**ADDIS ABABA UNIVERSITY**

**COLLEGE OF HEALTH SCIENCES**

**SCHOOL OF ALLIED HEALTH SCIENCES**

**DEPARTMENT OF MEDICAL LABORATORY SCIENCES**

# Association of *Helicobacter pylori* infection with atopy and allergic disorders in Ziway, Central Ethiopia

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# Addis Ababa University

## School of Graduate Studies

This is to certify that the thesis prepared by Mheret Tesfaye, entitled: Association of *Helicobacter pylori* infection with atopy and allergic disorders in Ziway, Central Ethiopia, and submitted in fulfilment of the requirements for the Degree in Master of Science in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology specialty track) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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## List of Abbreviations

| | |
|---|---|
| AD | Atopic dermatitis |
| Antibody | Ab |
| Antigen | Ag |
| CagA | Cytotoxin-associated gene A |
| DRERC | Departmental Research Ethical Review Committee |
| EDTA | Ethylene diaminetertacetic acid |
| ELISA | Enzyme Linked Immunosorbent Assay |
| GERD | Gastroesophageal reflux disease |
| HP-NAP | *H. pylori* neutrophil-activating protein |
| IgE | Immunoglobulin E |
| IgG | Immunoglobulin G |
| ISAAC | International Study of Asthma and Allergies in Childhood |
| MALT | Mucosal associated-lymphoid-type |
| NHANES | National Health and Nutrition Examination Survey |
| SOP | Standard operating procedure |
| SPT | Skin Prick Test |
| Th1 | T helper 1 |
| Th2 | T helper 2 |
| TLRs | Toll-like receptors |
| UBTC | urea breathe test |
| VacA | Vacuolating cytotoxin A |
| WAO | World Allergy Organization |

## Operational Definitions

**Atopic** - defined as positive skin prick test results against *Dermatophagoides pteronyssinusin* and cockroach allergen.

**Allergic condition-**defined as a positive response to one or more of asthma, wheezing, hay fever and eczema in the past 12 months

**Hematological parameters-** Total WBC and absolute eosinophil count

**Formal education –** refers to mothers, who can read, write or learned to higher levels,

**None-** refers to mothers who cannot read or write

**Past *H.pylori* infection** – Any past and/or current *H.pylori* infection determined by serum antibody test

**Current *H.pylori* infection**- *H.pylori* infection determined by stool antigen test

## Abstract

**Background:** Some epidemiological and experimental data point to the protective effect of *Helicobacter pylori* infection against the development of many extra-gastric diseases, including gastroesophageal reflux disease and its associated outcomes, childhood asthma and allergy. There is scarcity of data concerning this in Ethiopia.

**Objective**: To assess the association of *Helicobacter pylori* infection with atopy and allergic disorders in Ziway, Central Ethiopia

**Methods and materials**: Health facilities and school based cross sectional study was conducted from October 2016 to January 2017, Ziway, Ethiopia. A total of 461 children were enrolled in the study. Participant‟s socio-demographic and clinical information was collected using International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire. Skin prick test was done; blood and stool samples were collected from each participant. Automated WBC and manual eosinophil count were performed from EDTA blood while total serum IgE, *H. Pylori* stool antigen test and serum antibody test were determined using ELISA technique. Descriptive statistics were used to express the socio-demographic characteristics of the participants. Binary logistic regression was computed to assess association between variables using SPSS version 20 for windows. $P < 0.05$ were taken as statistically significant.

**Results**: The overall prevalence of past and current *H.pylori* infection was 70.3% and 5.2%, respectively. The overall prevalence of any sensitization was 2.4%. Our result also did not show the protective effect of the bacteria. There was no association between past *H.pylori* infection and atopic sensitization and allergic conditions (OR 1.58, 95% CI 0.44, 5.70 (P=0.48) and OR 0.96, 95% CI 0.56, 1.62 (P=0.86), respectively) and the current infection of *H. pylori* was not associated with allergic conditions with OR 1.81, 95% CI 0.53, 6.22 (P=0.35). In this study the IgE levels were elevated in current *H.pylori* infected atopic participants.

**Conclusion**: In this study we didn‟t find any significant association between past and current *H.pylori* infection with atopy or allergic conditions. Further studies with high quality longitudinal cohort are needed to map every possible correlates of the infection.



**Keywords: *Helicobacter pylori*, Atopy, Allergy, IgE**

# 1. Introduction

## 1.1. Background

*Helicobacter pylori* (*H. pylori*) is gram negative bacteria which colonises the human stomach and also been the focus of medical researches since its discovery in 1980s by Warren and Marshall [1].The bacterium has infected almost 50% of the world's population; although most infections are asymptomatic. The transmission could be through faeco-oral or oral-oral routes. [2]. It can also cause a chronic low-level inflammation of the stomach lining, if not treated which leads to clinical impediments like peptic ulcer, gastric cancer and gastro-oesophageal diseases [3].

Numerous test methods are used to detect *H.pylori* infection including invasive tests such as histology, rapid urease test and culture, and non-invasive tests such as C-urea breath test (UBT) and serology (stool antigen and serum antibody) tests [4].Prevalence of infection is lower in developed countries than that of developing countries. In developed countries; although overall incidence of infection in young children is < 10%, up to 50% of children living in deprived socio-economic conditions are infected [5].*H. pylori* prevalence in children all over the world is diverse and dependent on many factors. Lower prevalence rates are reported in communities with higher socioeconomic status and generally better environmental conditions, while the highest percentage of infected children is observed in developing countries [6].

Mostly *H. pylori* infection occurred early childhood, when the body immune system is developing. *H. pylori* successfully establish a persistent infection in its host in spite of the presence of vigorous innate and adaptive immune response. Bacterial virulence factors together with host factors evolved an array of mechanisms to evade both innate and adaptive immune responses [7].Accordingly a Th-1-directed immune response, induced by *H. pylori* infection, increases gastric inflammation and atrophy, whereas Th-2 redirection reduces them. Different pathways are responsible for the predominant *H. pylori* -induced mucosal Th-1 response [8]. Stimulation of human neutrophils, monocytes, and dendritic cells with *H. pylori* neutrophil-activating protein (HP-NAP) strongly up regulates both IL-12 and IL-23 production, via TLR2 activation.

In the gastric mucosa of *H. pylori* infected patients, a considerable proportion of Th cells that are specific for different *H. pylori* antigens, including HP-NAP, CagA, urease, VacA, and heat shock proteins, and HP-NAP drives the production of high levels of IFN-γ and TNF-α by gastric Th cells, thus promoting a polarized Th-1 response [9, 10].*H. pylori* infection also influences the Th1/Th2 balance via effects on gastric hormones. When levels of somatostatin are reduced and gastrin production is increased, this also inhibits Th2 cytokine release and promotes Th1 responses [11].

Prevalence trends have showed that *H. Pylori* burden in developed countries is decreasing while the incidence of childhood allergic and autoimmune diseases is increasing[12].There have been recent suggestions that infection with *H.pylori* is "protective" against gastroesophageal reflux disease (GERD), oesophageal adenocarcinoma, and possibly some allergic illnesses, so its elimination might cause unexpected outcomes [13].Allergic diseases are among the most common chronic diseases in children and adolescents [14]. The World Allergy Organization (WAO) defines Atopy as a personal and/or familial tendency, usually in childhood or adolescence, to become sensitized and produce IgE antibodies in response to ordinary exposure to allergens, usually proteins. As a consequence, these persons can develop typical symptoms of asthma, rhino conjunctivitis, or eczema with abnormally increasing total IgE levels [15, 16].

Atopic disease comprises the triad asthma, hay fever and eczema (atopic dermatitis). All are common conditions in paediatric practice. Atopic dermatitis (AD) is a chronic, highly pruritic (itchy) inflammatory skin disease. In fact, AD is often the initial step in the "atopic march" (the sequential development of allergic disease manifestations during early childhood), which leads to asthma and/or allergic rhinitis in the majority of afflicted patients [17].Asthma is a chronic inflammatory condition of the lower airways characterised by recurrent wheezing, breathlessness and coughing [18].Hay fever is a chronic inflammatory condition of the upper airways that can be described by watery rhinorrhoea, nasal obstruction, nasal itching, and sneezing[19].Atopic dermatitis has a prevalence that ranges from 15- 30% in children and from 2% to 10% in adults in developed countries.

