ ይችላሉ።

የጥናቱ ሂደት

የዚህ ጥናት ተሳታፊ ከሆኑ በሁለት ቀጠሮ ቀኖች የደም ናሙና ከክንዶ ላይ እንዲሰጡ ይጠየቃሉ። አሁን 4 ሚ.ሊ (2 የሻይ ማንኪያ የሚሆን)፣ እና ከሁለት ወር በኋላ 4ሚ.ሊ (2 ሻይ ማንኪያ የሚሆን) የደም ናሙና እንዲሰጡ ይጠየቃሉ። አነስተኛ ሂሞግሎቢን የደም ማነስ (አኒሚያ) ስለሚያሳይ ይህን ለማወቅ የሂሞግሎቢን ምርመራ ይደረግልዎታል ። በጣም አነስተኛ ሂሞግሎቢን ያለዎት ከሆነ ምንም ዓይነት ደም ከእርስዎ አንወስድም። በተጨማሪም ሆድ ውስጥ የሚገኙ ትላትሎችን ለመመርመር የሰገራ ናሙና በሁለቱም የቀጠሮ ቀኖች እንዲሰጡ ይጠየቃሉ። የሰንባ በሽታ ምልክቶችን ለማየት የሰንባ ራጅም አንድ ጊዜ ይወሰድልዎታል። ለሴቶች የእርግዝና ምርመራ ከሽንት ናሙና በሁለቱም የቀጠሮ ቀኖች ይደረጋል። ለኤች.አይ. ሺ ምርመራም በሁለቱም የቀጠሮ ቀኖች ይደረግላቸዋል። የምርመራ ውጤትዎም በጤና ባለሙያ ይነገርዎታል። ውጤቱ ፖዘቲቭ ከሆነ በአቅርባቢያዎ ከሚገኝ የህክምና እና ክትትል ማድረጊያ (ART Clinic) ማዕከል ጋር እንዲገናኙ ይደረጋል።

ደሙ ንፅህና በተሞላበት ጥንቃቄ በጤና ባለሞያ ይወሰዳል። ደምም በዚህ ጥናት የሰውነታችንን ከበሽታ የመከላከያ ህዋሳት እና ውስን በነዚህ ህዋሳት ላይ የሚከሰቱ ለውጦችን ለማጥናት ጥቅም ላይ ይውላል። ሁሉም የጥናት ተሳታፊዎች በሚሳተፉባቸው ቀናቶች በጥቅሉ ስለጤንነታቸው የሚገልጽ ጥያቄዎችን ተጠይቀው መልሶቹ (መረጃዎቹ) ለጥናቱ በተዘጋጀ መጠይቅ ይሰፍራሉ። ይህም ወደ 20 ደቂቃ ይፈጃል። በዚህ ጥናት መጨረሻ የተረፉ ናሙናዎች ወደፊት ላይ ለሚደረጉ ጥናቶች እንዲውሉ በአህጉር ይቀመጣሉ። ለዚህም ተጨማሪ የተለየ የስምምነት መጠይቅ እናደጋለን።

ስጋት

በዚህ ጥናት በመሳተፍዎ ደም በሚወሰድበት ጊዜ ከሌላ ጊዜ ተጨማሪ እና መለስተኛ የሆኑ የምችት መጓደል እና ህመም ሊገጥምዎት ይችላል። ይህን ስጋት ለመቀነስ አስፈላጊ ቅድመ ጥንቃቄ በጤና ባለሞያ ይደረግልዎታል።

ጥቅማጥቅሞች

በዚህ ጥናት መሳተፍዎ እርስዎን በቀጥታ ተጠቃሚ አያደርግዎትም። ሆኖም ግን የሳንባ በሽታ ምልክት ካለብዎት ለማወቅ በጤና ባለሞያ ይታያሉ፤ በተጨማሪም አንዱ የዚህ ጥናት ምርመራ አሁን ወይም ከዚህ በፊት በሳንባ በሽታ ተይዘው ከሆነ ለማወቅ ይረዳል፤ ይህ ምርመራ ከሌሎች ምርመራዎች ጋር ተጣምሮ የሳንባ በሽታን ለመለየት ይጠቅማል፤ ስለዚህ ከዚህ ምርመራ ሊጠቀሙ ይችላሉ፤ በተጨማሪም የዚህ ጥናት ውጤት ወደፊት ለሳንባ በሽታ የተሻለ ክትባት ለማግኘት ያግዛል።

ወጪ ስለመመለስ

በዚህ ጥናት ለመሳተፍ ክፍያ አይሰጥዎትም። ሆኖም ግን ለጥናቱ ሲባል ለሚያጠፉት ጊዜ እና ወደ ጤና ማዕከሉ ሲመጡ ያወጡትን የመጓጓዣ ወጪ ለመሸፈን የሚሆን 300 ብር በሁለቱም የተሳትፎ ቀጠሮዎች ይሰጥዎታል። ተጠርተው የራጅ ውጤት ለማድረስ ሲመጡ በተጨማሪ 300 ብር ይሰጥዎታል።

ሚስጥራዊነት

ለዚህ ጥናት የሚሰበሰበው መረጃ ሚስጥራዊነቱ የተጠበቀ ይሆናል። ማንነትዎን የሚገልፅ መረጃ ከተመራማሪዎቹ በስተቀር ማንም አያየውም። በስምዎ ፋንታ የጥናት ኮድ/ቁጥር እንጠቀማለን እና ይህንን

ቁጥር ተመራማሪዎች ብቻ እንዲያውቁት ይደረጋል። መረጃዎች የያዙ ሰነዶችም በሚስጥር ቁጥር እና በቁልፍ ይጠበቃሉ እና ፈቃድ ለተመራማሪዎች ብቻ ይሰጣል። እንደአስፈላጊነቱ ከስነምግባር ኮሚቴው እና ተቆጣጣሪ ባለስልጣን መስሪያቤቶች በስተቀር ፈቃድ ለሌላ ሰው አይሰጥም።

ውጤትን ማሳወቅ

በዚህ ጥናት የሚገኙ እውቀቶች/መረጃዎች በአካባቢያችን እና በአለም አቀፍ ደረጃ በሰፊው ለህብረተሰቡ በህትመት መልክ እና በኮንፈረንስ ላይ በማቅረብ ይተላለፋሉ። ሚስጥራዊ መረጃዎች አይተላለፉም።

ያለመሳተፍ ወይም ከተሳትፎ የመውጣት

መብት እርስዎ ካልፈለጉ በዚህ ጥናት አለመሳተፍ ይችላሉ። ተሳትፎዎንም በሚፈልጉበት ጊዜ ማቋረጥ ይችላሉ። አለመሳተፍ ወይም ተሳትፎዎን ማቋረጥ በዚህ የጤና ማዕከል ሊያገኙ የሚችሉትን ማንኛውም አገልግሎት አይለወጥም። በዚህ የጤና ማዕከል የሚያገኙት ጥቅማጥቅሞች እንደተጠበቁ ይቆያሉ።

