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Department of Microbiology, Immunology & Parasitology

**Maternal Intestinal Helminth Infection and Its Impact on the Neonatal and Maternal
Humoral Immune Response to MTB infection: A Study at Hana Maryam Health Center
Addis Ababa, Ethiopia**

Investigator: Meaza Zewde

**A thesis submitted to the Department of Microbiology, Immunology and Parasitology,
St. Paul's Hospital Millennium Medical College, in partial fulfillment of the
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Apr, 2024

Addis Ababa, Ethiopia

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Declaration

I, the undersigned declare that this thesis is my original work in partial fulfillment of the requirement for the degree of Master of Science in Medical Microbiology. I also declare that it has never been presented in this or any other university and that all resources and materials used in the thesis have been duly acknowledged.

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Abbreviation and Acronym

AHRI-Armauer Hansen Research Institute

BCG-Bacillus Calmette-Guerin

BmA- Brugia malayi Antigen

CBL- Cord blood lymphocytes

CBMC-Cord blood mononuclear cells

CD- Clustered differentiation

CLR- C-type lectin receptor

CS-Cesarean Session

ELISA- Enzyme linked Immunosorbant assay

HI- Helminth infection

IL-Interleukin

IRB- Institutional Review Board

LTBI-Latent Tuberculosis infection

MTB-Mycobacterium tuberculosis

PBMC- peripheral blood mononuclear cells

PHA-PhytoHemagglutinin

PPD- Purified protein derivatives

SSA-Sub-Saharan Africa

STH-Soil transmitted helminth

SWAP- Schistosoma worm antigens

SVD- spontaneous vaginal delivery

TB- Tuberculosis

TH1-T-helper 1

TH2-T-helper 2

WHO-World health organization

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Abstract

M. tuberculosis (MTB) and helminth Infections are the major health problems that are mostly found in unprivileged and underdeveloped areas of the world where both infections can co-exist. Helminth infection during pregnancy has an immunological effect on both the fetus and the maternal immune system. However, there exists limited data in Ethiopia, regarding the effect of maternal helminth infection on neonate immunity to MTB infection. In the present study, we hypothesized that neonates that are born to a helminth-infected mother have distinct humoral immunity than neonates from an uninfected mother.

Objectives: The present study aims to investigate maternal intestinal helminth infection and its impact on the neonatal and maternal humoral immune response to MTB infection

Methods: This study is a comparative cross-sectional study as part of an ongoing longitudinal cohort study working on innate immunity. A purposive sampling technique was used to select 75 study participants. Study-specific case report forms (CRF) were used to collect participant's socio-demographic, physical, and clinical information. Stool and venous blood samples were collected from eligible pregnant mothers at baseline and cord blood samples during delivery. Wet mount and Kato Katz stool examinations were performed to identify those with intestinal helminth infection. Plasma samples were analyzed for immunological parameters profiling of both the mother and neonates using ELISA. *Mycobacterial* culture filtrate protein (CFP) antigen-specific maternal and neonatal IgG antibodies and total IgE levels of neonates were measured. Non-parametric tests including Mann-Whitney U tests, and Spearman correlation were used for data analysis a P-value less than 0.05 was considered statistically significant.

Result: From purposively selected study participants of 33 helminth-infected mothers the predominant parasites identified were *Tania Spp* 12/33 (36%) and *Hookworm* 11/33 (33.3%). Of the 75 QFT tested participants' only 10 (13.3%) were QFT+ve and a significant number of 5(6.7%) were indeterminate results. Elevated plasma IgG levels were observed among QFT-positive mothers and no significant difference was observed among helminth-infected and non-infected groups. An increased plasma IgE level was observed in neonates born to helminth-infected mothers (P=0.03). Similarly, elevated IgE was also observed among neonates from QFT-negative mothers (P=0.02). However, an increased IgG level was observed among neonates from QFT-positive mothers (P=0.04) regardless of the helminth infection status of the mother.

Conclusion: The current finding showed maternal helminthic infection impacts the neonatal humoral immune response. An elevated total IgE was observed among neonates that were born to a helminth-infected mother. This showed maternal helminth infection affects neonatal IgE antibody levels, which could be due to an in-utero sensitization to helminth antigens. Neonates born from mothers with positive QFT tests have higher mycobacterial CFP antigen-specific IgG levels regardless of helminth infection status.

Recommendation: The study showed the impact of helminthic infection on neonatal humoral immunity response to MTB infection but further study should be initiated with a larger sample size to address the magnitude of the impact.

Keywords: Helminth infection, cord blood, pregnancy, neonates, in-utero priming, MTB

1. Introduction

1.1 Background

Tuberculosis (TB) is one of the major public health problems that exist as the 13th leading cause of death and the second leading infectious killer after COVID-19 (above HIV/AIDS). In 2021, an estimated 10.6 million people fell ill with TB and 1.6 million people died of TB globally (1) . On the other hand, soil-transmitted helminths (STH) are estimated to affect more than 1.5 billion people worldwide, amounting to 24% of the world's population, with the highest burden reported in people living in poor resource settings (2, 3). TB and helminth infections coexist in many African countries (4, 5).

Epidemiological studies suggest that co-infection of TB patients with helminths is associated with more severe disease and poor treatment outcomes. In Tb-endemic areas, a high proportion of adults harbor latent tuberculosis infections (LTBI), and individuals with LTBI may have circulating antigens and higher concentrations of Tb-specific antibodies in their system (6, 7).

On the other hand cross cross-placental transfer of Mtb antigen has been reported in murine models (8) indicating that maternal LTBI may lead to exposure to Mtb antigens in utero driving a modified profile of sensitization (9) or inducing immune tolerance in the fetus (10). Similarly, human studies also showed that there was cross-placental transfer of helminth antigens and creates in-utero sensitization (11). Besides, helminth infection also results in the susceptibility of Children to soil-transmitted helminth (12).

Gebreegziabiher et al,- conducted a study in the Tigray region and showed that both LTBI and Helminth infections are prevalent among pregnant women (13). Besides, studies have also shown helminth infection in pregnant women is sometimes associated with anemia in the case of the mother and makes the neonates susceptible to infections. Helminth infection during pregnancy also has immunological effects that may alter the baby's immune function to infection after birth (14-17).

Neonates are afforded some protection through passive immunity provided by maternal immunoglobulin (IgG) actively transferred across the placenta during pregnancy. Transfer of IgG across the placental barrier is thought to involve interaction between IgG and the neonatal Fc receptor (FcRn). Cross-placental IgG transfer is a crucial mechanism that protects the newborn from infections in early life while the infant's immune system gets mature enough to take over the role (18).

Apart from IgG, studies have also reported transplacental transfer of antigens from the mother to the fetus if the mother encounters infection during pregnancy. Such an antigen transfer may cause in-utero sensitization of neonatal B cells to make IgE and IgG antibodies (19). There are also reports on helminth-specific immune responses acquired during gestation persist into childhood and that this prenatal sensitization biases T-cell immunity with an antagonist effect on BCG vaccine immunogenicity (20). In addition to in-utero stimulation of the fetal immune system brought on by helminth antigens, there existed higher levels of cord blood IgE and CD-5 B cells (21). Similarly, infants born from *Schistosoma* and filarial worms infected mothers had Th2 biased immune responses, and increased regulatory T cells (Tregs) frequency in cord blood (22).

On the other hand, though helminth does sensitize in utero for parasite-specific immune responsiveness of the fetus and triggers production of various cytokines; those children did not display a dominating expression of immunity of either Th1 or Th2 (23).

The available data showed conflicting reports around supporting or against the potential of maternal helminth infection towards the infant's immune system, particularly on susceptibility to infection and vaccine responsiveness. The present study is designed to investigate whether maternal helminth infection determines neonatal and maternal humoral immune response to MTB infection.

1.2 Statement of the Problem

As different literatures reported TB and helminth infections are the most challenging health problems in areas where the two infections coexist, Ethiopia included (10). Pregnant women who were living in parasitic endemic areas like Ethiopia are among those who showed a higher prevalence of helminth infection with multiple parasite illnesses (17).

Due to the increased nutritional requirements and changing immune system, pregnant mothers are often prone to parasitic infections, which can have negative consequences for the fate of both the maternal and the newborn health (14, 17). Apart from that, helminth infection has had an impact on the immune status of both the infected mother and offspring and a study also evidenced that due to in-utero exposure to parasitic infection and neonatal sensitization to helminthic antigens create during gestation: children of mothers infected with STH may have an increased susceptibility to STH infection (12, 24).

Apart from that existing literature showed that helminth infection had a decline in the incidence of allergic and autoimmune diseases (25, 26) and is believed to have a therapeutic effect on inflammatory diseases (27). Besides, chronic helminth infections tend to skew the pro-inflammatory protective immunity to TB (28) and vaccine immunogenicity (22, 29).

Despite routine BCG vaccination at birth, TB remains a global public health challenge. The immune responses of the newborn child and how they are affected by maternal helminth infection are yet not fully understood. Immune response during pregnancy is shown to be skewed toward the Th2 type with anti-inflammatory profiles; however, it is the Th1 response with inflammatory cytokines that are required for protection against TB infection.

Literature evidenced a conflicting report on in-utero sensitization to helminth antigen and its impact on neonatal immunity. To better understand maternal intestinal helminth infection and its impact on maternal and neonatal humoral immunity to MTB infection, we propose to evaluate the response of *MTB* antigen-specific IgG antibody levels in both pregnant mothers and their neonates and the IgE response of the neonates.

1.3 Significance of the study

Helminths have evolved complex mechanisms for immune subversion, with effects on immune responses that lead to their long-term persistence. There is existing evidence of in-utero sensitization to maternal helminth infection. But, the mechanisms by which maternal infections sensitize offspring are still not fully established and the issue of how the fetal immune system responds to in-utero exposure to antigens remains a matter of ongoing controversy. The findings from this study will give insights regarding the impact of helminth infection on the immune status of neonates.

In areas where helminth infection is endemic, where Ethiopia is part of, the issue could have a significant contribution in designing strategies towards maternal and neonatal health. Thus, neonatal immunity to MTB infection and its correlation with maternal helminth infection may provide valuable input. Additionally, it provides insight into how helminth infection in the mother affects the humoral immunity of the mother and newborn against MTB infection. For individuals who wish to learn more, it is also insightful knowledge about this area.

2. Literature review

2.1 Maternal helminth infection

As different findings were reported there existed a significant number of helminth infections among pregnant women. A study conducted in Bogota, Colombia among pregnant women who were recruited from three selected district poor residential areas showed the overall prevalence of intestinal parasitism was 41% and 9 % of polyparasitism. While both *Giardia* and *Amoeba* were present in those subjects, the parasites *Blastocystis hominis* (25%) and *Endolimax nana* (8%), *Entamoeba coli* (8%), and *Lodamoeba Butschlii* were shown to be more prevalent (30).

Similarly, a cross-sectional study from Kenya showed 76.2% of the participating pregnant women were infected with at least one geohelminth: 52.3% with *Ascaris lumbricoides*, 39.5% with hookworm, and 29.0% with *Trichuris trichiura* (36). Another hospital-based survey from another district of Kenya, among 153 pregnant women showed overall prevalence of infection was 13.8%. Similar to the previous study, Ascariasis was the most prevalent at 6.5%, hookworm infection was 3.9%, and Trichuriasis was 1.3% (24).