After the first two years of life the prevalence of AD decreases while the prevalence of asthma, allergic rhinitis, and sensitization to inhalant allergens increases. This shows that it is essential to assess the related risk factors at the first presentation of atopic disease in order to predict its progression in later years [17, 20].Epidemiological and experimental data now point to a strong protective effect of *H.pylori* infection on the development of many extra-gastric diseases, including gastroesophageal reflux disease and its associated outcomes childhood asthma and allergy [21].Data from the fourth National Health and Nutrition Examination Survey(NHANES) covering 7412 individuals (out of 8969 enrolled participants), that included not only adults but children and teenagers, indicated that acquiring *H.pylori* infection earlier in life was correlated with a lower occurrence of allergies and asthma[22].

## 1.2. Statement of the problem

*H.pylori* is one of the commonest bacterial pathogens affecting half of the world`s populations. About 70% of the people are asymptomatic, whereas some of the infected individuals may develop duodenal or gastric ulcer. Prevalence of the infection is diffent among developing and developed countries [5]. In developing countries the infection occurs at younger ages and the prevalence reached 70% to >90%, that is many times greater than the prevalence in developed countries[23].Its prevalence is highly variable in relation to geography, ethnicity, age, and socioeconomic factors – higher in developing countries and lower in the developed world [24]. Hygiene hypothesis says an infection acquired early in life may protect from, contribute to, or simply act as a bystander with respect to the development of an atopic condition in adult age [25].Nevertheless, many scholars do not accept the hygiene hypothesis as cause of allergic diseases, rather than defensive elements: due to their ability in increasing the mucosal porousness, these infections may assisted the infiltration of allergens [26].

Even though there is few data on allergy and allergens in Africa, the symptoms of asthma, allergic rhinitis, and atopic eczema in adolescents have been increasing over years in the Region. This leads the World Allergy Organization to recommend the dedicated involvement of the scientific community to cover the global allergy epidemic and its epidemiology in different regions. As a country Ethiopia has very few studies on effect of *H.pylori* on allergy and allergens in children [27]. Since our country is on the track of development our lifestyle and environmental conditions (like air pollution, temperature increase) are changing which paves a way to allergy predisposing factors that makes this study essential. Genetic conditions are one of contributing factor which influences the prevalence of the outcomes. Due to these reasons it is appropriate to assess association of *Helicobacter pylori* infection with atopy and allergic disorders.

## 1.3 Significance of the study

The study explores the relationship between *H.pylori* infection and prevalence of atopy and allergic conditions, thus the study may help to narrow the gap between conflicting studies on the possible impact of *H.pylori* infection on atopic children who are genetically exposed for development of allergic conditions and on children who already developed allergic diseases in different places. Since very few studies have been done in Ethiopia, the finding helps to better understand the interaction of *H. pylori* with atopy and allergic conditions; thus, it could be used for future guide in the development of national policy for *H.pylori* treatment.

# 2. Literature review

## 2.1. *H. pylori* and hygiene hypothesis

The hygiene hypothesis of atopic disease suggests that environmental changes in the industrialized world have led to reduced microbial contact at early age and thus result in the growing epidemic of atopic eczema, allergic rhinoconjunctivitis, and asthma. Even though the newborn‟s mucosal and systemic immune responses are immature, the modern infant may lack critical interactions with bacterial strains through TLRs and CD14 which promote the development of an anti-inflammatory, tolerogenic immune environment maintained by mediators such as TGF-and IL-10 [28].Epidemiological data from a cross-sectional survey showed that in subjects with active *H.pylori* infection the prevalence of asthma, eczema, allergic rhinitis is 30% lower than in *H. pylori*-negative subjects [25].

## 2.2. *H.pylori,* Atopy and allergy

In 2015, a systematic review and meta-analysis was done by Taye *et.al*. The analysis was done to assess if *H.Pylori* infection is inversely associated with atopy. From 732 potentially related literatures only 16 of the studies were selected by giving the provided data in required format for the analysis. The pooled result of the analysis put forward *H. pylori* infection is associated with an estimated 18% reduction in odds of atopy. Lastly the authors underline the outcomes did not differ according to the population age (adult or children), methodological quality or study design [27].

Additional Meta -analysis was conducted by Elane *et al*. in 2014 to assess the relationship between *H.pyloi* and atopic diseases by reviewing the existing literatures on *H.pylori* infection and atopy/allergic diseases. Pooled results of case-control studies showed a significant inverse association of *H. pylori* infection exclusively with atopy, in contrast the pooled results of the cross –sectional studies reveal that there is significant association between *H.pylori* infection and allergic diseases only. The overall results suggested that there is some evidence of inverse association between *H.pylori* infection and atopy/allergic diseases which leads them to recommend the need of further studies [26].

The impact of the *H.pylori* on atopic disorders in childhood was studied cross sectionally in 2012by Holster *et al*. The study involved 545 Dutch children and atopy and asthma were asses by using questionnaire. The study tried to compare the prevalence of *H.pylori* between children with or without allergic rhinitis but the result shows no significant association (8.5% vs. 9.5%), atopic dermatitis (8.7% vs. 9.2%), and physician-diagnosed asthma (7.1% vs. 9.4%).In conclusion, no association was found between *H.pylori* and allergic rhinitis, atopic dermatitis, or asthma insight of some potential limitations of the study like some of the results largely relied on self-reported data and also the laboratory diagnostic techniques were not separately validated in Dutch children specifically [29].

The prevalence of *H.pylori* infection in developing countries was assessed by Bardhan and the infection in developing countries seems to be nearly universal, beginning in early childhood. Children become infected in the first few months of life; in some communities as many as 50% of the children were infected by the age of 5 years, and up to 90% are infected by the time they reach adulthood. In some developing countries with improvements in industrialization, socioeconomic conditions, and hygiene, infection rates are lower [21].The spectrum of African diseases includes infections, nutritional deficiencies, allergies and natural disasters. Allergic disorders in Africa and Africans were investigated by Mbugi*et al*. The result demonstrated that incidence of allergy in Africa is comparable to other selected infections by accounting to 20-30% of the disease burden. The result suggested a more balanced priority approach on tackling diseases [30].

A longitudinal birth cohort study was done to assess exposure to *H.pylori* infection in early childhood and the risk of allergic disease and atopic sensitization in Butajira, Ethiopia by Amberbir *et.al*. A total of 863 children were followed. *H.pylori* infection at age 3 was significantly associated with a decreased risk of incident of eczema between ages 3 and 5 (adjusted OR, 95% CI, 0.31; 0.10–0.94, P = 0.02). Cross-sectionally at age 5, *H. pylori* infection was inversely associated with skin sensitization (adjusted OR, 95% CI, 0.26; 0.07–0.92, P = 0.02).These findings of the study added further evidence to suggest that early-life exposure to *H. pylori* may play a protective role in the development of allergy [31].

### 2.3. *H.pylori* and Asthma

Wang *et.al* conducted meta-analysis on association between *H.Pylori* infection and asthma risk in 2012. The study used five case-control studies which comprises 770 cases and 785 controls. The assessment was done by estimating the odds ratio (OR) of the risk asthma with

status of *H. pylori* infection. The overall data did not show a significant association between *H. pylori* infection and asthma risk (OR=1.01; 95%CI=0.82-1.24) also no association was observed in the analysis regarding ethnicity, source of controls and CagA status. The authors concluded that the pooled data failed to propose a marked association between *H. pylori* infection and asthma risk [32].