ጥያቄ ካለዎት ማንን ያግኙ

ይህ ጥናት በአህራ-አለርት የምርምር ስነምግባር ኮሚቴ ተገምግሞ ፈቃድ ያገኛል። የዚህ ኮሚቴ ስራ የጥናት ተሳታፊዎች መብት መጠበቅ እና ከጉዳት መጠበቅን ማየት ነው።

ጥያቄ ካለዎት የጥናቱን ዋና ተመራማሪ ወይም የአህራ-አለርት የምርምር ስነምግባር ኮሚቴን በማንኛውም ጊዜ ፤ ጥናቱ ከጀመረ በኋላም መጠየቅ ይችላሉ። ለማግኘት የሚከተለውን መረጃ ይጠቀሙ፡

የጥናቱ ዋና ተመራማሪ ዶ/ር ፤ ማርታ ዘዉዴ ኢሜል፤ martha.zewdie@ahri.gov.et

የአህራ-አለርት የምርምር ስነምግባር ኮሚቴ፤ ስልክ ቁጥር 011 3 481289

ስለዚህ ምርምር ተጨማሪ ጥያቄዎች ካለዎት ሊጠይቁኝ ይችላሉ። ጥያቄ አለዎት?

ክፍል 2፡ የስምምነት ቅፅ

ከላይ የተገለፀውን መረጃ አንብቤአለሁ፡ ወይም ተነበልኛል። ጥያቄ ለመጠየቅ ዕድል ተሰጥቶኛል፡ እና ለጠየቅኳቸው ጥያቄዎችም አጥጋቢ መልስ ተሰጥቶኛል። ስለጤንነቱ እና አጠቃላይ ሁኔታዬ ጥያቄ እንደምጠየቅ፤ እና የደም ናሙና ከክንዴ ላይ በሁለት የቀጠሮ ቀኖች ፤ 4 ሚ.ሊ (2 የሻይ ማንኪያ የሚሆን) ፡

እና ከሁለት ወር በኋላ 4ሚ.ሊ (የ2 ሻይ ማንኪያ የሚሆን) ከሁለት ወር በኋላ: እንደሚወሰድ ተነግሮታል።
 የኤች.አይ. ቪ ምርራም በሁለቱም የቀጠሮ ቀኖች ይደረግልኛል። የሰገራ ናሙናም በሁለቱም የቀጠሮ ቀኖች
 እሰጣለሁ። የሳንባ ራጅ ይወሰድልኛል:

እኔ በዚህ ጥናት እንደሳተፍ ወላጄ/አሳዳጊዬ መስመማማት እንዳለበት ተረድቻለሁ። በዚህ ጥናት ለመሳተፍ
 በፈቃደኝነቴ እስማማለሁ።

የተሳታፊ ስም: _____ የተሳታፊ ፊርማ: _____

ቀን: _____ (ቀን/ወር/አ.ም.)

ማንበብ ለማይችሉ

ከላይ የተገለፀው መረጃ ለተሳታፊው በትክክል እንደተነበበ እና ተሳታፊው ጥያቄ የመጠየቅ እድል ተሰጥቶት
 በአግባቡ እንደተመለሰለት እመሰክራለሁ። ተሳታፊው ስምምነቱን በነፃነት እደሰጠ አረጋግጣለሁ።

የእማኝ ስም: _____ እና የተሳታፊ የጣት አሻራ

የእማኝ ፊርማ: _____



ቀን: _____ (ቀን/ወር/አ.ም.)

የጥናቱ የጤና ባለሙያ ስም: _____ ፊርማ: _____

ቀን: _____ (ቀን/ወር/አ.ም.)

ክፍል 3፡ የስምምነት ቅጽ (የተረፈ ናሙና ለማስቀመጥና ለወደፊት ጥናት ለመጠቀም)

ይህ ጥናት ከተጠናቀቀ በኋላ የሚተርፉ ናሙናዎች ይኖራሉ። የተረፉትን ናሙናዎች ለማስቀመጥ እና ወደፊት ለሚካሄዱ ጠቃሚ ጥናቶች ለመጠቀም ፈቃድዎን እንጠይቃለን። ይህን የምንጠይቅበት በፊት የሰጡን ስምምነት የሚፈቅድልን ናሙናዎችን ለዚህ ጥናት ብቻ እንድንጠቀም ስለሆነ ነው። የተረፉት ናሙናዎች ለወደፊት ጥናት እንዲውሉ ካልፈለጉ ፈቃድዎን አለመስጠት እና በዚህ ጥናት ብቻ መሳፍ ይችላሉ።

አሁን ናሙናዎቹ ከማን እንደተወሰዱ ማወቅ ይቻላል። ናሙናዎች ለረጅም ጊዜ ለወደፊት ጥናት ሲቀመጡ በዚህ ሁኔታ እንዲቀመጡ ወይም ማንነትዎን የሚገልጹ መረጃዎች አስወግዶ ማስቀመጥ ይቻላል። ናሙናዎቹ ማንነትዎን ከሚገልጹ መረጃዎች ከተለያዩ ወደፊት ከሚካሄዱ ጥናቶች የሚገኙትን መረጃዎች ለእርስዎ መንገር አይቻልም። ማንነትዎን የሚገልጹ መረጃዎች ተጣምረው ከቆዩ ለጤናዎ ጠቃሚ ሊሆኑ የሚችሉ መረጃዎችን ልንነግረዎ እንችላለን።

የተረፉት ናሙናዎች ትርፍ/ገቢ ለማግኘት ለማንም አይሰጡም/አይሸጡም። ሆኖም ግን አንድአንድ ከሰው የተወሰደ ናሙና የሚጠቀሙ ጥናቶች ተመራማሪዎችን የምርመራ ቁሳቁስ፣ መድሃኒት፣ ወይም ክትባት ለማግኘት እና በዘላቂነት ገቢ ሊያስገኙ ይችላሉ። ይህ በሚሆንበት ጊዜ እርስዎ ምንም አይነት ክፍያ አያገኙም ግን እርስዎ እና ተመሳሳይ ህመም ያደረባቸው የህብተሰቡ አባላት በውጤቶቹ ተጠቃሚ ሊሆኑ ይችላሉ። የተቀመጡትን ናሙናዎች ሊጠቀሙ ያቀዱ ጥናቶች በቅድሚያ በኢትዮጵያ የምርመራ ስነ ምግባር ኮሚቴ ይገመገማሉ።