Another cross-sectional study design in Ethiopia, Mekele which recruited 85 pregnant women and tested both parasitic and LTBI infections revealed that the prevalence of parasitic infections among the study participants was 27.1% (n = 23) and *Schistosoma mansoni* 20.0% was the most common infection followed by *Ascaris lumbricoides* (8.2%) (13).

Similarly, a study in Mecha district, northwest Ethiopia from a total of 783 pregnant women, reported that the prevalence of intestinal parasite infection among pregnant women was 70.6% [95% CI 67 -74%] and the predominant intestinal parasite species in this area of study was *Ascaris lumbricoides* (32.7%) and different risk factors were mentioned for the increased prevalence (17).

2.2 Latent TB infection in pregnant women

Similar to helminth infection LTBI is also prevalent among pregnant women and different studies are supporting this evidence. One of the studies from Uganda on 261 pregnant women irrespective of gestational age showed that LTBI prevalence was 37.9% (n = 99) (95% CI, 32.3–44.0). However, 24 (9.2%) subjects had indeterminate QFT-plus results. Among participants with LTBI, TB1 and TB2 alone were positive in 11 (11.1%) and 18 (18.2%) participants, respectively (31).

Another cross-sectional study from Tanzania among pregnant mothers who were tested for latent tuberculosis above 12 weeks of gestational age in resource-limited areas showed that the Prevalence of latent infection ranged between 26.2% and 37.4% (32).

Similarly, a study from Adama Ethiopia, among pregnant women living with Tb and HIV-endemic areas showed 33% (277/829) of participants were QFT positive ≥ 0.35 IU/ml in TB1 or TB2). Twenty-nine women (3.5%) had discordant results, of which 16 (1.9%) were TB1+/TB2- and 13 (1.6%) were TB1-/TB2+; 248 (30%) were concordant positive (33).

On the other hand, a study from Mekele reported that of a total of 85 study participants 26.8% were QFT-GIT positive and there exists a significant number of indeterminate results in 14 (17%) of QFT-tested pregnant study participants. The study also showed that this indeterminate result was associated with the presence of helminth infection among pregnant mothers (34).

2.3 *Mycobacterium* Tb infection and IgG antibody response

As literature showed IgG levels against different *MTB-specific* antigens were higher in TB patients than in endemic and non-endemic controls. The increased IgG level demonstrated the significance of MTB infection for humoral immunity generation and provided proof of B-cell protection (35).

According to Rajamanickam A et al, data showed a decrease in TB-specific IgG levels in those LTBI/SS infected people than LTBI alone and supported how helminth infection affected TB immunity (36).

Many studies support the existence of cross-placental transfer of IgG antibodies to the offspring and will keep the neonates from infection after delivery. A study from Gabon on the impact of helminth infection during pregnancy on vaccine immunogenicity of infants showed that significantly lower anti-tetanus toxoid immunoglobulin (IgG) was detected in the cord blood of infants born to helminth-infected mothers (37).

Another cohort study from Kenya also showed that Babies whose mothers had infections for *Schistosoma haematobium*, hookworm, or prenatal malaria had considerably lower levels of means of infant and maternal plasma antibody concentrations (cord/maternal ratio CMR). That showed there was a decrease in cross-placental transfer of IgG antibodies among neonates who were born from helminth-infected mothers than uninfected mothers (38).

In contradiction with the above finding, a study from Ethiopia, in Mekele by Gebreegziabiher *et al* reported that the mean concentration value of Tb-specific IgG in cord blood was significantly lower in helminth negative participants (12.3 ± 5.1) than positive study

participants (21.9 ± 7.9); $p=0.002$) and the report showed that Helminthic infection significantly increases the cross placental transfer of IgG antibodies (13).

2.4 Helminth infection and IgE antibody

Immunoglobulin E (IgE) is one of the body's five classes (iso-types) of antibodies which are produced by B cells and plasma cells like in the case of other Immunoglobulins. The concentration of IgE in circulation is very low than other Immunoglobulins. Immunoglobulin E in cord blood mostly measures less than 1 U/mL (1 U = 2.4 ng) (39). Even though the IgE antibody is found in a smaller amount of the circulation, this is the expected class of Immunoglobulin that is found both in allergy and helminth infections and has a protective role for human immunity (40).

2.4.1 Maternal helminth infection and IgE

The maternal helminth status and IgE level data showed those pregnant women with Helminth infection have an elevated IgE antibody level than Helminth non-infected pregnant mothers. The comparative study in Nigeria, on the serum levels of cytokines in pregnant women, preschoolers, and school-age children of helminth infected with a non-infected group showed significantly greater IgE levels were observed in helminth-infected women than non-infected women. Children of pre-school and school-aged children of helminth infected had significantly greater serum levels of IgE than did children in non-infected (41).

2.4.2 Maternal helminth infection and neonatal IgE

Similarly, in another report, there is an increased level of cord blood IgE that was frequently observed in the setting of maternal helminth infection and the existence of helminth-specific IgE in cord blood. The evidence also dictated that it was not a transplacental transfer of IgE from mother to fetus, but rather fetal immunological responses to parasite antigens that led to increased cord blood IgE (42).

Another report *Schistosoma haematobium* on Cord Blood IgE and CD5⁺ B cells in African Newborns showed that there was significantly increased Schistosome soluble egg antigen (SEA)-specific IgE in cord plasma of those born to mothers with Schistosome infections and correlations between fetal and maternal SEA-specific and *Filaria* antigen-specific IgE (21).

Similarly, a study from Mekele also indicated that the mean cord blood total IgE level was substantially lower in helminth-negative subjects compared to helminth-positive subjects ($p=0.042$). Each helminth species did not differ statistically, despite the collective effect of maternal helminth infection reaching a significant threshold (13).

Another study from Kenya, where Schistosomiasis, bancroftian filariasis, and geo-helminth infection are endemic, showed Schistosome and filarial Ags stimulated a 3 to more 100-fold

increase in the production of polyclonal IgE and parasite-specific IgG Abs by lymphocytes from 10 of 40 and 6 of 39 Kenyan newborns, respectively. CBL observed to have helminth Ag-driven B cell responses were more likely to be from newborns of Schistosome-or Filaria-infected mothers than from uninfected mothers (19).

Another case-control study from Nigeria by Olateru et al tried to measure the serum level cytokine and IgE levels of both the mother and the babies. As the data showed the mean concentration of IgE level were higher in babies born to HP mothers compared with babies born to HN mothers (43).

2. 5 Conceptual framework

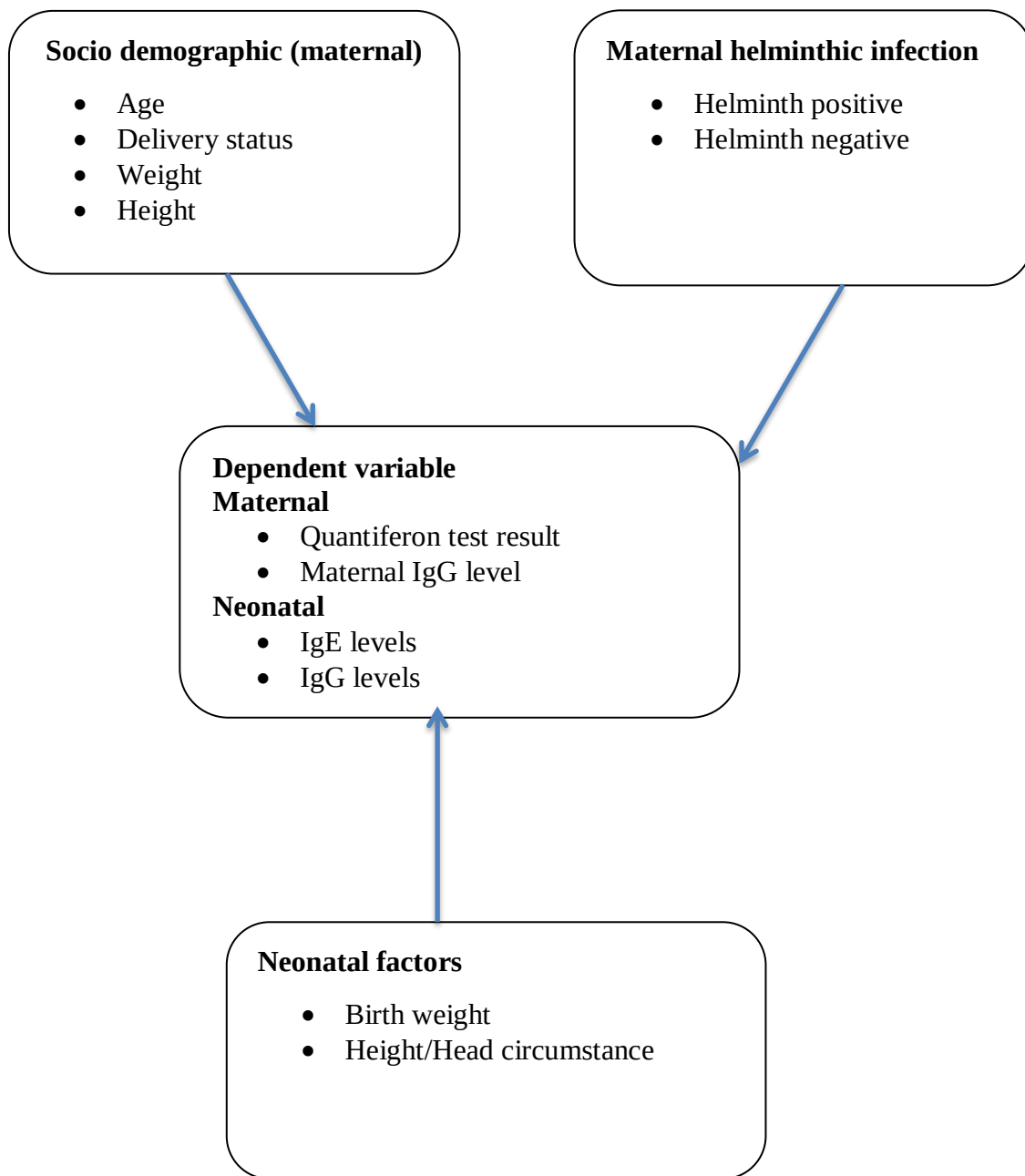


Figure 1 Conceptual framework (Maternal and Neonatal Immunological profile (dependent variable) and the independent variables)

3. Objectives

3.1 General Objective

To investigate the impact of Maternal Intestinal helminth Infection on Maternal and Neonatal humeral immune responses to MTB infection at Hana Maryam Health Center, Addis Ababa, Ethiopia

3.2 Specific Objectives

- To determine the LTBI rate of Helminth-infected and non-infected mothers
- To compare the level of Mtb antigen (CFP antigen) specific IgG antibody levels between Helminth-infected and non-infected mothers and their neonates
- To compare the total IgE antibody levels in neonates born from Helminth-infected and non-infected mothers

4. Material and Methods

4.1 Study period and design

The study was a comparative cross-sectional study as part of an ongoing longitudinal prospective cohort study working on the effect of helminth infection on the BCG vaccine response of the infant. The study period of the current study was from May 2022 to Nov 2023.