The correlation between Childhood Asthma and *H.pylori* Infection in Kashan was assessed by cross-sectional study which is done by Khamechian*et.al* in 2015. From a total of 300 children (5 to 18 years old), 138 of them were *H. pylori* positive, eight cases (5.8 %) were asthmatic while of the 162 *H. pylori* negative, 28 (17.3%) were asthmatic. This difference was statistically significant. The correlation between *H. pylori* and asthma was studied after controlling the confounding variables including, gender, age and family history. The results obtained for the above variables were significant showing that there is an inverse correlation between *H. pylori* and asthma [33].

The association between *H.pylori* colonization and childhood Asthma was assessed by Chen *et al*. in 2008.in a cross-sectional study, the data from 7412 participants in their National Health and Nutrition Examination Survey (NHANES) 1999–2000 were used. *H.Pylori* seropositivity was inversely associated with onset of asthma before 5 years of age and current asthma in children aged 3–13 years. Among participants 3–19 years of age, the presence of *H. pylori* was inversely related to ever having had asthma (odds ratio [OR], 0.69; 95% confidence interval [CI], 0.45–1.06), and the inverse association with onset of asthma before 5 years of age was stronger (OR, 0.58; 95% CI, 0.38–0.88). Among participants 3–13 years of age, *H. pylori* positivity was significantly inversely associated with current asthma (OR, 0.41; 95%CI, 0.24–0.69).*H. pylori* seropositivity also was inversely related to recent wheezing, allergic rhinitis, and dermatitis, eczema, or rash [34].

### 2.4. *H.pylori* and atopic dermatitis /eczema

In 2015, a study was done on *H.Pylori* infection and its potential role in childhood eczema by Ali *et al* involving 170 atopic cases and 80 controls. Of the 170 patients presenting with atopic dermatitis, 6 cases (3.5%) tested positive for serologic anti-*H. pylori* IgG antibodies and 12 cases (7.0%) tested positive *H pylori* Stool antigen; Of the 80 healthy controls, 12 cases (15.0%), 6cases (7.5.%) and 2cases (2.5%) were tested positive by serology, stool antigen and both Serology/Stool antigen tests, respectively.

The authors concluded that *H. pylori* infection is associated with childhood eczema in genetically predisposed atopic children. There was significant inverse association between atopic dermatitis and positive *H pylori* serologic testing [20].

Herbarth *et al* had done a research with a total of 2487 participants including a parent completed questionnaire on respiratory tract infections and a urea breathe test (UBT) result. The study was done to determine the effect of *H.pylori* colonisation on eczema. Prevalence rates related to 2487 children in this age group (mean age 6.3 years) were for eczema 16.9%, for *H pylori* colonisation 6.1%, for bronchitis 30.2% and for frequent colds23.4%. In all, 33.5% of the parents had allergic disorders, 16.4%were unemployed. Summarising, this analysis presented in a preschool population that a bacterial infection/colonisation of the gastro-intestinal tract may have a protective effect against eczema up to the age of school entry (6–7 years) [35].

*H. pylori* infection and it is potential role in childhood eczema was assessed Ali*et al*. in 2015.children 2 months to 7 years old were part of this case-control study. Of the 170 patients with atopic dermatitis, 6 cases (3.5%) were positive for anti-H. pylori IgG antibodies and 12 cases (7.0%) were positive for Stool antigen; Of the 80 healthy controls, 12 cases (15.0%), 6cases (7.5.%) and 2case (2.5%) were tested positive by serology, stool antigen and both Serology/Stool antigen tests respectively. (For serology testing, Odds Ratio = 0.2073, 95% CI =0.0748 - 0.5749 P value =0.0025).In heritably predisposed atopic children, *H.pylori* infection is associated with childhood eczema [36].

Since most studies reviewing the association between *H. pylori* infection, atopy and allergic disease have been using different study design the results were also conflicting. Inconsistency of the results might be caused by variation of the prevalence of *H.pylori* and atopy among developed and developing countries. Although it has been hypothpothesized that infections may play a protective role in allergic diseases, the role of *H.Pylori* is not clear. In this study we tried to define the link between *H.pylori* infection and allergic conditions. Though studies particularly those from low income countries, are remarkably limited, only few studies reported links in young children.

# 3. Objective

## 3.1. General objective

To assess the association of *Helicobacter pylori* infection with atopy and allergic disorders in Ziway, Central Ethiopia, from May 2016 to May 2017

## 3.2. Specific objectives

- To determine prevalence of *H.pylori* infection among children using stool antigen and serum antibody tests
- To assess the association between *H.pylori* infection, atopy, and allergic symptoms
- To compare total serum IgE, and peripheral eosinophil counts among *H.pylori* infected and non-infected children
- To assess the association between atopy, and allergic symptoms
- To assess associated risk factors of atopy and allergic symptoms in the study group

# 4. Hypothesis

- There is association of *H.pylori* infection with atopy and allergic symptoms among young children
- There is association between different factors and atopy and allergic symptoms among young children

# 5. Methods and materials

## 5.1. Study area

This study is done in Batu (Ziway) town, which is located in Oromia National Regional State, in East Shoa zone, Adami Tulu Jiddo Woreda, at a distance of 160 Km from Addis Ababa. Its astronomical location is 7º 56‟ North Latitude and 38º 43‟ East Longitude with an elevation of 1643 meters above sea level. Batu is one of the reform towns in the region and has a town administration, municipality and two kebelles. The city also has governmental and private health facilities; from them we use Batu hospital and the only private Sher hospital. The average number of patient flow under 15 years of age per day in Sher and Batu hospital was 35 and 23 respectively. The city also has both government and private schools of which 14 are primary schools from them Sher and Batu primary schools were included.

## 5.2. Study design

A cross sectional study design was used.

## 5.3. Study period

The study was conducted from May 2016 to October 2016 at Ziway, Ethiopia.

## 5.4. Population

### 5.4.1. Source population

Young children attending the selected schools and health facilities during the study period

### 5.4.2. Study population

Children (2-14 years) who fulfilled the selection criteria

## 5.5. Eligibility

### 5.5.1. Inclusion criteria

- ✓ Young children (2-14 years old)
- ✓ Willingness of the participants

### 5.5.2. Exclusion criteria

- ✓ Children who were taking antibiotics (like peptobismol and proton pump inhibitors Nexium or Prilosec) for the last one month.
- ✓ Children who were taking second generation antihistamines and antidepressants such as doxepin, other tricyclics, and tetracyclics which have antihistamine activity and may need to be withheld for 1-2 weeks or more.

## 5.6. Study variables

### 5.6.1. Dependent variables

- allergy symptoms
- Atopic status
- Total IgE profile
- Absolute eosinophil count
- Prevalence of *H.pylori* infection

### 5.6.2. Independent variable

- Socio demographic characteristics (sex, age ,area of residence ,maternal education)
- Familial factors (maternal and paternal history of allergy)
- Childhood factors (immunization, breastfeeding till year 3, number of siblings, child‟s use of antibiotic)
- Household characteristics (roof type, household size and child‟s sleeping place)
- Environmental factors (indoor cooking, indoor kerosene use, and insecticide use)

## 5.7. Sample size calculation and sampling technique

### 5.7.1. Sample size calculation

The minimum required sample size to determine a single population proportion was calculated using the following formula.

$$d = Z_{\frac{\alpha}{2}} SE$$

$$= Z_{\frac{\alpha}{2}} \sqrt{\frac{P(1-P)}{n}}$$

$$\Rightarrow \sqrt{n} = Z_{\frac{\alpha}{2}} \frac{\sqrt{P(1-P)}}{d}$$

$$\Rightarrow \boxed{n = Z_{\frac{\alpha}{2}}^{2} \frac{P(1-P)}{d^2}}$$

where;

n is the sample size to be determined, $Za/_2$ is 1.96

p =50% d= 0.05

$$n = 1.96^2 \times \frac{0.5\,(1-0.5)}{(0.05)^2}$$

$$n = \frac{(1.96)^2 \times 0.25}{0.0025}$$

n = 384 Even though our sample size is 384 we recruited 461 participants to increase the power of the study.

### 5.7.2. Sampling technique

Convenient sampling technique was used to include a minimum of 384 children.