የተረፉትን ናሙናዎች ተቀምጠው ወደፊት ለሚቀረጹ ጥናቶች እንዲውሉ መፍቀድዎ ለእርስዎ ጥቅም ላይኖረው ይችላል። መፍቀድዎም ለእርስዎ ጉዳት አያመጣም። በቆይታም ሆነ መረጃ በመለዋወጫ ሰአት፡ የጥናት ኮድ በመጠቀም እና ስምዎን ወይም ሌላ እርስዎነትዎን የሚገልጽ መረጃ ባለመጠቀም የመረጃዎ ሚስጥራዊነት ይጠበቃል። የተረፉት ናሙናዎች ተቀምጠው ወደፊት ለሚቀረጹ ጥናቶች እንዲውሉ ፈቃደኛ ካልሆኑ፡ ናሙናዎቹ በዘሂህ ጥናት ማለቂያ ይጣላሉ። የተረፉት ናሙናዎች ተቀምጠው ወደፊት ለሚቀረጹ ጥናቶች እንዲውሉ ፈቃደኛ ከሆኑ በአህጉሪ ላቦራቶሪ ውስጥ ቢያንስ ለ10 አመት ይቀመጣሉ እና ውስን በመስሪያቤቱ ፈቃድ ያገኙ ሰዎች ብቻ ናሙናዎቹን ወይም ተዛማጅ መረጃዎቹን ማግኘት ይችላሉ።

እባክዎ ከሚከተሉት አማራጮች ምርጫዎን ምልክት ያርጉበት

1. የተረፉኝን በኮድ የተቀመጡ ናሙናዎች ለማንኛውም አይነት የወደፊት ጥናት ላይ እንዲውሉ

_____ እፈቅዳለሁ። _____ አልፈቅድም። የተረፉኝ ናሙናዎች በዚህ ጥናት ማለቂያ ይጣላሉ፡

2. ወደፊት ለሚሰሩ ጥናቶች ስለ እኔ ተጨማሪ መረጃ ከተፈለገ፡ ተመራማሪዎቼ ወደፊት እንዲያገኙኝ

_____ ፈቃደኛ ነኝ። _____ ፈቃደኛ አይደለሁም።

የተሳታፊ ስም፡ _____ ፊርማ፡ _____ ቀን፡ _____

የእማኝ ስም፡ _____ ፊርማ፡ _____ ቀን፡ _____

የጤና ባለሙያ ስም፡ _____ ፊርማ፡ _____ ቀን፡ _____

Annex 3- Questionnaire

3.1 Candidate Participant Screening Tool

1.	Screening ID: IRTBHHC 1.1 Study group	_____ _____			
2.	Health Facility	_____			
3.	Initials of Data Collector	_____			
4.	Initials of participant	<table border="1" style="display: inline-table; vertical-align: middle;"><tr><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td><td style="width: 20px; height: 20px;"></td></tr></table>			
5.	Date of sampling (DD/MM/YY)	__ __ / __ __ / __ __ (G.C.)			
6.	Do you agree to participate in the study?	1. Yes 2. No			
	If NO- Not eligible for the study				
7.	How old are you?	_____			
	a. If the participant is younger than 15 years	Not eligible for the study			
	b. If the participant is between 15-18, has a parent /guardian given consent?	1. Yes 2. No			
	If yes Not eligible for the study				
8.	Are you feeling sick?	1. Yes 2. No			
9.	Temperature of participant?				
10.	Do you have the following symptoms				
	a. Cough				
	b. Sputum				
	c. Hemoptysis				
	d. Night sweats				
	e. Weight loss				
	f. Poor appetite				
	g. Dyspnea (shortness of breath)				
	Others: Specify: -				

11.	Is the candidate a TB suspect?	1. Yes 2. No
	If yes - Not eligible for the study	
12.	Are you willing to be tested for HIV?	1. Yes 2. No
	If yes- Not eligible for the study	
	a. If yes what is the result?	1. Positive 2. Negative
	If Positive- Not eligible for the study	
13.	If candidate is female, are you willing to have a pregnancy Test?	1. Yes 2. No
	If Yes, what is the result?	1. Positive 2. Negative
	If Positive- Not eligible for the study	
14.	Has a doctor or healthcare worker ever told you that you have any of the following conditions:	
	a. Diabetes	1. Yes 2. No
	b. Hypertension	1. Yes 2. No
	c. cardiovascular diseases	1. Yes 2. No
	d. kidney disease	1. Yes 2. No
	e. Liver disease (hepatitis)	1. Yes 2. No
	f. Any other infectious disease or communicable chronic disease? (others)	
	If any of the answer to Q-14 are yes end of the Questionnaire	

15.	Are you on any medications which are steroids or other immune-suppressants? IF yes, Specify	1. Yes 2. No
16.	Are you on any medication?	1. Yes 2. No
	IF yes, Specify: -	
17.	Have you ever been treated for TB before?	1. Yes 2. No
	If Yes, specify: - END (it is not eligible for study)	
18.	Decision: is the candidate eligible for the study?	1. Yes 2. No
	If NO - END the Questionary	
	If Yes, Give Participants Study code and Continue with Questionnaire. Participant Study Code: IRTBHHC	

3.2 Case Report from for House- Hold At visit one

1.	Health Facility			
2.	Initials of Data Collector			
3.	Initials of participant			
4.	Participant Study Code	IRTBHHC-		
5.	Participant Group	(CC or HHC)		
6.	Date of sampling (DD/MM/YY):	__ __ / __ __ / __ __		
Socio Demographic Information				
7.	Age (years)?			
8.	Sex?	1. Male 2. Female		
9.	Address	Sub city: - Woreda_-		
10.	Marital status?	1. Single 2. Married 3. Divorced 4. Widow/er		
11.	Education?	1. Illiterate/ can't read 2. Non-formal education 3. Grade 1-8 4. High school 5. Diploma 6. Degree and above		
12.	Occupation?	1. Unemployed 2. Housewife 3. Pensioned 4. Civil servant 5. Private business		
13.	Monthly Income in birr	1. Below 1,500 2. 1, 500 – 3000 3. 3,000 – 5000		

		4. Above 5,000 5. Can't estimate
Housing & Living condition		
14.	Number of rooms in your home	
15.	Does the house have windows	1. Yes 2. No
16	Number of People living in the same household?	
17	Are you regularly exposed to indoor firewood/charcoal smoke at home?	1. Yes 2. No
18	How often do you do physical exercise?	1. Once a month or less 2. 2-4 times a month 3. 2-3 times a week 4. 4 or more a week 5. Never
19	How often do you have a drink containing alcohol?	1. Once a month or less 2. 2-4 times a month 3. 2-3 times a week 4. 4 or more a week 5. Never
20	How many days a month do you chew a chat?	1. Never 2. Daily 3. 5-6 days per week 4. 3-4 days per week 5. 1-2 days per week
History of TB Exposure		
21.	Did you have a contact with TB Patient in your Household?	1. Yes 2. No
22.	If Yes to Question 21, How long have you lived with the TB patient?	_____ (Days/weeks/months)