4.2 Study Area

The study was conducted in the Nifas Silk Lafto sub-city at Hana Maryam Health Center, Addis Ababa, Ethiopia. The health center provides different health services for those who need medical care. Antenatal care and post-natal care are among the services that are given to pregnant women who need follow-up at the health center. During the start of this study, the health center's six months (July-December) data showed a total of 2050 pregnant mothers visited the health center for the first time, and out of that 1453 came to follow and only 724 mothers delivered at the health center. Besides normal follow up the health center does have a referral system to, Zewditu Memorial Hospital, for those pregnant women who need further follow-up, special clinical care, and cesarean session.

4.3 Study Population

All pregnant women were coming to Hana Maryam Health Center for normal antenatal check-ups

4.4 Subject of Study

Pregnant mothers with gestational age of 35 weeks and above and their newborns, at Hanna Mariyam Health Center in Addis Ababa. Clinicians at the ANC would invite pregnant mothers who are coming for a regular ANC visit and are in the third trimester to participate in the study after providing the necessary information about the study. If the mother is willing to take part in the study the study clinician would do medical record, clinical and medical evaluation as well as laboratory testing as per the inclusion and exclusion criteria below. Socio-demographic, physical, and clinical data were collected using a well-structured data collection tool. Stool samples were collected following proper instruction and guidance by the research nurses. Study participants were assigned into two groups based on helminth infection status: group one-helminth positive (cases) and group two-helminth negative (controls).

4.5 Inclusion criteria/ Exclusion criteria

4.5.1 Helminth-infected group 1

Inclusion

- Pregnant mothers with a gestational age of 35 weeks and above
- Pregnant mother with gestational age of 35 weeks and above who are given consent to participate in the study for themselves and their newborn baby
- Helminth-infected pregnant women

Exclusion

If one of the criteria listed below is observed in mothers or the newborn baby

- HIV seropositive
- HBV positive test result
- Evidence of clinical anemia and hemoglobin concentration below 110g/L
- Evidence of immunosuppressive disease conditions (diabetes, hypertension, kidney disease, malignancy, etc.)
- Individuals on immunosuppressive treatment
- Overt signs of acute infection (fever above 38.5°C; eg. UTI, RTI etc.)

4.5.2 Helminth non-infected- group 2

Inclusion

- Pregnant mother with gestational age of 35 weeks and above who are given consent to participate in the study for themselves and their newborn baby
- Helminth non-infected (no ova or parasite seen)

Exclusion

All exclusion criteria for the Helminth-infected group observed in mothers or newborn babies were also applicable for this group too

4.6 Sample Size Determination

This was a study with precious samples (cord blood), considering normal sample size calculation is not feasible for different reasons: One of the reasons is that managing a large sample size requires a lot of time and resources (44). Due to this, the study used the sample consideration of an already established longitudinal cohort study currently conducted in the study area. Mothers coming to the health facility for regular antenatal care with a gestation age of 35 weeks and above were invited to take part in the study. To minimize re-infection and lost follow-up we recruited pregnant mothers above 35 weeks. Stool samples were collected for stool examination to identify intestinal parasite infection at baseline. Venous

blood samples were also collected at the same time to investigate the immune response of the mother and neonates to the underlying intestinal helminth infection. A total number of 167 study participants were recruited in the main project from which 75 pregnant mothers were purposely selected for the current study based on the availability of cord blood samples. Then 33 study participants were from the helminth-infected group and 42 from the helminth non-infected group were selected since there were only 33 helminth-positive neonatal cord blood during sample selection.

4.7 Study variables

Dependent variables

- Maternal QFT and MTB-specific IgG levels
- Neonatal Mtb antigen-specific IgG levels and Total IgE levels

Independent variables

- Maternal helminth infection
- Age
- Maternal Weight
- Maternal Height
- Delivery status
- Birth Weight of the neonates
- Head circumference of the neonates

4.8 Operational definitions

Cord blood sample- infant's blood collected from the umbilical cord at the time of normal delivery (SVD) or Cesarean session (CS)

Helminth infection: identification of intestinal parasites in stool sample by wet mount or Kato Katz

4.9 Sample collection and processing

Stool samples from eligible mothers were collected to group the study participants by maternal helminth status. Maternal helminth infections were screened with direct wet-mount as well as a Kato-Katz stool examination kit to determine the parasite load using egg count as a parameter. In addition to the stool sample, 5-6 ml of blood with lithium heparin tube was collected and then incubated overnight with four Quantiferon tubes (Nil, TB1, TB2, and Mitogen) to stimulate T cell immune responses. The TB1 tube contains an MTB antigens ESAT-6 and CFP-10 cocktail which are dedicated to stimulating T cells specifically CD4+. Similarly, TB2 tubes contain similar antigens with additional elements to stimulate CD8+ cells. And then three 10 ml of EDTA tubes each were used to collect 20-30 ml of maternal and umbilical cord blood of newborns at the time of delivery. Since the current project took the sample collected for longitudinal cohort study; from the collected 20-30 ml blood we only used a few amounts of plasma for the current experiments. After sample collection, the samples were transported at ambient temperature within three hours of collection at the Armauer Hansen Research Institute (AHRI) lab to process and analyze the sample. During sample processing, the investigator harvested the plasma and stored the sample at -80⁰c until the experimental day.

Laboratory assays

We addressed the proposed study objectives and performed a comprehensive analysis of the assays listed below.

1. Wet mount and Kato Katz stool examination

After a stool sample was collected from the study site and arrived at the AHRI lab then four slides were prepared as per the sample amount. Two slides for wet mount preparation and two slides for Kato Katz (a total of four slides) for every individual were prepared and then observed by two personnel separately and the average egg count of the helminth was considered. The Kato-Katz technique is a copro-microscopic method for the diagnosis of soil-transmitted helminths (STH) currently considered the gold-standard method for the diagnosis of these parasites. It facilitates the detection and quantification of helminth eggs that infected subjects pass on their stools. In this method, stools are sieved through a mesh screen to remove large particles and transferred to a standard hole of a template (41.7 mg) on a slide. After filling the hole, the template is removed, and the remaining sample is covered with a piece of cellophane soaked in glycerol. The glycerol clears the fecal material around the eggs.

The eggs are then counted under a light microscope and the infection rate is calculated as eggs per gram of stool (EPG) (45).

2. Enzyme linked Immunosorbant assay (ELISA)

The current study used indirect ELISA for measuring plasma IgG antibody levels of the mother and their neonates and sandwich ELISA for measuring total IgE antibody levels of the neonates and maternal Quantiferon status (46). IgG antibody levels were measured using Mtb specific antigen, which was given by bei RESOURCE. Then, for both groups' IgG and IgE antibody analyses, a commercially available kit called Mabteck was utilized. Following the measurement of the antibody levels using an ELISA reader (Soft Max Pro), the observed OD values were converted to concentration using software called Micro Plate Manager (MPM v.4). To translate the OD values into concentration, the programmer took into account the plate's standard and blank readings. Then, it multiplied each value's concentration by the dilution factor. To ascertain the quantitative outcome of the measured OD values for the QFT state, we used Quantiferon TB Gold Plus (v.2.71.2) software. Following that, SPSS-V8 and Graph Pad Prism were used to evaluate and interpret the data.

3. Laboratory assay optimization

The current study examined the suggested experiments by the laboratory setup and the kit insert methodology before we began the real experiment. We employed the Mtb antigen-specific culture filtrate protein (CFP) to detect the amounts of IgG antibodies during the ELISA experiment for both maternal and cord blood antibody analysis of IgG antibodies. A CFP antigen was coated on a high-binding plate during the ELISA experiment at two distinct concentrations: 10ug/ml and 5ug/ml. The latter dose functions flawlessly. Sample dilution for antibody testing was optimized in addition to antigen concentration. The kit suggests a sample dilution of 1:100 for IgG antibody level; however, we used 1:2 dilutions to test various concentrations (1:100, 1:200, 1:400, and 1:800). To address the outliers, we further diluted with a 1:2 dilution of (1:250 and 1:500). This works well, and we utilized it to obtain the predicted absorbance measurement because some of the results were dipping below and above the detection limit. While evaluating the total IgE in the cord blood, we were also optimizing for IgE during standard preparation in addition to IgG.

4. Quantiferon TB Gold ELISA

Maternal LTBI status was assessed during an overnight (16–20 hours) incubation of a 1ml heparin blood sample for each tube (Nil, Tb1, Tb2, and Mitogen) using QFT-plus kits. It is an in vitro diagnostic tool for indirectly detecting MTB infection. Using a peptide cocktail of MTB antigens called ESAT-6 and CFP-10, the QFT-Plus test activates T cells in heparinized

whole blood, and an ELISA was used to evaluate the in vitro IFN- γ response. The supernatant was removed from overnight incubation and kept at -80⁰c until the experiment day. To ensure that the reagents were performing at their best, the kits were left at room temperature while the plasma was thawed during the experimentation day. According to the manufacturer's instructions, conjugate Ab which had been diluted with green diluents was applied to the plate. A sample volume of 50ul/well for (Nil, TB1, TB2, and Mitogen) and a serially diluted standard were added and incubated for 2 hrs. After adding the substrate and rinsing the plate six times with a washing buffer, the color development took place after 20 minutes (47). Following the application of a stopping solution to halt the reaction, the soft-Max pro-ELISA reader was used to measure the color and optical density intensity at the preferred wavelength. Then QuaniFERON-TB Gold Plus software was used to evaluate the OD readings (48).

5. Mtb antigen specific IgG ELISA

Antigen-specific IgG antibodies were examined using plasma from both mothers and newborns. In indirect ELISA, the first antibody is indirectly conjugated to the enzyme using a secondary antibody as a linker. High-binding plates were coated with a CFP antigen concentration of 5ug/ml (100ul/well) of TB-specific antigen, and the plates were then incubated at 4 ⁰c for a whole night. The plate was locked for an hour and cleaned with PBS the following day. Then 1:500 diluted samples and a1:2 serially diluted standards were added, and the plate was incubated for one hour. Following another plate wash, detection antibodies that were diluted 1:1000 were added, and the mixture was incubated for two hours. The plate was rinsed after incubation, TMB substrate was applied, and color development was allowed to occur for 15 minutes. The reaction was halted with 2N H₂SO₄ after color development, and the optical density was measured at 405 nm using a Soft Max Pro ELISA reader, which can subtract a reference wavelength of 570–650 nm (49). Following that, the OD readings were converted to concentrations and quantified in units of ug/ml (**Annex-5**).

6. Total IgE ELISA

Using a commercially available Mabtech ELISA kit, total IgE antibodies were analyzed from cord blood newborn plasma. The analyte is encased between two antibodies in a sandwich ELISA. The ELISA plate is immediately covered with the antibody (capture antibody), which binds to the antigen/analyte. After adding the secondary antibody, an enzyme-conjugated or detection antibody is added. The addition of substrate starts the reaction, and the signal's absorbance is used to measure the product that is produced. A 100 ul/well of capture mAb coated in a high binding plate was used for the experiment's first day, and it was incubated at

4°C overnight. Subsequently, the plate was blocked with 0.1% BSA containing 0.05% Tween 20 with PBS for an hour (300ul/well) and cleaned three times with PBS the next day. Following plate washing, a 50 ul/well sample and serially diluted standard were added, and the plate was incubated for two hours. After another wash, detection antibodies that were diluted 1:1000 were added to the plate and incubated for one hour. After incubation, the plate was cleaned again, and a 1:1000 diluted streptavidin-HRP was added. The mixture was then incubated for an hour. Following the prior plate washing procedure, the TMB substrate was placed, and color development occurred over 15 minutes. The SoftMax pro-ELISA reader was used to measure the optical density at 450 nm after the reaction was stopped with 2N H₂SO₄ (50). As previously mentioned, MPM software was used to convert the OD values into a concentration of pg/ml (*Annex-6*).