## 5.8. Data collection and processing

**Questionnaire based data collection:**

Information like socio-demographic characteristics together with child‟s sex, maternal education was collected by using questionnaire after informed consents were signed by the participants/parents/ guardians. The questionnaire was based on International Study of Asthma and Allergies in Children (ISAAC) included information about wheeze (in the last 12 months),asthma (IN the last 12 months), hay fever (history of problems with sneezing or running nose (when not affected by cold or flu), or problems with itchy watery eyes in the last 12 months) and eczema ( having an itchy skin rash which has affected the skin creases, e.g. front of the elbow, behind the knees, the front of the ankles, around the neck, or around the eyes in the last 12 months).Other questions were also included on the questionnaire about other potential confounders comprising familial factors (maternal and paternal history of asthma and allergy); childhood factors (immunization, breastfeeding status, number of siblings, child‟s use of antibiotic); household characteristics (roof type, household size and child‟s sleeping place and materials); and environmental factors (indoor cooking, kerosene use, and insecticide use).

**Laboratory data collection**

Skin-prick test to *Dermatophagoides pteronyssinusin* and cockroach allergen (immunotek) was done by using skin-prick lancets.Blood and stool sample: venous blood was collected with ethylene diaminetertacetic acid (EDTA) vacutainer tube using multisample needles and stool samples were collected by clean, leak proof and wide-mouthed standard caps. The past and current *H.pylori* statuses were defined by *H.pylori* serum/blood Antibody test kit and *H.pylori* stool antigen test kits, respectively and ELISA technique was used to detect the total IgE from the samples. MINDRAY BC-3000Plus automated haematology analyser was used to determine haematological parameters and thin blood films were prepared to do manual eosinophil count using wright stain .

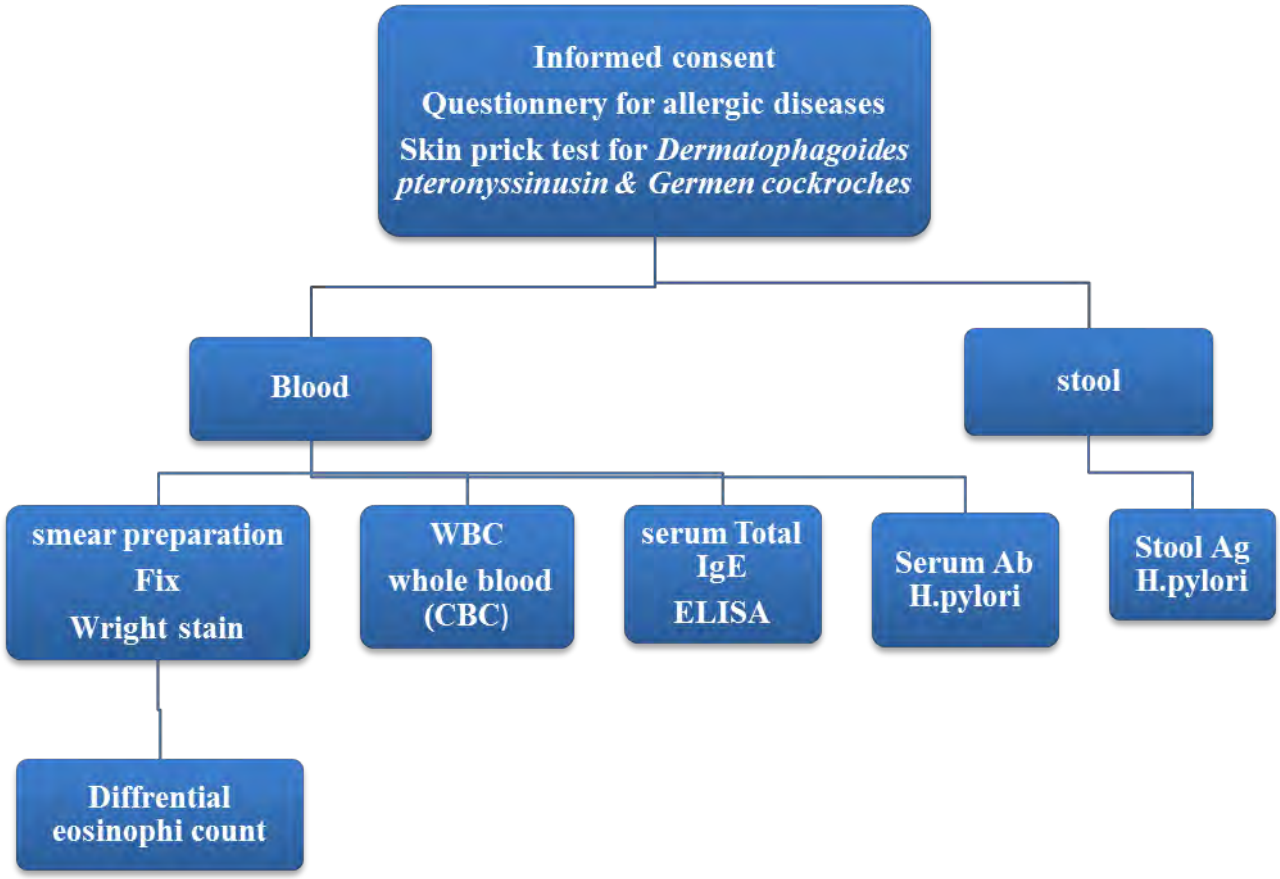



**Figure.1** Data collection procedure and analysis

### 5.8.2. Skin Prick Test (SPT)

Skin prick testing provides information about the presence of specific IgE to protein and peptide antigens (allergens). Small amounts of allergen are introduced into the epidermis and non-vascular superficial dermis and interact with specific IgE bound to cutaneous mast cells. Histamine and other mediators are released, leading to a visible "wheal-and-flare" reaction peaking after about 15 minutes [37]. Frequently used site for skin prick test is volar surface of forearm then there will be marks (with the initial letters of each allergens being tested) on the skin then place one drop of each allergen solutions on the marked places and prick and wait for 15 minutes. Positivity of the test result was defined by a wheal of 3mm greater than the negative control [31].

### 5.8.3. Serum Total IgE

The Diagnostic IgE Quantitative Test is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The test specimen (serum) is added to the IgE monoclonal antibodies immobilized on polystyrene microtiter wells (solid phase) and incubated with the Zero Buffer. If human IgE is present in the specimen, it will combine with the antibodies on the well. The well is then washed to remove any residual test specimen, and goat anti-IgE in the antibody-enzyme (horseradish peroxidase) conjugate reagent is added. The conjugate reagent will bind immunologically to the IgE on the well, resulting in the IgE molecules being sandwiched between the solid phase and the enzyme-linked antibodies. After incubation at room temperature, the solid phase is washed with water to remove unbound labelled antibody. A solution of 3, 3‟, 5, 5‟-Tetramethylbenzidine (TMB) is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped and the resulting yellow color is measured spectrophotometrically at 450 nm. The concentration of IgE is directly proportional to the color intensity of the test sample. The test was done by following the manufacturer instruction as detailed on the annex

### 5.8.4. *H.pylori* stool Antigen rapid test

The *H.pylori* Ag Rapid test is used to detect current *H.pylori* infection by using a sandwich lateral flow chromatographic immunoassay. When an adequate volume of extracted faecal specimen is dispensed into the sample well of the test cassette, the specimen migrates by capillary action across the cassette. *H.pylori* antigens if present in the specimen will bind to the anti-*H.pylori* conjugates. The immunocomplex is then captured on the membrane by the

pre-coated antibody, forming a burgundy coloured T line, indicating *H.pylori* positive test result. Absence of the T line suggests that the concentration of H. pylori antigens in the specimen is below the detectable level .The test was done following the manufacturer instruction as detailed on the annex

### 5.8.5. *H.pylori* Antibody rapid test

The *H.pylori* Ab Rapid test is used to detect any past or current *H.pylori* infection; however the results do not distinguish between the two.A sandwich lateral flow chromatographic immunoassay principle was used. When an adequate volume of extracted specimen serum/plasma/whole blood is dispensed into the sample well of the test cassette, the specimen migrates by capillary action across the cassette. The specimen will bind to the anti-*H.pylori* conjugates if antibodies (IgG, IgM or IgA) present. The immunocomplex is then captured on the membrane by the pre-coated *H.pylori* antigens, forming a burgundy coloured T line, indicating *H.pylori* antibody positive test result. Absence of the T line suggests a negative test result. The test was done by following the manufacturer instruction as detailed on the annex.