23.	Relationship to index case	1. Family member 2. Relative 3. Close friend 4. Other, Specify_____
24.	Over the past three months on average how many days per week do you spend in or around this residence?	_____ (days)
25.	Do you ever stay in another residence? (Over the past three months)	1. Yes 2. No
26.	If Yes to Q25, how many days per week?	_____ (days)
27.	Over the past three months, how often did you come in contact with the index case?	1. <1 day per week 2. 1-3 days per week 3. 4-6 days per week 4. Daily 5. None 6. Don't recall
28.	On the days you had contact with the index case, how much time did you spend with the index case?	1. <1hr a day 2. 2-6 hrs a day 3. 7-12 hrs a day 4. 13-18 hrs a day 5. Over 18 hrs a day 6. Don't recall
29.	What was the nature of contact with the index case? (You may tick more than one)	1. Share Meals 2. Sleep in the same room 3. Sleep in the same bed 4. Caretaker
30.	Has anyone else in your family been treated for TB before?	1. Yes 2. No
	If 'Yes' when was TB treatment completed?	___ / ___ / ___

Physical Examination				
31.	Is BCG scar present? (Examine the participant's Right arm)		Present Absent Indeterminate	
32.	Does the participant have a vaccination card?		1. Yes 2. No	
33.	Surface Temperature (degree Celsius)?			
34.	Pulse rate (per minute)			
35.	Systolic BP (in mm Hg)			
36.	Diastolic BP (in mm Hg)			
37.	Weight in Kg			
38.	Height in CM			
39.	BMI			
40.	MUAC (mid- upper arm circumference in cm)			
41.	TB Clinic ID No of the Index TB patient:			
42.	Smear Microscopy and/or GeneXpert result of the index TB patient?			
		Smear#	Positive	Negative
		1		
		2		
		3		
History of COVID19 exposure/vaccination				
43.	Have you been vaccinated for COVID19?		1. Yes 2. No	

44.	If Yes, Name the vaccine(s) and number of doses and date of last vaccine.	Vaccine type: _____ No of doses: _____ Last vaccination date: _____
45.	Have you been diagnosed with COVID-19 N/P test?	1. Yes 2. No
	What was the result of COVID-19 test	1. Positive 2. Conformed Negative
46.	Has anyone in your household been diagnosed with COVID19?	1. Yes 2. No
Laboratory test results		
47.	Hemoglobin test result	
48.	HIV Test Result	
49.	Pregnancy Test result	
50.	Stool test result	

3.3 Case Report Form for House-hold contacts at visit two

1.	Health Facility	
2.	Collector (Initials)	
3.	Initials of participant	
4.	Participant Study Code	IRTBHHC- _____
	Participant Group	(CC or HHC) _____
5.	Date of sampling (DD/MM/YY)	__ __ / __ __ / __ __ (G.C.)
6.	Visit No.	1 2
7.	Are you feeling sick?	1. Yes 2. No

	If yes, explain the symptoms: -	
8.	Do you have the following symptoms?	
	a. Cough	
	b. Sputum	
	c. Hemoptysis	
	d. Hemoptysis	
	e. Weight Loss	
	f. Poor appetite	
	g. Night sweats	
	h. Dyspnea (shortness of breath)	
	Other: Specify: -	
9.	Has a doctor or healthcare worker ever told you that you have any of the following conditions:	
	a. Diabetes	1. Yes 2. No
	b. Hypertension	1. Yes 2. No
	c. cardiovascular diseases	1. Yes 2. No
	d. kidney disease	1. Yes 2. No
	e. Liver disease (hepatitis)	1. Yes 2. No
	f. Any other infectious or non-communicable chronic disease? (others)	1. Yes 2. No

10.	Are you on any medication?	1. Yes 2. No
11.	Are you on medication which is steroids or other immunosuppressant? (Please refer a list of immunosuppressing drugs provided)	1. Yes 2. No
	if yes, specify:-	
Physical Examination		
12.	Surface Temperature (degree Celsius)?	
13.	Pulse rate (per minute)	
14.	Systolic BP (in mm Hg)	
15.	Diastolic BP (in mm Hg)	
16.	Weight in Kg	
17.	Height in CM	
18.	BMI	
19.	MUAC (Mid- upper arm circumference in cm)	
History of COVID19 exposure or vaccination in the past 8 weeks		
20.	Did you receive a COVID19 vaccination in the past 8 weeks (since Visit 1)?	1. Yes 2. No
21.	If Yes, Name the vaccine(s) and number of doses and date of last vaccine.	Vaccine type: _____ No of doses: _____ Last vaccination date: _____
22.	Have you been diagnosed with COVID19?	1. Yes 2. No
23.	Has anyone in your household been diagnosed with COVID19?	1. Yes 2. No
Laboratory test results		
24.	Hemoglobin test result	
25.	HIV Test result	
26.	Pregnancy Result	

27.	Stool result	
Contact with TB patient in the past 8 weeks		
28	In the past two months or since your first visit have you come in contact with a TB patient?	1. Yes 2. No
	a. If yes, specify duration	

3.4 Case Report from for Community contacts at Visit 1

1.	Health Facility				
2.	Initials of Data Collector				
3.	Initials of participant				
4.	Participant Study Code	IRTBHHC-			
5.	Participant Group	(CC or HHC)			
6.	Date of sampling (DD/MM/YY):	__ __ / __ __ / __ __			
Socio Demographic Information					
7.	Age (years)?				
8.	Sex?	1. Male 2. Female			
9.	Address	Sub city: - Woreda_-			
10.	Marital status?	1. Single 2. Married 3. Divorced 4. Widow/er			
11.	Education?	1. Illiterate/ can't read 2. Non-formal education 3. Grade 1-8 4. High school 5. Diploma 6. Degree and above			
12.	Occupation?	1. Unemployed 2. Housewife 3. Pensioned 4. Civil servant 5. Private business			
13.	Monthly Income in birr	1. Below 1,500 2. 1, 500 – 3000 3. 3,000 – 5000			

		4. Above 5,000 5. Can't estimate
Housing & Living condition		
14.	Number of rooms in your home	
15.	Does the house have windows	1. Yes 2. No
16	Number of People living in the same household?	
17	Are you regularly exposed to indoor firewood/charcoal smoke at home?	1. Yes 2. No
18	How often do you do physical exercise?	1. Once a month or less 2. 2-4 times a month 3. 2-3 times a week 4. 4 or more a week 5. Never
19	How often do you have a drink containing alcohol?	6. Once a month or less 7. 2-4 times a month 8. 2-3 times a week 9. 4 or more a week 10. Never
20	How many days a month do you chew a chat?	6. Never 7. Daily 8. 5-6 days per week 9. 3-4 days per week 10. 1-2 days per week
History of TB Exposure		
21.	Did you have a contact with TB Patient in your Household?	1. Yes 2. No
22.	Has anyone else in your family been treated for TB before?	1. Yes 2. No