4.10 Data management and statistical analysis

A comprehensive data management plan was developed before the start of data collection. All participants' data was coded and kept confidential. Socio-demographic, clinical examination findings, medical history, and laboratory results were recorded in case report forms, entered into the project-specific database on Redcap, and securely stored on AHRI's server. The relevant clinical and laboratory results were compared among the study groups using the median at 95% confidence interval, frequency, and standard error of the mean. Non-parametric tests Kruskal-Wallis and Mann-Whitney U tests and Spearman correlation coefficient were used since we used small sample sizes and the data was not expected to be normally distributed. Then SPSS and Graph pad prism were used for data analysis and depending on the nature of the data, $P < 0.05$ is considered statistically significant. Graphs and tables were used to present the main findings of the study.

4.11 Ethical considerations

This study was part of an ongoing project that has already got both the city government of Addis Ababa Health Bureau/National Ethics and Institutional Review Board (IRB) approval. Then the study received ethical approval from St. Paul's Hospital Millennium Medical College (SPHMMC) IRB and AHRI-ALERT Ethics Review Committee (AAERC). Then after ethical approval, the study was conducted after delivering an information sheet to the participants and obtaining informed consent of the mother for taking part in the study for themselves and their neonates. All participant recruitment, sample collection, and laboratory assays were performed by qualified health professionals, and study protocol-specific job

training was also given to those who were involved in the study to make sure that all the procedures were handled as per the protocol.

4.12 Dissemination of the result

The result of the study will be disseminated to the respective stakeholders who are involved in this study and in favor of this research, including SPHMMC, Hana Mariyam Health Center, Institutional review boards, and the sponsor. The data will be published in peer-reviewed journals and presented in local as well as international seminars that will help to be delivered to everyone who needs to explore more and those concerned bodies that need the results for future experiments.

4.13 Challenges and Limitations of the Study

Since the study was conducted on pregnant women and their neonates, there were challenges that we faced including a high number of lost to follow-ups. Among the various reasons linked to it: failure to collect the cord blood while the mothers were referred to the hospital not linked to the study site, home delivery, change of the mother to another health facility by her interest, mother's refusal to continue with the study and poor cord blood sample quality among the reasons that challenge us for lost follow up. The COVID-19 mass vaccination campaign was also another challenge for the study since mothers were not volunteers to take the vaccine and were denied to come to the clinic for normal follow-up.

5. Result

5.1 Study Participant's Characteristics

From those purposely selected seventy-five study participants, n=33 mothers were helminth positive, and the rest n=42 mothers were helminth negative. The mean age of study participants was $25 \pm$ (SD=3.89) and helminth infections were relatively observed among the age group of 24-29 yrs. (46.7%). Of the seventy-five mothers' 90.6% (n=68) of neonates were born with normal delivery and 9.3% (n=07) of neonates were delivered with Cesarean section (**Table 1**)

Table 1. Study Participants' Characteristics

Variables	Frequency, N (%)
Helminth status	
Negative	42 (56.0)
Positive	33 (44.0)
Maternal age group	
18-23	31 (41.3)
24-29	35 (46.7)
30-35	7 (0.9)
Delivery type (count)	
C-section	7 (9.3)
Normal	68 (90.7)

The mean weight and height of the mother were $62.8 \text{ kg} \pm$ (SD=14) and $161\text{cm} \pm$ (SD=16.29) respectively. In addition, the mean IgG level of the mothers was $2347.5 \text{ ug/ml} \pm$ (SD 1766.33). The mean weight and head circumference of the neonates were $3285.1\text{gm} \pm$ (SD=449.9) and $41.378\text{cm} \pm$ (SD 5.85) respectively. The mean cord blood plasma IgE and IgG levels were $6.96 \pm$ (SD 14.59 pg/ml) and $2600 \pm$ (SD=2255 ug/ml) respectively (**Table 2**).

Table 2. Participant's Physical and Hormonal data

Variables	Mean ($\pm$ SD)
Maternal	
Weight (Kg.)	62.8 ($\pm$ 14)
Height (mt.)	161.4 ($\pm$ 16.3)
Venous blood IgG level (ug/ml)	2347.5 ($\pm$ 1766.33)
Neonatal	
Weight (gm.)	3285.1 ($\pm$ 449.9)
Head circumference (cm)	41.4 ($\pm$ 5.9)
Cord blood	
IgG (ug/ml)	2600 ($\pm$ 2255)
IgE (pg/ml)	6.96 ($\pm$ 14.59)

We used both wet mount and kato-katz to identify the helminth status of the mother and isolate specific parasite types. Except for *S. stercolaris*, which is found in the larval stage and possibly not isolated by the kato-katz method, the following parasites were so far identified by both methods. From the total of seventy-five study participants 44% (n=33) were helminth positive and the predominant parasite were *Teania Spp* 36% (n=12) and *H. worm* 33.3% (n=11). *Strongloid stercolaris* and *Ascaris lumbricoides* account for 12.1% and 6% respectively which is followed by *Schistosoma mansoni*, *Hymenolepis nana*, and *Trichuris Trichiura* each accounting for 3.03% (n=01) that were identified separately (**figure.2**).

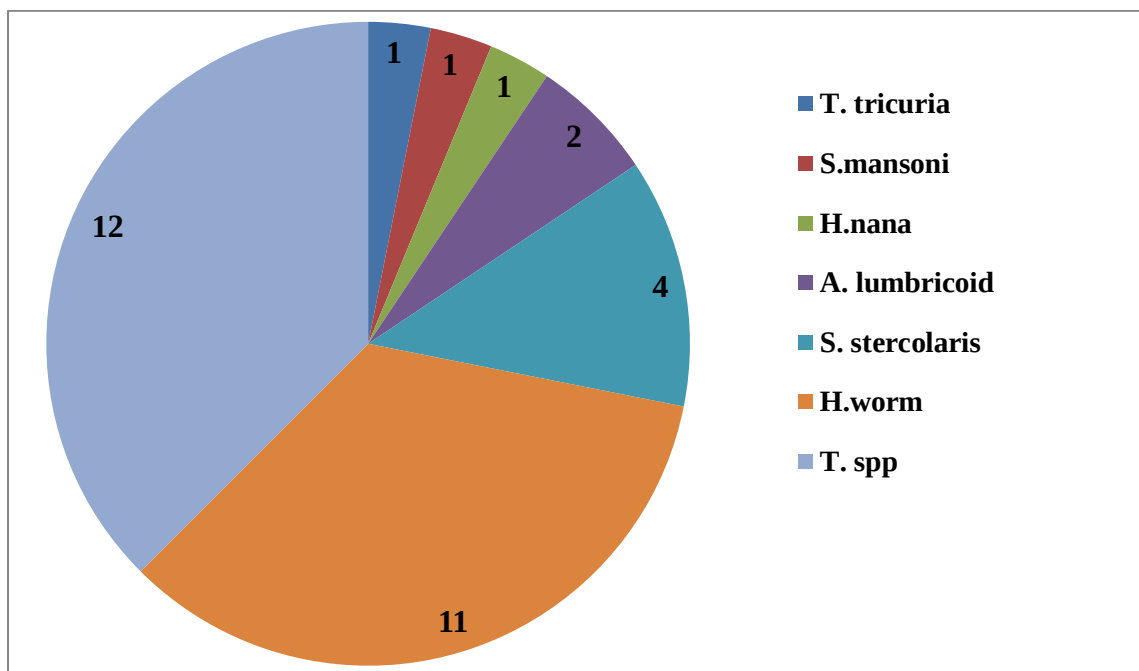


Figure 2. Maternal intestinal helminth species types

Maternal helminth infection and predominant parasite T.spp (light blue color) and H. worm parasite (orange color). This is followed by S. stercolaris (light green) and A. lumbricoides (grapes color).

5.2 Maternal helminth infection and QFT result

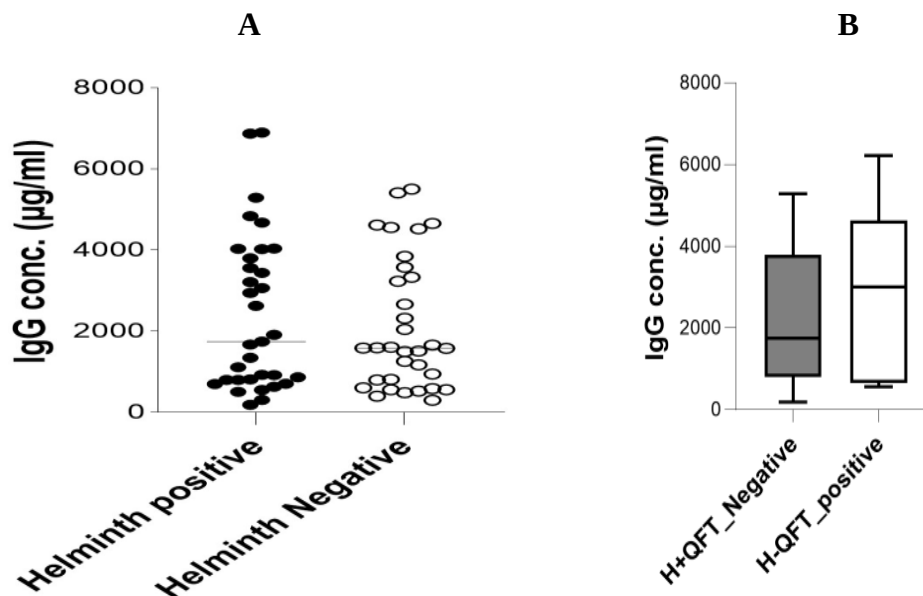
As shown in Table 2, below the Quantiferon status of pregnant mothers was 13.3% (n=10) QFT+ve and 80% (n=60) were QFT-ve and the rest 6.7% (n=5) were indeterminate Quantiferon results. Of participants with QFT test-positive results, only 2 were from the helminth-positive group. There are an increased number of indeterminate results were observed in this study (**Table 3**).

Table 3. Helminth infection and QFT test results of the mother

		QFT- Gold Plus test	
		QFT+	QFT-
		n (%)	n (%)
Helminth Infection	Positive (n=33)	2 (6%)	29 (88%)
	Negative (n=42)	8 (19%)	31(74%)

5.2 CFP antigen-specific IgG response of mothers with helminth infection

Maternal IgG plasma concentration of the mother about helminth infection shows no significant difference between the two groups (**Fig. 3a**). When we looked further and see if there was a link between the QFT test result and IgG concentration about helminth infection, we observed an elevated IgG level of MTB antigen-specific IgG response in mothers who were helminth negative but Quantiferon positive (**Fig.3b**). The IgG levels of mothers as per their QFT status and Helminth infection levels. But, we had very few (n=02), observations for both helminth-infected QFT positive participants.