### 5.8.6. Principle of MINDRAY BC-3000 Plus hematology analyzer

MINDRAY BC-3000 Plus is a 3-part differential haematology analyzer which accurately counts and sizes cells by detecting and measuring changes in electrical resistance when a particle (such as a cell) in a conductive liquid passes through a small aperture. Each cell suspended in a conductive liquid (diluent) acts as an insulator. As each cell goes through the aperture, it momentarily increases the resistance of the electrical path between the submerged electrodes on either side of the aperture. This causes a measurable electronic pulse. For counting, the vacuum used to pull the diluted suspension of cells through the aperture must be at a regulated volume. The number of pulses correlates to the number of particles. The height of the electrical pulse is proportional to the cell volume.

### 5.8.7. Differential white cell count

A differential white cell count provides information on about the different white cells present in the circulating blood, i.e. neutrophils, lymphocytes, monocytes, eosinophils, basophils (rarely seen). Providing the total WBC count is known, the absolute number of each white cell type, i.e. number of each cell per litre of blood, can be calculated and an assessment made of whether the number of a particular cell type is increased or decreased (compared with the accepted reference range. Thus, the absolute eosinophil count was computed from percent eosinophil obtained from the manual differential count multiplied by total WBC count.

## 5.9. Statistical Analysis

Data were double checked and SPSS version 20 software was used for statistical analysis. The data were cleaned, coded and merged to be ready for analysis. Mean and standard deviation (SD) and simple frequencies were done to show the distribution of the socio-demographic and clinical characteristics of the participants. As the prevalence of *cockroach and D.pteronyssinus* allergen was low to analyse separately, the combined variable „any sensitization" was made to state to sensitization to either *D. pteronyssinus* or *cockroach* allergen. The prevalence of wheeze, eczema, hay fever and asthma in the past 12 months and sensitization to *D.pteronyssinus* and cockroach allergen was calculated. The association of early and current effect of *H.pylori* on each outcome was determined by computing crude odds ratios with 95% confidence intervals. Non parametric independent samples test was used to compare the medians across groups to assess the exposure and outcome of the study in relation to total IgE and peripheral eosinophil count. Crude odds ratio was used to determine the distribution and association of potential confounders. P- Value < 0.05 was considered as statistically significant.

## 5.10. Data Quality Assurance

Standard operating procedure (SOP) was followed during pre-analytical, analytical, and post analytical phases of the study.

### 5.10.1. Pre-analytical data quality assurance

- ✓ An interviewer administered questionnaire which included (the Socio-demographic and symptoms related to allergic diseases) was collected by the principal investigators.
- ✓ Skin prick test was performed by principal investigators under supervision of a senior advisor.
- ✓ Venous blood and stool specimens collected from participants were properly labelled and processed on time.
- ✓ All reagents were checked for their expiry date and prepared and handled according to manufacturer"s instructions

### 5.10.2. Analytical data quality assurance

- ✓ Appropriate reagents and Standard protocols were used for each laboratory tests.
- ✓ MINDRAY BC-3000Plus haematological analyser was checked for its performance using quality control materials

### 5.10.3. Post- analytical data quality assurance

- ✓ The data were rechecked on daily basis.
- ✓ Each laboratory test results were recorded and documented properly

## 5.11. Ethical consideration

Ethical clearance was obtained from departmental research and ethics review committee of the department of medical laboratory science of Addis Ababa University, College of Health Sciences. The proposal was also reviewed by ethical review board of Oromia Health Bureau. Written informed consent (signed or thumb print) was acquired from each parent/guardian and assent from their children aged 12-14 years. Confidential identifiers were used to code participant‟s identities.Participants were informed of their right to refuse to participate in the study or to withdraw at any time from the study. In addition invasive procedures, though minimal, were fully explained. Results and any information regarding patients were kept confidential during and after the completion of the research project by password protected electronic and locked hard copy files. We tried to make promising condition to get drug from pharmacies for *H.pylori* antigen test positive children.

# 6. Results

## 6.1. Socio demographic characteristics

A total of 461 eligible study participants were recruited from four sites: Batu Hospital (10, 2.2%), Sher Hospital (103, 22.3%), Sher Elementary School (160, 34.7%) and Batu Elementary School (188, 40.8%). Just over half were female (50.8%) and majority (96.1%) were from urban areas. The mean age was 8.8 +/-2.96 (the age range being between 2 and 14 years) (97.9%) were vaccinated. About 57.9% of the participants‟ mothers‟ have formal education and 43.8% are housewives.Table1 showed the detailed characteristics of the study participants.

**Table1: Socio demographic characteristics of young children, Ziway, Ethiopia, 2016**

| Variables | | Number | Percent |
|---|---|---|---|
| **Sex(N=461)** | Male | 227 | 49.2 |
| | Female | 234 | 50.8 |
| **Age*(N=458)** | <5 | 37 | 8.1 |
| | 5-9 | 229 | 50.0 |
| | 10-14 | 192 | 41.9 |
| **Residence(N=461)** | Rural | 18 | 3.9 |
| | Urban | 443 | 96.1 |
| **Maternal education (N=461)** | Formal | 267 | 57.9 |
| | None | 194 | 42.1 |
| **Maternal occupation (N=461)** | Farming and related | 92 | 20.0 |
| | Trading and related | 73 | 15.8 |
| | Government employee | 12 | 2.6 |
| | Housewife | 202 | 43.8 |
| | Others | 86 | 18.7 |
| **Vaccination history (N=461)** | Never been vaccinated | 10 | 2..2 |
| | Vaccinated | 451 | 97.8 |

*Ethiopia mini demographic and health survey 2014.Center of Statistical Agency. Addis Ababa, Ethiopia.2014.Age was categorized according to the EDHS classification.

## 6.2. Prevalence of *H.pylori* infection

Past and current *H.pylori* infection among the study participants was determined using a rapid *H. pylori* serum/blood antibody and *H.pylori* stool antigen tests, respectively. As displayed in Table 2, the prevalence was 70.3 % and 5.2% for the antibody and antigen *H.pyloi* infection respectively and only 18 participants were positive for both tests.

**Table 2: Frequency of *H.pylori* infection among young children, Ziway, Ethiopia, 2016**

| | **Number** | **Percent** |
|---|---|---|
| ***H.pylori* positive with antigen test** | 23/ 445 | 5.2 |
| ***H.pylori* positive with antibody test** | 296/421 | 70.3 |
| **Positive for both tests** | 18/421 | 4.3 |

### 6.3. Allergic sensitization and self-reported allergic symptoms

Of 454 participants who were tested for skin prick test for the two allergens, 1.1% were sensitized to house dust mites (*Dermatophagoides pteronyssinus)* and 1.5% were sensitive for German cockroach (*Blattella germanica*). Sensitization to either of the allergens was 2.6%. A single participant was sensitive to both allergens. Based on self-reported allergic outcomes for the last 12 months, 3.7% of the children had wheeze, 2.2% had asthma, 13.2% had eczema and 6.9% had hay fever. Only 5 participants‟ asthmatic status was confirmed by a doctor (Table 3)

**Table 3: Self-reported allergic symptoms and skin sensitization test results of young children, Ziway, Ethiopia, 2016**

| Variables | Number | percent |
|---|---|---|
| **Self-reported allergic symptoms (N=461)** | | |
| **Wheeze** | 17 | 3.7 |
| **Asthma** | 10 | 2.2 |
| **Hay fever** | 32 | 6.9 |
| **Eczema** | 61 | 13.2 |
| **∞Any allergy symptoms** | 94 | 20.4 |
| **Skin Prick Test (N=454)** | | |
| **HDM (*Dermatophagoides pteronyssinus)**** | 5 | 1.1 |
| **German cockroach (*Blattella germanica*)** | 7 | 1.5 |
| **Sensitization to both allergens** | 1 | 0.22 |
| **Any sensitization** | 11 | 2.4 |
| **Both atopy and allergy** | 5 | 1.1 |