Physical Examination		
23.	Is BCG scar present? (Examine the participant's Right arm)	Present Absent Indeterminate
24.	Does the participant have a vaccination card?	1. Yes 2. No
25.	Surface Temperature (degree Celsius)?	
26.	Pulse rate (per minute)	
27.	Systolic BP (in mm Hg)	
28.	Diastolic BP (in mm Hg)	
29.	Weight in Kg	
30.	Height in CM	
31.	BMI	
32.	MUAC (mid- upper arm circumference in cm)	
History of COVID19 exposure/vaccination		
33.	Did you receive a COVID19 vaccination in the past 8 weeks (since Visit 1)?	1. Yes 2. No
34.	If Yes, Name the vaccine(s) and number of doses and date of last vaccine.	Vaccine type: _____ No of doses: _____ Last vaccination date: _____
35.	Have you been diagnosed with COVID-19 N/P test?	1. Yes 2. No
36.	Has anyone in your household been diagnosed with COVID19?	1. Yes 2. No
Laboratory test results		
37.	Hemoglobin test result	

38.	Stool test result	
39.	Pregnancy test	
40.	HIV test	
41.	Chest X- ray test result	
42.	Did the participant Consent form for storage and future use of the samples?	1. Yes ☐ 2. No ☐

3.5 Case Report Form for Community Visit 2(CC-V2)

1.	Health Facility	
2.	Collector (Initials)	
3.	Initials of participant	
4.	Participant Study Code	IRTBHHC- _____
	Participant Group	(CC or HHC) _____
5.	Date of sampling (DD/MM/YY)	__ __ / __ __ / __ __ (G.C.)
6.	Visit No.	1 2
General Health Condition		
7.	Are you feeling sick?	1. Yes 2. No
	If yes , explain the symptoms:-	
8.	Do you have the following symptoms?	
	i. Cough	
	j. Sputum	
	k. Hemoptysis	
	l. Hemoptysis	
	m. Weight Loss	
	n. Poor appetite	
	o. Night sweats	
	p. Dyspnea (shortness of breath)	
	Other :Specify:-	
9.	Has a doctor or healthcare worker ever told you that you have any of the following conditions:	

	g. Diabetes	1. Yes 2. No
	h. Hypertension	1. Yes 2. No
	i. cardiovascular diseases	1. Yes 2. No
	j. kidney disease	1. Yes 2. No
	k. Liver disease (hepatitis)	1. Yes 2. No
	l. Any other infectious or non-communicable chronic disease? (others)	1. Yes 2. No
10	Are you on any medication?	1. Yes 2. No
11.	Are you on medication which is steroids or other immunosuppressant? (Please refer a list of immunosuppressing drugs provided)	1. Yes 2. No
	if yes, specify:-	
Physical Examination		
12.	Surface Temperature (degree Celsius)?	
13.	Pulse rate (per minute)	
14.	Systolic BP (in mm Hg)	
15.	Diastolic BP (in mm Hg)	
16.	Weight in Kg	
17.	Height in CM	
18.	BMI	
19.	MUAC (Mid- upper arm circumference in cm)	

20.	Did you receive a COVID19 vaccination in the past 8 weeks (since Visit 1)?	1. Yes 2. No
21	If Yes, Name the vaccine(s) and number of doses and date of last vaccine.	Vaccine type: _____ No of doses: _____ Last vaccination date: _____
22.	Have you been diagnosed with COVID19?	1. Yes 2. No
23.	Has anyone in your household been diagnosed with COVID19?	1. Yes 2. No
Laboratory test results		
24.	Hematological test result	
25.	Stool result	
26.	Chest X- ray test result	
27.	Did the participant Consent form for storage and future use of the samples?	1. Yes ☐ 2. No ☐
Contact with TB patient in the past 8 weeks		
28.	In the past two months or since your first visit have you come in contact with a TB patient?	1. Yes 2. No
	a. If yes ,specify duration	

3.2 ለየቤተሰብ አባላት እና የማህበረሰብ አባላት መጠይቅ

1	በጥናቱ ለመሳተፍ ተስማምተዋል?	1. አዎ 2. አይ
2	እድሜህ ስንት ነው? (በአመታት)	
3	ተሳታፊው ከ 15 ዓመት በታች ከሆነ-	መጠይቁን አቁም
4	ተሳታፊው በ15-18 መካከል ከሆነ፣ ወላጅ/አሳዳጊ ስምምነት ሰጥቷል?	1. አዎ 2. አይ. መልሱ አይ ከሆነ መጠይቁን አቁም
5	የሚከተሉት ምልክቶች አሉዎት?	ሳል : አክታ : ደም ማሳል : በምሽት ማላብ : የክብደት መቀነስ:ደካማ የምግብ ፍላጎት : ትንፋሽ ማጠር : ግለሰቡ የሳንባ ነቀርሳ (ቲቢ) ተጠርጣሪ ነው? 1. አዎ 2. አይ. አዎ ከሆነ መጠይቁን አቁም
6	ከሚከተሉት በሽታዎች ውስጥ የትኛውም እንዳለህ ሐኪም ወይም የጤና ባለ ሙያ ነግሮህ ያውቃል? 6.1 የስኳር በሽታ 6.2 የደም ግፊት 6.3 የልብ እና የደም ቧንቧ በሽታዎች 6.4 የኩላሊት በሽታ 6.5 የጉበት በሽታ (ሄፓታይቲስ) 6.6 ሌላ ማንኛውም ተላላፊ ወይም የማይተላለፍ ሥር የሰደደ በሽታ? (ሌሎች)	1. አዎ 2. አይ. ለ ጥያቄ 6 ከተሰጡት መልሶች ውስጥ አንዳቸውም አዎ ከሆኑ፣ ምልመላውን ያቁሙ

7	ስቴሮይድ ወይም ሌላ የበሽታ የመከላከልን እቅም የሚያዳክሙ የሚወስዱት መድሃኒት አልዎት?	1. አዎ 2. አይ አዎ ከሆነ ይግለጹ
8	ከዚህ በፊት በቲቢ ታክመው ያውቃሉ? አዎ ከሆነ መጠይቁን አቁም	1. አዎ 2. አይ አዎ ከሆነ መጠይቁን አቁም
9	ኤች አይቪ ለመመርመር ፍቃደኛ ኖት? አዎ ከሆነ ውጤት	1. አዎ 2. አይ አይ ከሆነ መጠይቁን አቁም

ውሳኔ፡ ግለሰቡ ለጥናቱ ብቁ ነው?