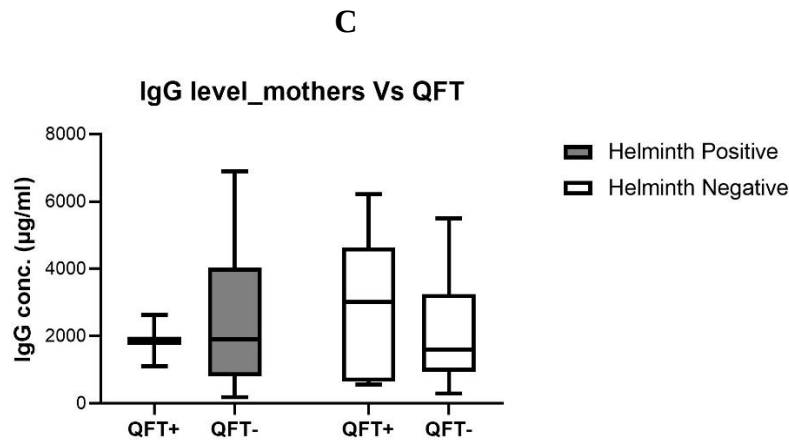


Figure 3. Plasma IgG concentration in pregnant mothers

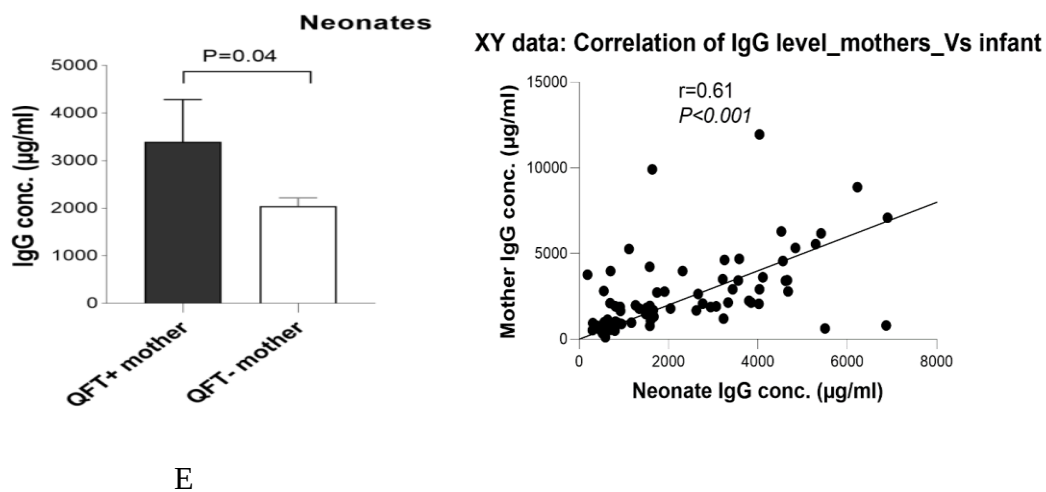
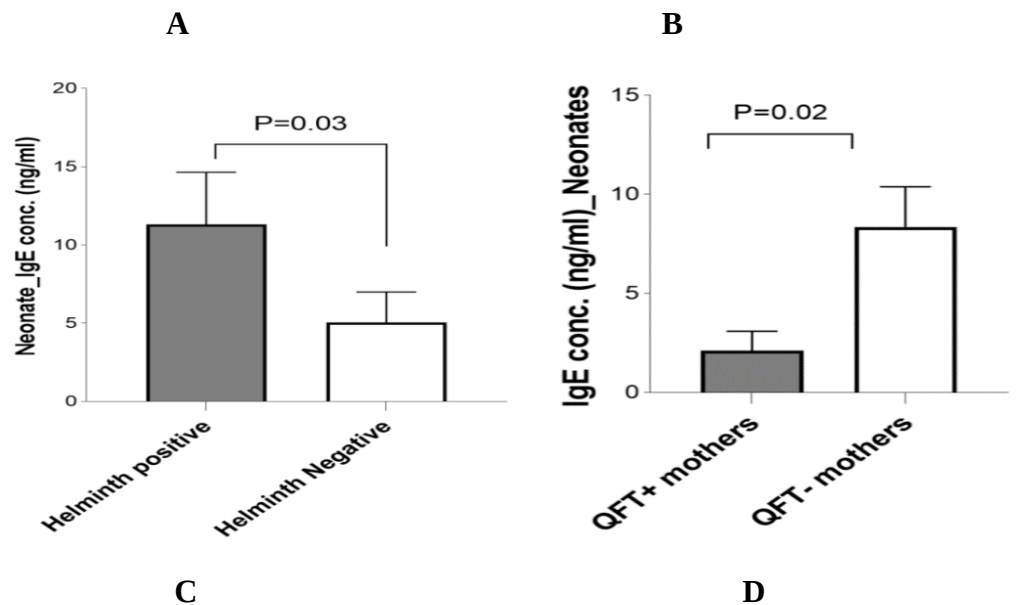
As the graph pad prism outcome showed IgG concentration in helminth-infected mothers (dark filled dots) and those with no helminth infection (open dots) (A) and in regards to helminth infection showed no significant difference. As QFT status of the participant regarding helminth infection – compared with helminth positive QFT negative (Grey filled box and whisker plot) helminth negative QFT positive (open white box and whisker plot) (B), showed an elevated IgG level. The IgG levels of the mother as per their QFT test and helminth infection status (C).

5.3 Effect of maternal helminth infection on neonatal humoral response

As the data from cord blood plasma IgE and IgG showed those neonates that were born from helminth-infected mothers had an increase in IgE levels than neonates from helminth non-infected mothers ($P=0.03$) (Fig. 4a). Similarly, there existed an elevated IgE concentration on those neonates from QFT negative mothers than neonates from QFT-positive mothers ($P=0.02$), (Fig 4b). This result might be an indicator of the presence of in-utero sensitization to maternal helminth antigens and results in the elevation of total IgE which is produced by the neonatal immune system.

Alike IgE levels the result from neonatal IgG and maternal helminth infection showed there is no significant difference between the two groups. Regardless of their maternal helminth status, those neonates that were born from QFT-positive mothers have an increase in IgG concentrations than neonates from QFT-negative mothers ($P=0.04$) (Fig.4c). Mtb antigen-specific plasma IgG levels in the mother's venous blood and the neonate's cord blood collected at delivery result showed that there is no statistically significant difference among both. This suggests that the two groups have similar distributions. The Spearman correlation coefficient of ($r=0.61$) indicates a strong positive correlation between the two variables. As the IgG level in the mother's blood increases, the IgG level in the neonate's blood also tends

to increase (**Fig 4d**). Regarding helminth infection there existed no strong correlation between the helminth-positive mother and their neonates ($r=0.39$). In contrast, a relatively strong correlation of ($r=0.73$ $p<0.0001$) *Mtb* antigen-specific IgG levels was observed among helminth non-infected mothers and their neonates (**Fig 4e**).



XY data: Correlation of IgG level_H- mothers_Vs infant H-

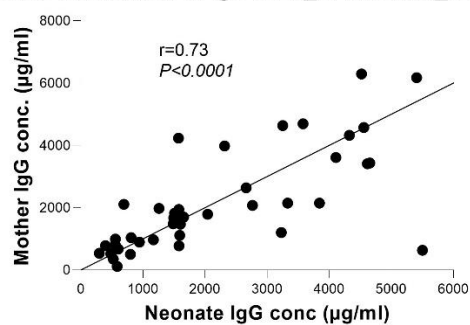
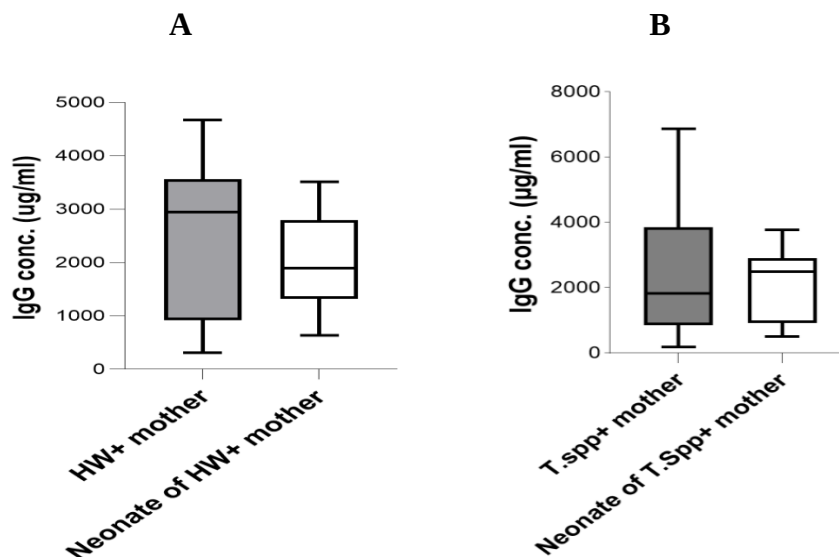


Figure 4 Maternal helminth and QFT status vs. neonates' IgG and IgE levels

IgE concentration of Neonates from helminth-infected mothers (dark filled bar) showed an elevated IgE level than Neonates from helminth-non-infected mothers (white bar) **(A)**. IgE level of neonates as compared with neonates from QFT positive mothers (small white bar) those Neonates from QFT negative mothers showed increased IgE levels (large white bar) **(B)**. Regardless of helminth infection IgG levels of neonates from QFT positive mother (dark bar) had an increased IgG level than those neonates from QFT negative mother (white bar) **(C)**. Presence of a strong positive correlation of maternal IgG levels (Dark spot) and neonatal IgG levels (open spot) **(D)**. Similarly, increased correlation between IgG levels of Helminth-negative Mothers (dark circles) and their Neonates (E) (open dots) Mann Whitney U test).

5.4 Maternal and neonatal helminth species specific humoral response

In this study, the predominant parasites that were so far identified were *Teania* and hookworm. Even though we have little observation regarding strongloid infection, our result from *Teania* Spp and hookworm showed neither of the two parasites has any difference regarding the IgG levels of mothers and their neonates **(Fig 5a, b)**. As hookworm parasite-specific correlation of maternal and neonatal IgG levels data showed, there existed a significantly strong correlation ($r=0.85$, $p=0.001$) among *Mtb* antigen-specific IgG levels of hookworm infected mothers and their Neonates **(Fig 5c)**.



C

XY data: Correlation of IgG level_of infant from HW+ Vs HW+ mother

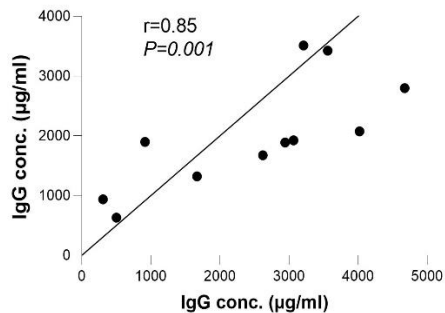


Figure 5. Humoral Immune response vs maternal HW (a) and T.spp infection (b)

IgG concentration of mothers and their neonates regarding helminth infection of the mother, hookworm infected mother and their neonate's IgG concentration (grey box) (A) IgG concentration of T.spp infected mother and their neonates (grey box) (B). A positive correlation between hookworm-infected maternal (dark circle) and their Neonate's IgG levels (white circle) C.