*HDM – house dust mite

∞ Any allergic symptoms was defined as a positive response to one or more of asthma, hay fever and eczema in the past 12 months

## 6.4. Association of *H. pylori* infection with atopic sensitization andallergic diseases

The exposure variables of past and current *H.pylori* infection and the outcomes like any sensitivity (positive to either *D. pteronyssinus* or cockroach allergen) and any allergic conditions (a positive response to one or more of asthma, hay fever and eczema in the past 12 months) among the children did not show statistically significant association. However, those with negative test result for past infection are 1.58 times more likely to have atopic sensitization with (OR 1.58, 95% CI 0.44, 5.70). Those negative for current infection were 1.81 times more likely to have allergic conditions, though it was not stastically significant with (OR 1.81, 95% CI 0.53, 6.22) as it is summarized on tables 4 and 5.

**Table 4: Associations between *H.pylori* infection and any sensitivity to allergens in young children, Ziway, Ethiopia, 2016**

| Variables | Any sensitivity [φ] | | | Crude OR (95 % CI) | P-value |
|---|---|---|---|---|---|
| | Overall N (%) | Yes n (%) | No n (%) | | |
| **Past *H.pylori* Infection(Ab test)** | | | | | |
| **Negative** | 125(29.7) | 4(3.2) | 121 (96.8) | 1.58(0.44,5.70) | 0.48 |
| **Positive** | 296(70.3) | 6(2.0) | 287(98.0.) | 1 | |
| **Current *H.pylori* Infection(Ag test)** | | | | | |
| **Negative** | 420(94.8) | 10(2.4) | 410 (97.6) | 0(0) | 1.0 |
| **Positive** | 23 (5.2) | 0 (0) | 23 (100.0) | 1 | |

[φ]Any sensitivity was defined as positivityto either *D. pteronyssinus* or cockroach allergen

**Table 5: Associations between *H.pylori* infection and Allergic conditions in young children, Ziway, Ethiopia, 2016**

| Variables | Any allergic conditions[¥] | | | Crude OR (95 % CI) | P-value |
|---|---|---|---|---|---|
| | Overall N (%) | Yes n (%) | No n (%) | | |
| **Past *H.pylori* infection(Ab test)** | | | | | |
| **Negative** | 125(29.7) | 24 (19.2) | 101(80.8) | 0.96(0.56,1.62) | 0.86 |
| **Positive** | 296(70.3) | 59 (19.9) | 237(80.1) | 1 | |
| **Current *H.pylori* infection(Ag test)** | | | | | |
| **Negative** | 422(94.8) | 90 (21.3) | 332(78.8) | 1.81(0.53,6.22) | 0.35 |
| **Positive** | 23 (5.2) | 3 (13.0) | 20 (87.0) | 1 | |

[¥]'Any allergic condition was defined as a positive response to one or more of asthma, hay fever and eczema in the past 12 months

## 6.5. Association between atopy and allergy symptoms

As shown in Table 6, almost significant association was found between atopy and any allergy conditions. Individuals with a positive SPT response to any of the two tested allergens were more likely to have any allergy symptom [OR 3.32 (95% CI: 0.99, 11.1), P = 0.052].

**Table 6: Associations between atopy and Allergic conditions in young children, Ziway, Ethiopia, 2016**

| | | Allergy symptoms | | | | |
|---|---|---|---|---|---|---|
| **Variable** | | Overall N | Yes n(%) | No n(%) | Crude OR (95 % CI) | P-value |
| **Atopy** | Yes | 11 | 5 (45.5%) | 6 (54.5%) | 3.32 (0.99, 11.1) | 0.052 |
| | No | 443 | 89 (20.1%) | 354 (79.9%) | | |

### 6.6. Distribution of Total IgE and Eosinophil among the different groups

Since there were outliers in eosinophil distribution, independent sample median test was used to ensure if there was a significant median difference of among the different *H.pylori*/allergy and *H.pylori*/atopy groups. The only significant total IgE concentrations distribution median difference was seen among current *H.pylori*/allergy groups. Multiple comparisons were not possible to assess the significant difference across variables since more than 20% of the cells have expected values less than 5.The rest groups did not show significant median difference.

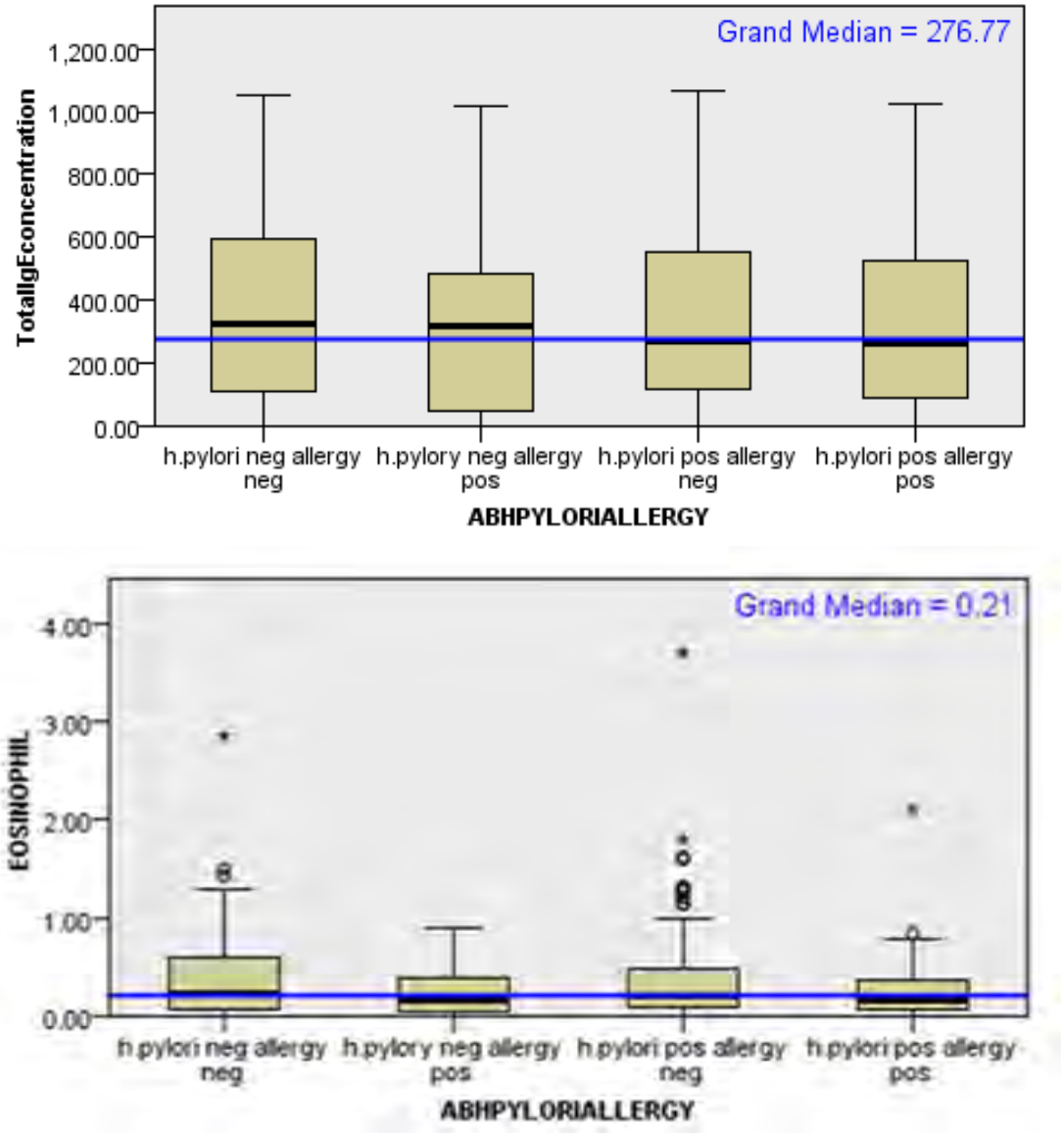
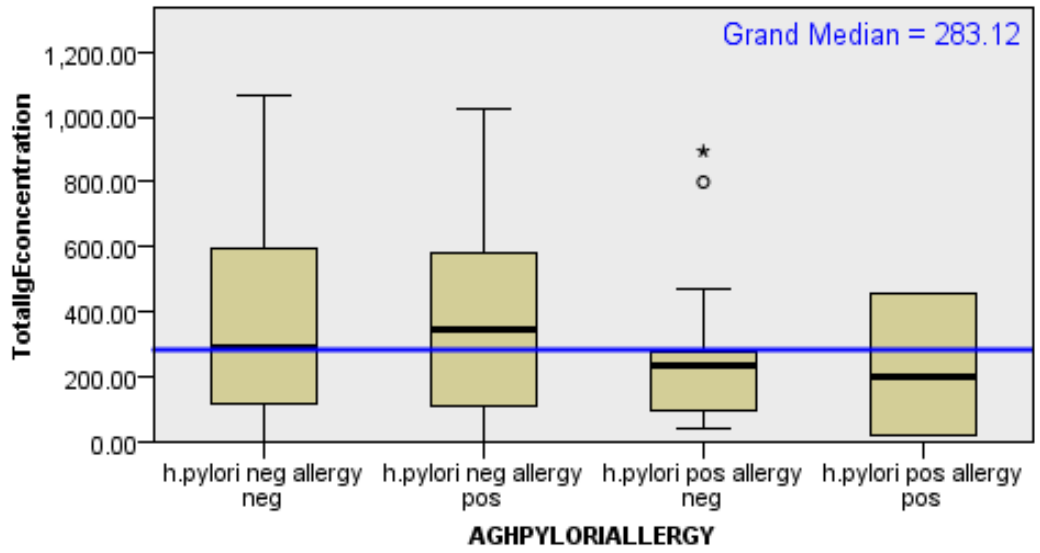