ከሆነ፣ በመጠይቁ ይቀጥሉ።

	የጤና ተቋም -----	የ መረጃ ሰብሳቢ ስም-----
1.	የተሳታፊዎች ስም 3 የመጀመሪያ ፊደሎች	
2	የሕክምና መዝገብ ቁጥር	
3	መለያ ቁጥር	
4	ናሙና የተወሰደበት ቀን (ቀን / ወር / አመት)	/ / (በኢትዮጵያ የቀን አቆጣጠር)

ማህበራዊ እና ኢኮኖሚያዊ መረጃ

5.	ዕድሜ (በዓመት) ?	
----	--------------	--

6.	ጾታ?	1) ወንድ 2) ሴት
7.	አድራሻ	ክፍለ ከተማ: ----- የቤት ቁጥር: ----- ስልክ ቁጥር: ----- ቀበሌ: -----
8.	የጋብቻ ሁኔታ?	1. ማንበብና መጻፍ የማይችል 2. መደበኛ ያልሆነ ትምህርት 3. ከ1-8ኛ ክፍል 4. ሁለተኛ ደረጃ ትምህርት ቤት 5. ዲፕሎማ 6. ዲግሪ እና ከዚያ በላይ
9	የትምህርት ደረጃ?	1. ማንበብና መጻፍ የማይችል 2. መደበኛ ያልሆነ ትምህርት 3. ከ1-8ኛ ክፍል 4. ሁለተኛ ደረጃ ትምህርት ቤት 5. ዲፕሎማ 6. ዲግሪ እና ከዚያ በላይ
10	ሥራ?	1. ሥራ አጥ 2. የቤት እመቤት 3. ከስራ ተባረረ 4. የመንግስት ሰራተኛ 5. የግል ንግድ
11	ወርሃዊ ገቢ?	1. < 1,500 ብር 2. > 1, 500 ብር 3. > 3,000 ብር

		4. > 5,000 ብር 5. መገመት አልቻልም
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የቤት እና የመኖሪያ ሁኔታ

12	በቤትዎ ውስጥ ያሉት የክፍሎች ብዛት ?	
13	ቤቱ መስኮቶች አሉት?	1.አዎ 2. አይ
14	በአንድ ቤተሰብ ውስጥ የሚኖሩ ሰዎች ብዛት?	
15	በቤት ውስጥ ለቤት ውስጥ ማገዶ/ከሰል ጭስ በየጊዜው ይጋለጣሉ?	1.አዎ 2. አይ
16	ምን ያህል ጊዜ የአካል ብቃት እንቅስቃሴ ያደርጋሉ?	በወር አንድ ጊዜ ወይም ከዚያ ያነሰ - በወር 2-4 ጊዜ - በሳምንት 2-3 ጊዜ - በሳምንት 4 ወይም ከዚያ በላይ ጊዜ - አላደርግም
17.	አልኮል ያለበት መጠጥ ምን ያህል ጊዜ ይጠጣሉ.?	- በወር አንድ ጊዜ ወይም ከዚያ ያነሰ - በወር 2-4 ጊዜ - በሳምንት 2-3 ጊዜ - በሳምንት 4 ወይም ከዚያ በላይ ጊዜ - አልጠጣም
18	ጫት ምን ያህል ጊዜ ይቅማሉ.?	-በሳምንት 5-6 ቀናት -በሳምንት 3-4 ቀናት -በሳምንት 1-2 ቀናት

		- በየቀኑ - አልቅምም
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የአካል ምርመራ

19	የቢሲጂ (ለሳንባ ነቀርሳ በሽታ ሚሰጥ) ክትባት ጠባሳ አለ።? (የተሳታፊውን ቀኝ ክንድ ይመልከቱ)	1 አለ 2 የለም 3 አይታወቅም
20	ተሳታፊው የክትባት ካርድ አለው??	1. አዎ 2. አዩ
21	የሰውነት ሙቀት (ዲግሪ ሴልሲየስ) ?	
22	የልብ ምት መጠን (በደቂቃ)	
23	ሲስቶሊክ የደም ግፊት	
24	ዲያስቶሊክ የደም ግፊት.	
25	ክብደት በኪ.ግ	
26	ክብደት በኪ.ግ	

Annex 4. Laboratory Principle and procedure:

Personnel

The Principal Investigator and health workers gathered the data while skilled and trained research technologists conducted all laboratory tests related to this study.

4.1 Blood specimen collection procedure

1. Prepare the patient: Have the patient sit or recline in a comfortable position and expose their arm. Wipe the antecubital area with antiseptic solution to remove any foreign material and allow it to dry completely.
2. Take sterile gloves and clean your hands with approved antiseptic solution thoroughly before proceeding.
3. Apply tourniquet around arm above elbow to distend veins then palpate for a suitable vein – usually hand veins may be preferable for venapuncture
4. Place gauze beside vein as precaution if there were any major bleeding event during or after venepuncture that can be contained easily when done properly before inserting needle into vein gently while slowly pushing on plunger of syringe until some blood enters LiHep tube.
5. Release tourniquet, wipe up excess blood (if any) using cotton wool swab, cover insertion site with fresh gauze patch and finally attach plaster over insertion site
6. Label sample tube correctly immediately after obtaining filled syringe by marking date time and sample source accurately. Discard used syringe safely into prearranged sharps bin.
7. Transport the sample as soon as possible to the AHRI

4.2 QFT Testing and Supernatant Storage

Introduction: Quantiferon (QFT) diagnostic testing, then storing the remaining plasma for future analyses. There are four tubes collected as part of the QFT testing: (1) nil (negative control); (2) PHA (mitogen or positive control); (3) TB Ag 1; (4) TB Ag 2. Supernatant from each tube will be stored at -80°C.

Principle: The QFT system uses specialized whole blood collection tubes that contain antigens representing certain *M. tuberculosis* proteins or controls. Incubation of the blood, collected via venepuncture, occurs in the tubes for 16-24 hours. Following incubation, plasma is harvested and tested for the presence of IFN- γ produced in response to the peptide antigens. Plasma leftover

after completion of QFT diagnostic testing can be frozen and stored at -80°C for future experiments, such as analysis for cytokine levels.

Procedure:

QFT Diagnostic Testing—Sample Receipt and Plasma Collection adapted from Qiagen QuantiFERON-TB Gold ELISA Package Insert

1. Receive LiHep tube from clinic containing whole blood. Do not refrigerate or freeze tube.
2. Take 1mL of blood from the tube and place it in four QFT tubes, labeling each one with a Sample ID and date of draw. Gently mix by inverting 10 times. Discard LiHep tube.
3. Incubate QFT tubes at 37C for 16-24 hours, upright position (no CO2/humidification).
4. After 18-24 hours, take out QFT tubes and centrifuge for 15 minutes at 2000 x g to separate plasma layers.
5. Label 4 2mL cryovials with Nil, PHA, TBAg1, or TBAg2 accordingly to the QFT Tubes labels.
6. Transfer plasma layers of QFT tubes into appropriately labeled cryovials (approx 500uL) without disturbing red blood cell layer at the bottom of tubes.
7. Store isolated plasma samples at 4°C for up to 28 days or batch test multiple participants' QFT plasma with ELISA materials.