5.5 Maternal helminth egg count and plasma IgG and IgE levels

Apart from the above findings parasite egg count has had a specific effect on the humoral immune response of the Neonates. Neonates that were born from Teania-infected mothers with an egg count above the median and below the median level showed a marginal significance for IgE antibodies ($p=0.05$) (Fig 6a). Similarly, neonates that were born from hookworm-infected mothers with egg counts above the median egg count and below the median egg count showed a borderline significant level for IgG antibody ($p=0.05$) (Fig 6b)

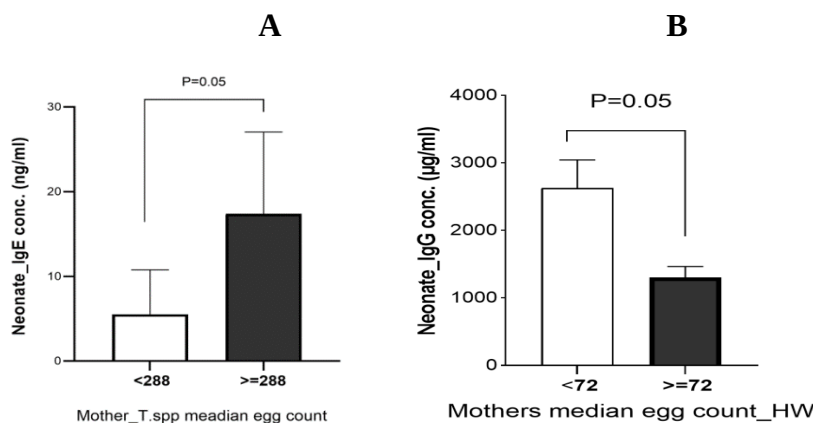


Figure 6. Parasite egg count and IgG and IgE levels of Neonates

IgG concentration of Neonates with regards to maternal Teania Spp infection, IgE levels from Teania infected mother below the median egg count (white bar) and Neonates above the median egg count (dark bar) (A). IgG of Neonates from hookworm-infected Mothers above median egg count (dark bar) and below median egg count (white bar) B.

6. Discussion

In this study, 10/75 (13.3%) of study participants were positive Quantiferon TB test. Similarly, studies from Ethiopia and North Tanzania reported 17% and 24-37% LTBI rates among pregnant women (32-34). In comparison, our findings are lower than these reports. The low LTBI rate observed in this study could be associated with the difference in the current study participants' characteristics that were healthy pregnant mothers, free from acute and chronic illness with Hgb<110g/L to minimize confounding factors that may explain the findings.

Relatively higher indeterminate Quantiferon TB test result (6.7%) was observed in this study. Our data doesn't allow a comparison of indeterminate results between helminth-infected and non-infected participants attributed to a low number of observations. However, previous studies have demonstrated the role of helminth infection in increasing indeterminate QFT results (34, 51). However, another report investigated that treatment for Hookworm infection did not change the LTBI status of the participants and that showed hookworm infection reduced the risk of *Mtb* infection (52). However, to present a potential explanation, more research in this area with a bigger sample size should be done. One of the limitations of our study is the small number of participants who were infected with helminths and had a positive QFT test (n=2). This sample size is insufficient to draw any definitive conclusions about the association between helminth infection and QFT test results in pregnant women. However, this low prevalence of QFT positivity among helminth-infected women may be explained by the Th2/Treg biased immune phenotype observed by both pregnancy and helminth infection. This immune profile may impair the ability to mount a Th1 response to *Mtb* antigens, leading to false-negative QFT results (53). Therefore, our participants faced a double burden of immunological challenges that could affect their QFT test outcomes.

IgG antibodies are among the most abundant immunoglobulin that play an important role in the immune response. Besides, it can cross the placenta of a pregnant woman to her developing fetus and provide passive immunity to the newborns (18). Due to this, the present study measured MTB antigen-specific plasma IgG levels of both the mother and their neonates to rule out whether maternal helminth infection had an impact on IgG antibody levels. As the present finding showed there existed no significant difference in CFP antigen-specific IgG levels among helminth-infected and non-infected pregnant women. On the contrary, there was an elevated CFP antigen-specific plasma IgG level of helminth non-infected and Quantiferon-positive pregnant mothers. This is a common illustration that had

been observed an elevated IgG antibody among MTB-infected groups against different Mtb antigens compared to non-infected groups (35). Even though our study participants were pregnant women and a relatively different characteristics from the general population the finding still supports the evidence. In contrast, a study in different health institutes of Addis Ababa showed no significant difference were observed in IgG levels of women with LTBI and neither pregnancy nor HIV affected the IgG levels (54). This could be due to the difference in the infection status of the mother and the infection type which infects the mother.

To know the cross-placental transfers of maternal IgG, MTB antigen-specific neonatal IgG was measured. Regardless of maternal helminth infection elevated MTB antigen-specific IgG levels were observed in neonates born from QFT-positive mothers compared with neonates born from QFT-negative test results indicating the possibility of cross-placental transfer of Mtb antigen-specific IgG or fetal immune response for MTB antigen exposure at prenatal age. This finding is in line with a study from Mekele that showed an increased neonatal IgG level from a QFT test-positive mother (13). Similarly, a study from Uganda showed PPD antigen-specific IgG responses declined between birth in cord blood and six weeks (55). Even though we used a different antigen, the finding showed how MTB antigen-specific exposure resulted in elevated IgG antibody levels in neonates that were born from QFT-tested-positive mothers than neonates from QFT-negative mothers.

On the contrary, it is also described in global literature that a decrease in IgG level in cord blood of neonates born from *S. pneumonia* and SARS-COV-2 infected mothers indicates a low level of transplacental transmission. A study from Kenya showed that pre-natal parasitic infections decrease the transfer of maternal IgG antibodies to infants for several serotypes of *S. pneumonia* (38). Similarly, maternal SARS-COV-2 infection also results in a decrease in the cross-placental transfer of antigen-specific IgG antibodies (56). Different reasons were mentioned for the variation of neonatal IgG and cross-placental transfer of IgG, maternal infection status and IgG sub-class were among the most listed (18).

The level of MTB-specific plasma IgG levels in the mother's venous blood and neonatal are positively correlated ($r=0.61$) meaning as the IgG level in the mother's blood increases, the IgG level in the infant's blood also tends to increase. The value indicated that this correlation is not due to random chance. Regarding helminth infection there existed no strong correlation between helminth-positive mother and their neonates ($r=0.39$). In contrast, a relatively

strong correlation of *Mtb* antigen-specific IgG concentration was observed among helminth-infected mothers and their neonates.

In summary, the results suggest that the IgG levels in the mother's blood and the neonate's blood are not only similar but also closely related. As the mother's IgG level changes, the neonate's IgG level is likely to change in the same direction. This could be due to the transplacental transfer of antibodies from the mother to the infant during pregnancy. However, further research would be needed to confirm these reports to get a reasonable and credible conclusion.

Immunoglobulin E (IgE) is predominantly associated with allergen exposure and plays a crucial role in protective immunity against helminth parasites (57). The total IgE result showed an elevated IgE antibody concentration among neonates born from helminth-infected mothers than those born from no helminth infection. This finding was comparable with a study from Mekele (13), Nigeria (43), and Mexico (58) that reported an increase in neonatal IgE antibodies of helminth-infected pregnant mothers. Furthermore, studies from India and Kenya also showed neonates from filaria-infected and helminth-infected mothers had increased cord blood IgE levels as compared to neonates from non-infected mothers (42, 59).

Therefore, an increase in IgE antibodies could be due to in-utero sensitization for helminth antigen. This was evidenced by an article review that showed an elevated IgE antibody in offspring from mothers who are infected with parasitic infection (60). In addition, another study also reported an in-utero sensitization to helminth antigens will result in B cells producing helminth antigen-specific IgE antibodies (20). Correspondingly, the current study reported an increase in IgE levels among neonates of helminth-infected mothers. This supports our hypothesis that neonates that were born from helminth-infected mothers have a distinct immune profile. On the contrary, an elevated total IgE was observed among neonates from Quantiferon-negative mothers in comparison to neonates from Quantiferon-positive mothers. This finding might be illustrative and supportive of the evidence that how immune response against helminth infection modulates the immune response to *Mtb* infection.

Apart from maternal helminth infection higher number of previous pregnancies, delivery season, Caesarean type of delivery, and a history of allergy during pregnancy are among the factors that were associated with an elevated neonatal IgE antibody (61). On the other hand, another study mentioned the presence of a difference in cord blood IgE levels among

neonatal sexes only (62). Yet our study does not give a conclusive report in this regard due to the smaller number of groups that we had to present such evidence.

In this study the effect of helminth parasite load, as determined by the egg count on the humoral response of the neonates was assessed. Our finding indicated a relatively similar proportion of plasma IgE antibody levels among neonates that were born from *Tenia*-infected mothers above and below the median egg counts. Similarly, a borderline significance level was observed in IgG antibody levels among neonates born from hookworm-infected mothers above and below the median egg count indicating no significant impact.

7. Conclusion

The current finding showed maternal helminth infection impacts neonatal humoral Immune response. An elevated total IgE was observed among neonates that were born from a helminth-infected mother. This showed maternal helminth infection affects neonatal IgE antibody levels, which could be due to an in-utero sensitization to helminth antigens. Neonates born from mothers with positive QFT tests have higher mycobacterial CFP antigen-specific IgG levels regardless of helminth infection status.

The impact of this immune profile difference on the fetal/Neonatal health or immune on the development should be elucidated further.

8. Recommendation

The current finding observed an impact of helminth infection on neonatal immunity response but further study should be commenced with a larger sample size to address the magnitude of the impact.

Maternal pre-screening for helminth and LTBI infection could be a good approach to managing maternal infection on neonatal immunity

A maternal deworming program is another possible way out mechanism to manage the impact of helminth infection on neonatal immune response.

Health education for pregnant women about helminth infection and LTBI infection will be helpful to them to protect themselves and their babies from further immunological complications.

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Annexes

Annex-1 Participant information sheet

Project Title: Maternal Intestinal helminth Infection on immunity to TB at Hana Maryam Health Center, Addis Ababa, Ethiopia

Investigators: Meaza Zewde

Background: This was a study that was conducted here in Addis Ababa, Ethiopia on TB and helminthic infections. Tuberculosis and helminth infections are coexisting and highly prevalent in many African countries. Epidemiology links helminth infections with higher susceptibility to develop latent or active TB, but the underlying mechanisms are poorly understood.

Objective: To investigate the effect of Maternal Helminth Infection on Humeral and Cell-mediated immune responses to MTB

Methodology: You are invited to participate in this study because you are suspected of having a helminth infection. If you agree to participate in this study, you will be asked to sign a consent form and give relevant information as per the questionnaire developed for this project.

You will also be asked to undergo a stool examination to determine whether you have a helminth infection or not and to document the type of helminth. If you are helminth-negative, you still take part in the study as a helminth-negative participant. In addition, you will also be tested for latent TB infection using blood-based laboratory assays. HIV testing will be given to you with pre-and post-test counseling as per the national guidelines. The results of the HIV test will be kept strictly confidential. The results will be given to you only when you wish to know. If you are HIV positive and you are willing, you will be referred to the ART center to obtain the standard care provided in the center.