**Figure 1**: Distribution of Total IgE and absolute esosinophil count among different past *H.pylori* allergy groups Ziway, Ethiopia, 2016.



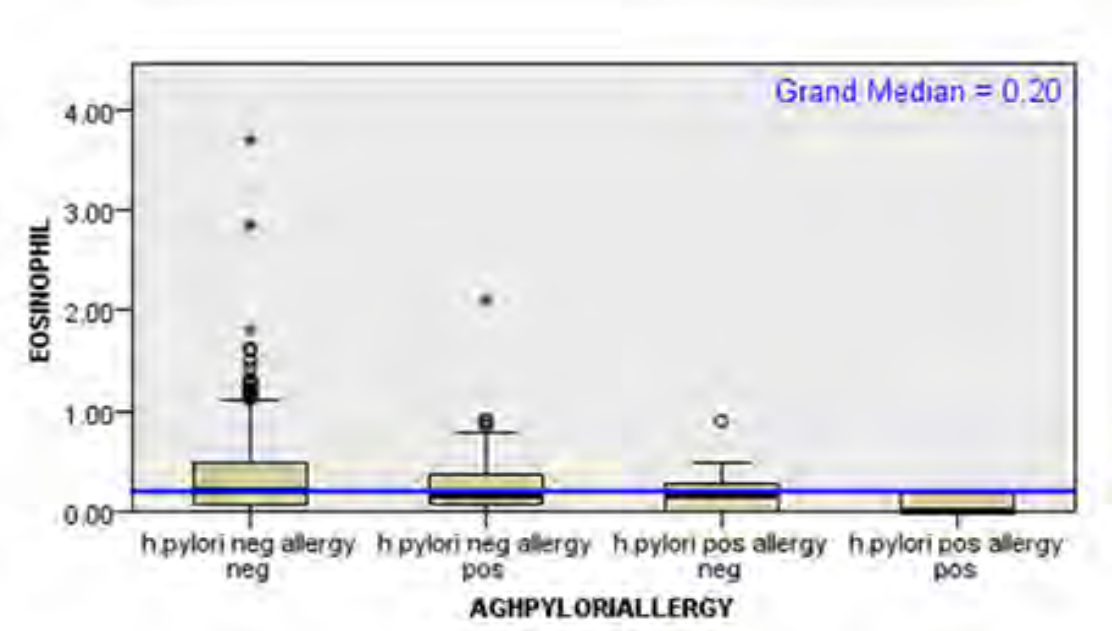




**Figure 2:** Distribution of Total IgE and absolute eosinophil count among different current *H.pylori* allergy groups Ziway, Ethiopia, 2016.

Distribution of absolute eosinophil count among current *H.pylori*/atopy groups showed significant median difference among the groups. Multiple comparison was not possible to assess the significant difference across samples because more than 20% of the cells have expected values less than 5. Other groups did not show significant median difference.

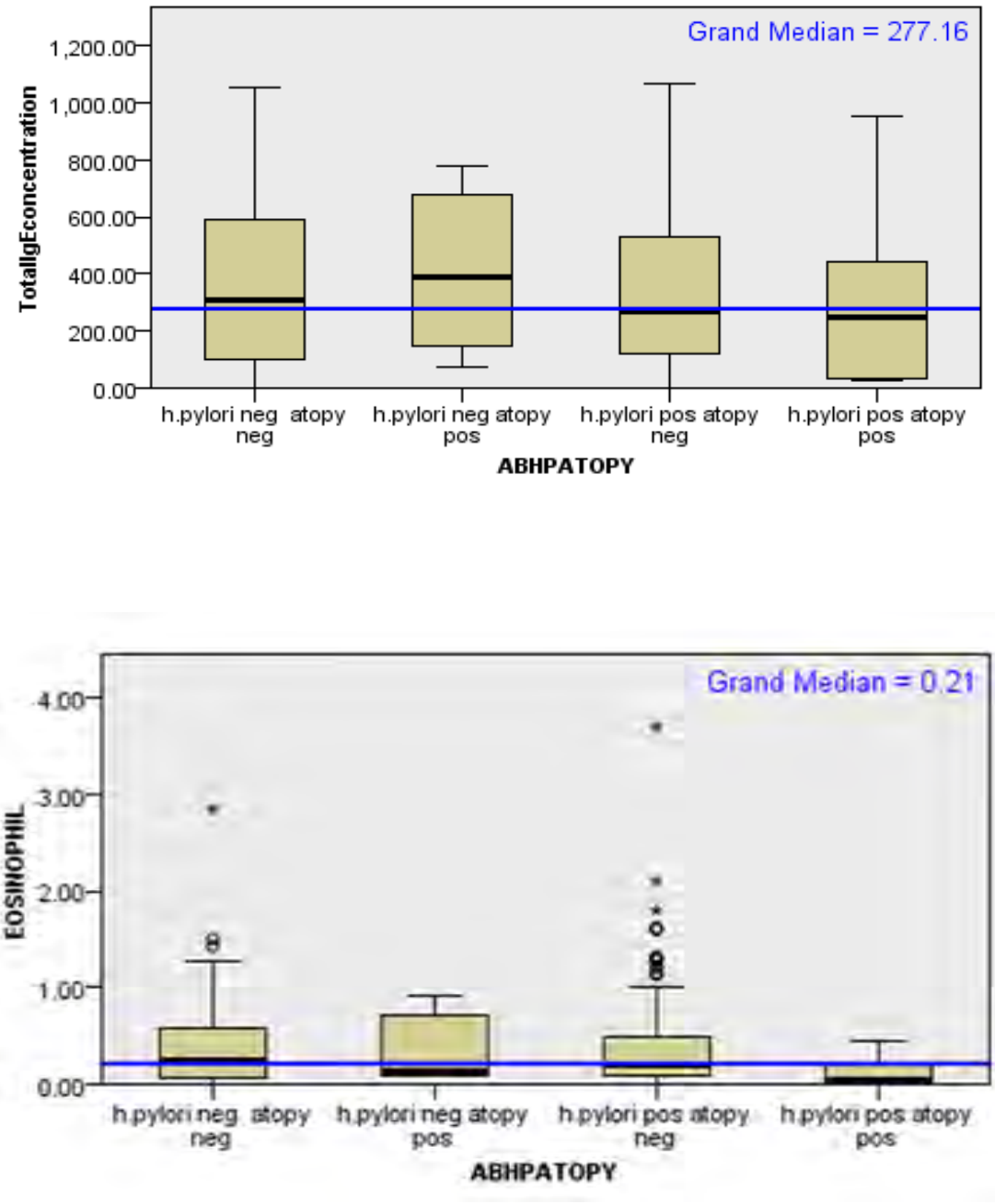
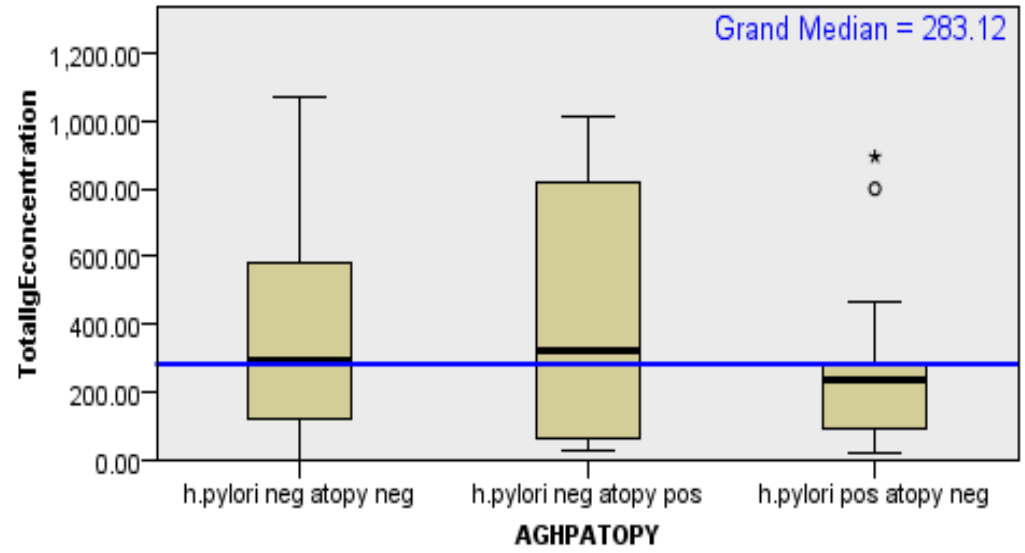