QFT Diagnostic Testing-QFT ELISA

1. Bring all samples and reagents (except for the conjugate 100x concentrate) to room temperature and let sit for 60 min.
2. Remove unused strips from the frame, seal in foil pouch, and store in refrigerator.
3. Mix deionized/distilled water into IFN- γ standard vial until specified volume is reached; mix gently to avoid frothing and dissolve completely; produce 8 IU/ml solution.
4. Dilute conjugate 100x concentrate by mixing with 0.3 ml of deionized/distilled water; mix gently to avoid frothing and dissolve completely.

5. Thoroughly mix plasma sample if it was stored frozen or more than 24 hours prior to assay, then add 50 µl of working strength conjugate to required wells using multichannel pipette; add 50 µl of plasma sample to appropriate wells with a multichannel pipette too; shake micro plate shaker for 1 minute 500 - 1,000 rpm's; cover each plate with lid and leave at room temperature for 120 minutes
6. Combine one-part wash Buffer 20x concentrate with 19 parts of deionized/distilled water in separate container; mix thoroughly to make 2 liters of working buffer solution; use 400 µl of solution per well for at least 6 cycles, an automated plate washer is suggested; soak 5 seconds between each cycle. Disinfect effluent reservoir and decontaminate any potentially infectious material according to standard lab procedure.
7. Lay plates down on absorbent towel face down so residual wash buffer can be removed; add 100µl enzyme substrate per well, and shake microplate shaker until mixed thoroughly; cover plates with lids and incubate at room temperature for 30 minutes (in dark place);
8. Add 50µl enzyme stopping solution per well, mix and measure Optical Density (OD) of each well within 5 minutes using 450nm filter on a microplate reader with 620-640nm reference filter ; OD value will calculate results

4.3 Human Cytokine Magnetic 35-Plex Panel

Principal

Human Cytokine Magnetic 35-Plex Panel analysis is to measure the concentration of various human cytokines in a plasma supernatant sample. This panel of analytes can be used to identify proteins that are secreted in response to an immunological stimulus, changes in cell composition and/or development, or other sources of inflammation. Measurement of cytokines can provide valuable information regarding the activity and state of the immune system, including a better understanding of disease progression or the effects of treatments. Cytokine analysis may also be used to assess health and immunity status in various populations or for research purposes. The Human Cytokine Magnetic 35-Plex Panel is designed for the quantitative determination of EGF, Eotaxin, FGF basic, G-CSF, GM-CSF, HGF, IFN- α , IFN- γ , IL-1ra, IL-1 α , IL-1 β , IL-2, IL-2r, IL-

3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p40/p70), IL-13, IL-15, IL-17A, IL-17F, IL-22, IP-10, MCP-1, MIG, MIP-1 α , MIP-1 β , RANTES, TNF- α , and VEGF in plasma supernatant .

Reagents preparation:

- Allow all reagents to warm to room temperature before use

General guideline:

- Do not invert the plate during the assay unless the plate is on the magnetic separator.
- The fluorescent beads and RPE reagents are light sensitive, and should be protected from extensive exposure to light.
- Handle all blood components and biological materials as potentially hazardous.
- Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
- Set the orbital shaker to a speed providing optimum agitation without the liquid splashing onto the lid. For an orbital shaker with a 3-mm orbital radius, a speed between 500 and 600 rpm is recommended.
- The magnetic beads settle rapidly. It is therefore important to mix well prior to use

Type of sample

Sample type	Amount required	Transport and storage
QFT supernatant plasma	25ul	-15°C to -30°C

Preparation of wash solution

- Prepare 1X wash Solution by adding 50 mL of Wash Solution Concentrate (20X) to 950ml of deionized water. Mix Well. This wash solution stable for 2 weeks at room temperature.

Sample Preparation

- Freeze samples Thaw completely and mix well prior to analysis.
- Clarify samples by centrifugation (12,000- 16,000 x g for 10 minutes at 2°C to 8°C) prior to analysis.

- Dilute plasma sample in Assay Diluent (1:2 or as appropriate)

Reconstitute lyophilized standards

- Reconstituted protein standards within 1 hour of use and cannot be stored for future use
- When using QFT supernatant , reconstitute the standard with Assay Diluent provided
- **Do not vortex**; to avoid the formation of foam when mixing or reconstituting protein solutions
- Mix and reconstitute one vial each of Human 20- Plex Standard, Human 25-Plex Standard and Human 4- Plex Standard as follows
 1. Add the volume of appropriate diluent as indicated on vial label of analysis.
 2. Gently mix the standard to ensure complete reconstitution, and incubate at room temperature for an additional 5 minutes.
 3. After all 3 vials are reconstituted, combine the contents of the 3 vials (total volume of 1ml)

Washing guidelines

- Incomplete washing adversely affects assay results. Perform all wash step with the 1X Wash Solution

Magnetic Plate separator

- Place the 96- well flat bottom plate containing beads and 200ul of 1X wash solution onto the magnetic separator.
- Allow the beads to settle for 30 – 60 seconds.
- Blot excess liquid on a stack of dry paper towels.
- Separate the plate from the magnetic separator before adding wash solution or any reagent to the plate

Assay Procedure

Analyte capture

1. Determine the number of wells in the 96-well plate to be used in the assay.

2. Vortex the 1X Antibody Beads for 30 seconds, then sonicate for 30 seconds immediately before use.
 3. Add 25 μ L of 1X Antibody Beads into each well. Protect the plate from light once the beads have been added.
 4. Wash assay wells twice with 200 μ L of 1X Wash Solution. (see “Washing guidelines”).
 5. Add 25 μ L of Incubation Buffer into each well.
 6. Add 50 μ L of diluted standards into standard wells.
 7. Add 50 μ L of your blank into blank/background wells.
 8. Add 25 μ L of Assay Diluent followed by 25 μ L of sample in sample wells.
 9. Cover the plate with an opaque lid and incubate the plate for 2 hours at room temperature under agitation on an orbital plate shaker. Note: (Optional) the plate can be incubated under agitation on an orbital shaker overnight at 2°C to 8 °C
- N.B: Always Keep Beads and plate protected from light.

Analyte detection

10. Prepare 1X Biotinylated Detector Antibody in conical tube
For single assay well: Add 50 μ L of Biotin Diluent and 5 μ L of 10X Biotinylated Antibody
11. Decant liquid and wash two times with 200 μ L 1X wash solution
12. Add 50 μ L 1X Biotinylated Detector Antibody to each well. Cover and incubate for 1 hour on shaker at room temperature
13. Prepare 1X Streptavidin RPR solution in conical tube
For single assay well:
 - ❖ Add 50 μ L of RPE- Diluent and add 50 μ L of 10X Streptavidin – RPR (Protect from light)
14. Decant liquid and wash twice with 200 μ L 1X wash Solution.
15. Add 50 μ L 1X Streptavidin- RPE solution to each assay well. Temperature on an orbital plate shaker.
16. Decant liquid and wash the wells 3 times with 200 μ L 1X solution
 - ❖ Dilution factor is 1:11 for extra pipetting volume.