To investigate alterations of Neonatal immunity cell expression from pregnant women infected with helminths, 5-6 ml of venous blood from you and 30 ml (equivalent to 6 teaspoons) of the umbilical cord blood during delivery will be collected.

Risks: You may feel minor pain and discomfort from the needle while blood is drawn from your vein. An experienced health professional will carry out the procedure with a standard aseptic and sterile condition.

Benefits: There will be no monetary benefit for participation, yet you only receive a modest amount of money, equivalent to 100 Ethiopian birr, at each visit to cover transportation costs and compensate for the time you spent with us. You may benefit from laboratory

investigation for helminth infection, if the test result is positive, you will be treated accordingly.

Confidentiality: The results of this study and related information will only be used for this study. Information collected from you will be coded and your names will not be used to Label specimens or questionnaires. All study documents bearing participant information including HIV test results will be kept confidential.

Dissemination of study outcomes: Data from this study will be analyzed anonymously and published in peer-reviewed journals and /or presented in local as well as international seminars. In so doing, your identity will not be revealed and no information containing your name will be disclosed.

Right to refuse or withdraw: You have full right to refuse participation or withdraw from participating in this study at any time and this will not affect the medical care you receive at this health facility now or anytime in the future.

The findings of this study will provide relevant data on how helminths could modulate immune response to tuberculosis.

Contact person: In case of any questions regarding the study or related issues, you can contact the following individuals;

Principal Investigator: Dr Meseret Habtamu: Tel: 0921 623485,

Investigator: Meaza Zewde: Tel: 0913118359

AHRI/ALERT Ethics Review Committee: Tel: 011 321 13 34

Finally: we would like to say thank you for taking the time to consider participation in this study.

የጥናት ተሳታፊ መረጃ ወረቀት:

የጥናቱርዕስ፣ የሆድቃ ትላትል ካለባቸው እናቶች የሚወለዱ ልጆች በደምህዋሳት ላይ የሚገኙ ተቀባይ ሞለኪውሎች ያላቸው ሁነታ እና የቲቢ በሽታ አምጪ ተሃዋስን የመከላከል አቅም መረዳት

ተመራማሪ: መዓዛ ዘውዴ

መግቢያ:

ይህ ጥናት የሆድቃ ትላትል እና የቲቢ ኢንፌክሽን ላይ ሃኖማርያም ጤና ጣቢያ ኢትዮጵያ የሚደርግ ነው። የቲቢ እና የሆድቃ ትላትል በሽታዎች በብዙ የአፍሪካ አገራት በአንድነት የሚከሰቱበት አጋጣሚ በርከት ያለ ነው። የበሽታ ስርጭት የተመለከቱ ጥናቶች እንደሚያሳዩት፣ የቲቢ ተጋላጭነትን ከሆድ እቃ ትላትል ጋር እንደሚገናኝ ቢገልጽም ስለ ክስተቱ ያለን መሠረታዊ እውቀት ግን አሁንም ዉስን ነው።

የጥናቱዓላማ፡ የዚህ ጥናት አላማ የሆድቃ ትላትል ካለባቸው እናቶች የሚወለዱ ልጆች በደምህዋሳት ላይ የሚገኙ ተቀባይ ሞለኪውሎች ያላቸው ሁነታ እና የቲቢ በሽታ አምጪ ተሃዋስን የመከላከል አቅም።

የጥናቱሂደት፡ እዚህ ጥናት ላይ ለመሳተፍ የተጋበዙት በሆድቃ ትላትል ኢንፌክሽን ሊኖርበት ስለሚችል ሲሆን በጥናቱ ላይ ለመሳተፍ ፈቃደኛ ከሆኑ የሚያስፈልገውን ትክክለኛ መረጃ እንዲሰጡ ይጠየቃሉ። የሆድቃ ትላትል ተጠቂ መሆንዎን ለማጣራት እና የትላትሉን አይነት ለመለየት የሰገራ ምርመራ እንዲያደርጉ ይጠየቃሉ። ከትላትል ነፃ ቢሆኑም፣ ለአሉታዊ ንፅፅር ያልተጠቁ ተሳታፊዎች ቡድን ዉስጥ ይሳተፋሉ። ለቲቢ በሽታ አምጪ ተዉሳክ የተጋለጡ መሆንዎን ለማወቅ የደም ናሙና ምርመራ ይደረግሎታል ከቅድመ እና ድህረምክር በኋላ የኤች አይ ቪ ምርመራ እንዲያደርጉ ይጠየቃሉ። ውጤቱም በጥብቅ ሚስጥር ይያዛል። ኤች አይ ቪ በደም ውስጥ ካለና ፍቃደኛ ከሆኑ ወደ ART ማዕከል ተልከዉ አስፈላጊው ክትትል ይደረግሎታል።

በደም ህዋሳት ላይ የሚገኙ ተቀባይ ሞለኪውሎች በሆድቃ ትላትል የተጠቁ እናቶችና ከእነርሱ ተወልደዉ ጫቅላ ህፃናት ላይ የሚኖረውን ተጽዕኖ ለመመርመር ከወሊድ በፊት ከእናቶች ከደም ስር ደምን በመቅዳት የሚወሰድ 5-6ሚ.ሊ (የሚሆን የደም ናሙና እና ከእትብት ገመድ 30ሚ.ሊ (6 የሻይ ማነኪያ) የደም ናሙና ይወሰዳል። የደም ናሙና ወደ አህሪ ቤተ ሙከራ ተወስዶ በተገቢዉ ሁኔታ ምርመራ የሚከናወን ይሆናል።

የምችትመጓደልናተጋላጭነት፡ ደም በሚወሰድበት ጊዜ በመርፌ ከመወጋት ጋር በተያያዘ በተወሰነ ደረጃ ህመምና የምችት መጓደል ሊከሰት ይችላል። ሁሉም ተግባራት ደረጃውን በጠበቀተዉ ሳክአልባ በሆነ መልኩና ልምድ ያላቸዉ የጤና ባለሙያዎች እንዲሰሩ በማድረግ የተጠቀሱትን አላስፈላጊ ጉዳዮች ለመቀነስ ጥረት ይደረጋል። ከጥናቱ ጋር በተያያዘ የሚከሰት ማንኛዉም ጉዳት/የምችት ማጣት ተገቢዉን ህክምና የሚያገኙ ይሆናል።

ተጠቃሚነት፡ በጥናቱ በመሳተፍዎ የሚያገኙት ቀጥተኛ የገንዘብ ክፍያ አይኖርም። ነገር ግን በምርመራ ውጤት የሆድቃ ትላትል ከተገኘ ተገቢዉን ህክምና በማግኘት ተጠቃሚ ይሆናሉ። ከጥናቱ ጋር በተያያዘ ለጠፉት ጊዜና ለትራንስፖርት ወጪ ማካካሻ የሚሆን 100 ብር በየጉብኝቱ ያገኛሉ።

ሚስጥራዊነት፡ ከጥናቱ የሚገኙ መረጃዎች ለዚህ ጥናት ጠቀሜታ ብቻ ይዉላሉ። የእርሶስም ለናሙና መለያነት ወይም በማንኛዉም የዚህ ጥናት ዉጤት ሪፖርት ላይ አይጠቀስም። በጥናቱ መጀመሪያ ለተሳታፊዎች የመለያ ሚስጥር ቁጥር ይሰጣል። ይህም ለናሙናዎችና በጥናቱ ጥቅም ላይ ለሚዉሉ ቅጾች መለያነት ይዉላል። የእርሶ ማንነት ከጥናቱ የሚስጥር ቁጥር ጋር የሚያገናኘዉን መረጃ ማወቅ የሚችሉት የጥናቱ ዋና ተመራማሪዎች ብቻ ይሆናሉ።

የጥናቱን ዉጤት ይፋ ስለ ማድረግ፡ የጥናቱ ዉጤት በጥቅል ተፈትሾ በሳይንሳዊ መጽሕፍት ይታተማል፤እንዲሁም ሃገር ዉስጥና በአለማቀፍ ሳይንሳዊ ስብሰባዎች ላይ ይቀርባል። ይህ በሚሆንበት ጊዜ የእርሶ ማንነት አይገለጽም፤ስም የያዙ መረጃዎችም አይወጡም።

ከጥናቱ እራሱን የማግለል/ያለመሳተፍ መብት፡ተሳታፊው በማንኛውም ሰዐት እራሱን ከጥናቱ የማግለልና መውጣት ሙሉ መብት አለው።ይህ ዉሳኔዎች በጤና ተቋሙ አሁንም ሆነ ወደፊት ማግኘት የሚገባዎት ግልጋሎት ላይ ምንም አይነት ተጽዕኖ አይኖረዉም።

የጥናትዉጤት፡ከዚህ ጥናት የሆድቃ ትላትል ኢንፌክሽን የደም ህዋሳት የቲቢ በሽታ አምጭ ተዉሳክን የመለየት አቅም በተመለከተ አዳዲስ መረጃዎችን ያወጣል።

ጥያቄ የመጠየቅ ነጻነት፡ ማንኛውም ጥናቱን የሚመለከት ወይም ተዛማጅ ጥያቄ ካለዎት በየትኛዉም ጊዜና ሁኔታ ከታች በተመለከቱት አዳራሾች ማግኘት ይችላሉ።

ዋና ተመራማሪ ዶ/ር መሰረት ሃብታሙ፡ ስቁ፡ 0921623485

ተመራማሪ፡መአዛ ዘውዴ ስቁ፡ 0913118359

አህሪ/አለርትኮሚቴ፡ ስቁ፡ 011 321 13 34

በመጨረሻም፡ በዚህ ጥናት ለመሳተፍ ለመወሰን እንዲያስችሎት ከኛ ጋር ለወሰዱት ጊዜ እናመሰግናለን፡
:

Annex 2 Consent form

Project title: Maternal Intestinal Helminth Infection and Its Impact on the Neonatal and Maternal Immune Response to Tuberculosis: A Study at Hana Maryam Health Center, Addis Ababa, Ethiopia

Participant Group: Helminth infected____ Helminth non infected____

Health Centre/Hospital:_____

Participant Study ID:_____

I have been informed that a project to investigate the ‘effect of helminth infection conducted here in Addis Ababa (Ethiopia) on TB and helminthic infections’ is being conducted by researchers I have been asked to give consent to participate in this study. I am aware that the study requires 30 ml of venous blood and cord blood during the time of birth and stool examination for intestinal parasites. I will also be tested for TB infection using a blood-based test.

I have been asked to have voluntary counseling and testing (VCT) for HIV and if I am HIV positive and willing, I will be referred to the ART centers to obtain the standard care provided in the centers for me and my child. I understand that all of the laboratory tests will be conducted in Ethiopia and I have also been told that there are no major risks associated with the study except for minor pain at the site of injection during blood collection. I have been informed that blood collection will be done by experienced health professionals according to the standard clinical care procedure. I am informed that participation is voluntary, and it is my right to refuse or withdraw myself and my child from the study at any time without explaining the reason. I have been informed that confidentiality will be protected by using study codes on documents and giving access to documents to only the investigators in this study. I had been given enough time to consider participation in this study before I gave my informed consent by signing this form. It is therefore with full understanding of the situation that I give my informed consent to participate myself and my child in this study.