Assay reading

17. Add 150 ul 1X wash solution to each well and place on orbital shaker for 2-3 minutes prior to analysis.

- ❖ If the plate cannot read on the day of assay, cover and store the plate in the dark at 2°C to 8°C. the next day to read bring back to room temperature on an orbital plate shake then remove then follow step 17.

18. Insert the plate to Luminex 100/200, MEGAPIX instrument and analyses the sample. Determine the concentration of sample from the standard curve using fitting software.

X-Ponent software results

- Confirms if all target bead counts for each analyte have been achieved to ensure accurate data acquisition.
- Shows the standard curve and individual statistics for each analyte.
- Generates an analyte PDF report with standard curves through the "reports" tab under results.
- Qualifies the standard points by checking for inaccurate points due to excessive plateauing or bottoming out using % Recovery limits and net MFI (MFI- background) checks.
- Uses Invalidate, Analyze, and Save to remove inaccurate data points from plateaus or bottoming out while reviewing the %CV of replicates.
- Please note that the %CV of replication calculation is based on concentration, not MFI data.
- Close the results and export the data to Excel using the "export CSV" function.

4.4. Direct Stool Examination

Procedure of Direct Stool Examination

1. Collect a sample of stool from the patient: The best way to do this is to have the patient collect the sample in a clean container that has been provided by the laboratory.
2. Label the sample container: Make sure to include information such as your name, date and time of collection, type of specimen and any other necessary identifying information specified by the laboratory.
3. Transport the sample to the lab: If transported in person, make sure that it is kept at room temperature sure not to contaminate with others
4. At the laboratory, record all relevant data: Details such as patient's name, age and gender must be recorded on an appropriately labelled form or sheet prior to conducting any tests.
5. Take a small portion of the sample and place it onto a microscope slide: Depending on what type of microscopic examination you are carrying out (e.g., for red cells or parasites).
6. Place a coverslip over the slide.
7. Observe under a microscope using appropriate illumination and magnification settings.
8. Record findings: the presence or absence of parasites, mucus, white and red blood cells.

4.5 Complete blood cells (CBCs) testing

CBCs testing procedure

1. Collect a venous or capillary blood sample in an appropriate anticoagulant tube.
2. Place the tube into the hematological analyzer, which is designed to count red and white blood cells, measure hemoglobin content in red blood cells, and evaluate platelets.
3. Enter patient information into the system to provide proper tracking within the laboratory records.

4. The instrument will then draw in the sample of blood and layer it into a very thin film along a channel inside the machine where optical detectors precisely measure the size, shape and other characteristics of cells in a matter of seconds

5. The analyzer processes this information electronically and then delivers readings for each type of cell or component under evaluation on a computer display or printed report.

Complete Blood Cells Count (CBCs)	Reference Range
WBC (white blood cell)	4,300 and 10,800 calls per cubic millimeter (cmm)
RBC (red blood cell)	4.2 to 5.9 million cells per cmm
Hemoglobin (Hbg)	13.8 to 17.2 grams per deciliter (g/dL) for men and 12.1 to 15.1 g/dL for women
Hematocrit (Hct)	45% to 52% for men and 37%-48% for women
Mean corpuscular volume (MCV)	27 to 32 picograms (a small fraction of a gram)
Mean corpuscular volume (MCV)	80 to 100 femtoliters (a fraction of one-millionth of a liter)
Mean corpuscular hemoglobin (MCH)	27 to 32 picograms (a small fraction of a gram)
Red cell distribution width (RDW) size and shape	11 to 15
Platelet count	150,000 to 400,000 per cmm
Mean platelet volume (MPV)	6 to 12 femtoliters (a very small fraction of a liter)

4.5 HIV Antibody rapid test

HIV Antibody rapid test procedure

1. Open the package and collect all the necessary items from inside it, including the test cassette, buffer solution and sample collection pipette.
2. Gently squeeze a drop of blood onto the sample region on the test cassette, making sure none of it runs off.
3. Add three drops of buffer solution to the test cassette from the dropper provided in the kit.
4. After 5 minutes, the results will be visible on both windows of the test cassette: "C" stands for control region, while "T" stands for test result browser. A positive result is one confirmation line in both "C" and "T" browsers with no other lines present, while a negative result is two distinct lines in both browsers with no other lines present.

4.6 Pregnancy HCG test

Pregnancy HCG test procedure

1. Allow the Pregnancy Test Strip and urine sample to reach room temperature (15-30°C) before opening the foil pouch.
2. Remove the Pregnancy Test Strip from the pouch and use it as soon as possible.
3. Place the test device on clean and level surface. Hold the dropper vertically and transfer 3 full drops to the specimen well and start the timer. Avoid air bubble formation.
4. Wait for the colored line(s) to appear. Read the result after 5 minutes. Do not read the result after 15 minutes.

Result interpretation

If two colored lines appear on the strip, it is pregnant

If one colored line appears on strip on control, it is no pregnancy

4.7 Hemoglobin Concentration test

Hemoglobin concentration measurement using Hemo Que procedure

Hemoglobin concentration measurement using Hemo Que

1. Prepare the Hemo-Que instrument by loading it with a new testing cartridge, connecting all power cords to the appropriate port, and ensuring that the printer is connected properly.
2. Calibrate the instrument by pressing the "Calibrate" button and waiting for the system to prompt for a successful calibration. Once complete, you must enter your ID and select a test for processing.
3. Take a blood sample from the patient's fingertip and place it onto the testing strip provided with the Hemo-Que kit. Place this in the designated slot of the machine to begin testing.
4. The Hemo-Que will now use optical sensors to measure both hemoglobin and hematocrit/hematological parameters such as red cell count (RBC) or white cell count (WBC). The results are displayed instantly onscreen as well as printed out onto a report if desired.
5. Record these data into your patient's records and discard any used materials appropriately according to protocol guidelines.

Result interpretation

Hemoglobin is a protein in red blood cells that carries oxygen throughout the body. A normal hemoglobin result is typically between 11.5 and 16.5 grams of hemoglobin per deciliter (g/dL) of blood for men, and 12 to 15.5 g/dL for women. Results that fall slightly below or above this range may indicate mild anemia or other health problems, while values that are much higher or lower can indicate more serious health concerns.

Annex 5. Statement of declaration

The undersigned agrees to accept responsibility for the scientific, ethical and technical conduct of the research project and for provision of required progress reports as preterm and conditions of the research and publications.

Investigator name

Signature

Date

Ziyada Rahimeto

Approval of the advisor

Advisors' name

Signature

Date

Addisu Gize

Dr.Anteneh Mehari

Dr. Martha Zewdie

Dr. Kidist Bobosha

Co – Advisor name

Signature

Date

Nuhamin Zena

Aproval of the Examiners

Signature

Date

Dr. Wondwosen Tsegaye

Professor Mengistu Legesse