Name of Participant:_____ Sign:_____ Date:_____

Name of Partner _____ Sign:_____ Date:_____

Name of Witness_____ Sign:_____ Date:_____

Name of Nurse:_____ Sign:_____ Date:_____

የስምምነትቅጽ፡

የጥናቱርዕስ፡የሆድቃ ትላትል ካለባቸው እናቶች የሚወለዱ ልጆች በደም ህዋሳት ላይ የሚገኙ ተቀባይ ሞለኪውሎች ያላቸው ሁነታ እና የቲቢ በሽታ አምጪ ተሃዋስን የመከላከል አቅም

ተሳታፊዎች፡በየሆድቃ ትላትል የተጠቁ በየሆድቃ ትላትል ያልተጠቁ

ሆስፒታል፡ _____

ለጥናቱ የተሰጠ የተሳታፊ መለያ ቁጥር፡ _____

በደም ህዋሳት ላይ የሚታዩ ተቀባይ ሞለኪውሎች በየሆድቃ ትላትል ወይም በቲቢ ኢንፌክሽን ምክንያት የሚያሳዩት ለዉጥና የቲቢ በሽታ አምጪ ተሃዋስን ለመለየት የሚያስችላቸውን መጠን መወሰን ላይ ያተኮረ ጥናት ዉስጥ እንድሳተፍ በቂ መረጃ ተሰቶኝ የስምምነት ቃሌን እንድስጥም ተጠይቂያለሁ።የሰገራ ምርመራ እንዲሁም የደም ናሙና በመጠቀም የሚሰራ የቲቢ ምርመራ እንደሚደረግልኝ ተገልጾልኛል።የኤች አይ ቪ የፈቃደኝነት ምክርና ምርመራ በማድረግ ሻይረሱ በደሜ ውስጥ ከተገኘ እና ፍላጎቴ ከሆነ ወደ ፀረ ኤች አይ ቪ ህክምና ማዕከል ተልኬ ህክምናዉን ለራሴና ለልጄ እከታተላለሁ። በሌላ በኩል ትላትሉ በምርመራ ከተገኘብኝ የፀረ-ትላትል መድሀኒት እንደሚሰጠኝ ተረድቻለሁ።ለዚህ ጥናት ከደም ስር ደምን በመቅዳት የሚወስድ 30ሚ.ሊ (6 የሻይማንኪያ) የሚሆን የደም ናሙና እንደምሰጥ ተነግሮኛል።በተጨማሪም ከወሊድ በሁላ ከእትብት ገመድ ደም እንደሚወሰድ ተነግሮኛል። ሁሉም የቤተ-ሙከራ ምርምር በኢትዮጵያ እንደሚካሄድ ነገር ግን የናሙናው የተወሰነ ክፍል ወደ ጀርመን ሀገር ለተጨማሪ ምርመራ እንደሚላክ ተረድቻለሁ። ውጤቱም ሚስጥራዊ እንደሚሆን ተገንዝቢያለሁ።በተጨማሪም በደም ናሙና ቅጂ ወቅት ደም የተቀዳበት ቦታ ላይ ከትንሽ ህመም ውጪ ምንም አይነት ትልቅ ችግር እንደማይኖር ተገልጾልኛል። የደም ናሙና ቅጂውም ልምድ ባላቸው የህክምና ባለሙያዎች መደበኛ የህክምና ሂደቱን ተከትሎ እንደሚካሄድ ተገልጾልኛል።

ተሳትፎ በፍቃደኝነት እንደሆነ እና በማንኛውም ሰዐት እራሴንም ሆነ ልጄን ከጥናቱ ማግለል መብቴ እንደሆነ፤ምክንያቱንም መግለጽ እንደማይጠበቅብኝ ተነግሮኛል።ለጥናቱም የምሰጠው መረጃ፤ አጠቃላይ ምርመራና የላቦራቶሪ ዉጤት መለያ ቁጥር በመጠቀም በሚስጥር እንደሚያዝና መረጃውም ለተመራማሪዎች ዕይታ ብቻ እንደሚውል ተነግሮኛል።ጥናቱ ላይ ለመሳተፍ ስምምነቴን ከመግለጹ በፊት በቂ የማሰቢያ ጊዜ ተሰቶኛል።ሁኔታውን በደንብ ስለተረዳሁ ጥናቱ ላይ ለመሳተፍ ስምምነቴን ለልጄ እና ለራሴ በፈረማዬ አረጋግጣለሁ።

የተሳታፊውስም፡ _____ ፊርማ፡ _____ ቀን፡ _____

የትዳርአጋርስም፡ _____ ፊርማ፡ _____ ቀን _____

የእማኝስም፡ _____ ፊርማ፡ _____ ቀን፡ _____

የነርስ ስም፡ _____ ፊርማ፡ _____ ቀን፡ _____

Annex 3 Data acquisition Pregnant Mothers (Visit 0)

Participant Group: Helminth Infected Helminth Uninfected

If performed at the health facility)

General Information

Name of health facility _____

Mother's Initials _____

Mother's project ID _____

Health facility card number _____

Telephone Number _____

II. Clinical and physical

Age (in years) _____

Weight (kg) _____ Height (cm) _____

Evidence or history of allergic reaction: Yes _____ No _____

If yes; specify _____

III. Stool Examination: Helminth positive Helminth Negative

If positive; parasite type (types): _____

Expected Due date of the mother: ____/____/____ (dd/mm/yyyy)

Data collected by Date/____ (dd/mm/yyyy)

Annex-4 Data Acquisition-during childbirth (Mother and New-born)(Visit 1)

Participant Group: Helminth Infected_____ Helminth non-infected_____

I. General Information

Name of health facility_____

Mother's Initials

Mother's project ID

Infant's Project ID

Mother's hospital card number_____

Infant's hospital card No_____

Telephone Number

II. Clinical and physical assessment of the mother

1. Type of delivery: Normal C-Section Other_____

2. Medication given while giving birth Yes No If Yes, specify _____

Sign of acute infection of the mother Yes No If Yes, specify _____

III. Clinical and physical assessment of the newborn

Apgar score of the newborn (1-10):

Note: Apgar score: Activity (muscle tone), Pulse (heart rate), Grimace (reflexes),

Appearance (how does the skin color look) skin problems (eczema), Respiration (breathing)

4. Anemia, Congenital anomalies, allergy: Yes No If yes (specify) -----

5. Baby: A. Weight (g)_____ B. Head circumference (cm)

6. BCG vaccine strain is given to the baby: Danish Japan USA Other (Specify)

Next appointment (6 weeks after BCG vaccination): _____/(dd/mm/yyyy)

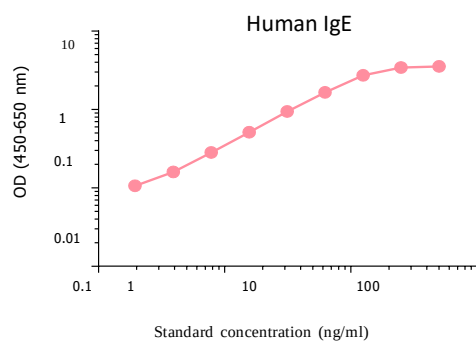
Annex-5 ELISA IgE Protocol

Day 1

1. Add 100 μ l/well of capture mAb 107 diluted to 2 μ g/ml in PBS, pH 7.4. Use high protein binding ELISA plates. Incubate overnight at 4-8 $^{\circ}$ C.

Day 2

2. Empty the plate and add 200 μ l/well of PBS with 0.05% Tween 20 and 0.1% BSA (incubation buffer) to block the plate. Incubate for 1 hour at room temperature. Wash the plate 5 times with PBS containing 0.05% Tween 20 (300 μ l/well).
3. Add 100 μ l/well of samples or standards diluted in incubation buffer or ELISA diluent, Include assay background control, i.e. wells without standard. Incubate for 2 hours at room temperature.
4. Wash as above.
5. Add 100 μ l/well of detection mAb 182-biotin diluted to 0.3 μ g/ml in incubation buffer or ELISA diluent. Incubate for 1 hour at room temperature.
6. Wash as above.
7. Add 100 μ l/well of Streptavidin-HRP diluted 1:1000 in incubation buffer. Incubate for 1 hour at room temperature. Please note that sodium azide used in buffers will inhibit HRP activity.
8. Wash as above.
9. Add 100 μ l/well of TMB substrate (product code: 3652-F10) and incubate at room temperature, protected from direct light for 15 minutes.
10. Add 100 μ l/well of 0.2 M H_2SO_4 to stop the reaction.
11. Measure the optical density in an ELISA reader at 450 nm within 15 min. Preferably, use a reader capable of subtracting a reference wavelength of between 570 and 650 nm. A representative standard curve is shown below.



Annex 6 IgG ELISA protocol

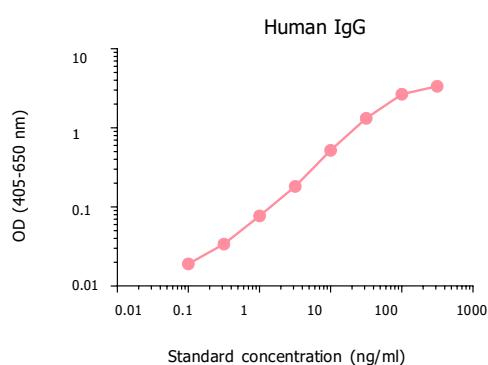
Protocol

Day 1

1. Add 100 μ l/well of capture mAb MT145 diluted to 2 μ g/ml in PBS, pH 7.4. Use high Protein binding ELISA plates. Incubate overnight at 4-8 $^{\circ}$ C.

Day 2

2. Empty the plate and add 200 μ l/well of PBS with 0.05% Tween 20 and 0.1% BSA (incubation buffer) to block the plate. Incubate for 1 hour at room temperature.
3. Wash the plate 5 times with PBS containing 0.05% Tween 20 (300 μ l/well)
4. Add 100 μ l/well of samples or standards diluted in incubation buffer Include assay background control, i.e. wells without standard. Incubate for 2 hours at room temperature.
5. Wash as above.
6. Add 100 μ l/well of detection mAb MT78-ALP diluted 1:1000 in incubation buffer. Incubate for 1 hour at room temperature.
7. Wash as above.
8. Add 100 μ l/well of pNPP substrate (product code: 3652-P10) and incubate the plate for approximately 60 minutes.
9. Measure the optical density in an ELISA reader at 405 nm. Preferably use a reader capable of subtracting a reference wavelength of between 570 and 650 nm. A representative standard curve is shown below.



ASSURANCE OF PRINCIPAL INVESTIGATOR

I agree to accept all the responsibilities for the scientific and ethical conduct of the research project. I will provide a timely progress report to my advisor and seek the necessary advice and approval from my primary advisors in the course of the research. I will communicate timely to my advisors and all stakeholders involved in the study including any resource of funding for this research.

Name of the student:

Meaza Zewde: _____

Signature _____

Date_____

Approval of the Primary Advisor

Name of the first Advisor: _____

Signature _____

Date_____

Approval of the Co-Advisor

Name of the primary advisor:_____

Signature _____

Date_____