**Figure 3:** Distribution of Total IgE and absolute esosinophil count among different past *H.pylori* atopic groups Ziway, Ethiopia, 2016.



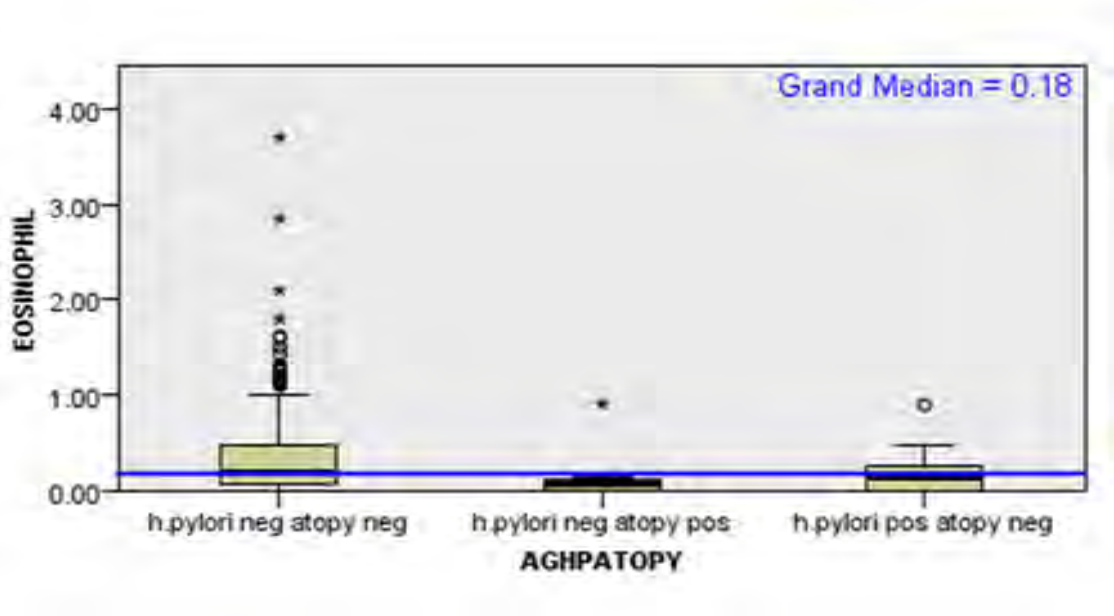




**Figure 4:** Distribution of Total IgE and absolute esosinophil count among different current *H.pylori* atopic groups Ziway, Ethiopia, 2016.

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## 6.7. Potential confounders of Atopy and Allergic conditions

Association between exposure variables and outcomes (any sensitivity and allergic conditions) was measured by computing odds ratios with 95% confidence intervals (Table 7). Our result showed no significant association between outcome variables and gender or status of maternal education among the study group. Keeping animals in the house was 3.72 times increased the possibility to develop atopy [OR=3.72(95%CI=1.06, 13.1) P=0.041].In addition both maternal and paternal history of allergy showed increased risk of developing allergic symptoms with [OR=3.22 (95% CI, 1.62, 6.41) P=0.001] and [OR=3.90(95% CI, 1.98, 7.68) P=0.000] respectively. Surprisingly, our result showed using charcoal everyday as a source of fuel decreases the risk of developing allergic symptoms [OR=0.50 (95% CI, 0.29, 0.84) P=0.009].Multivariate analysis was done for variables with p-value less than 0.2 with allergic symptoms. From them maternal and paternal history of allergy increased the risk to develop any allergy symptoms by 1.97 and 2.89 times respectively with [adjusted OR=1.97 (95%CI=0.89, 4.38) P=0.094], [adjusted OR=2.89 (95%CI=1.34, 6.24) P=0.007] (Table 8).

**Table 7: Association of potential confounders with Atopy and allergic outcomes of young children, Ziway, Ethiopia, 2016***

| Confounding variables | n% | Any sensitization OR (95 % CI) | P-value | Any allergy symptoms OR (95 % CI) | P-value |
|---|---|---|---|---|---|
| **Maternal education (formal vs. none)** | 267(57.9) | 1.27 (0.37,4.39) | 0.709 | 1.29 (0.81,2.10) | 0.287 |
| **Maternal allergic history** | 38 (8.3) | 1.09 (0.14,8.77) | 0.93 | 3.22(1.62,6.41)* | 0.001 |
| **Paternal allergic history** | 39 (8.5) | 2.5(0.52,12.01) | 0.253 | 3.90(2.00,7.70)* | 0.000 |
| **Breast fed till age 3** | 262(64.5) | 0.56 (0.39,5.70) | 0.562 | 1.24(0.73,2.09) | 0.429 |
| **Older siblings N=455** | | | | | |
| **0** | 174(38.2) | 1.23(0.14,10.79) | 0.853 | 1.90 (0.75,4.82) | 0.176 |
| **1-3** | 238(52.3) | 0.89 (0.10,7.86) | 0.92 | 1.36 (0.54,3.43) | 0.514 |
| **4-10** | 43 (9.5) | 1 | | 1 | |
| **Proper latrine N=460** | | | | | |
| **None, bush ,filed** | 6 ( 1.3) | 1 | | 1 | |
| **Traditional pit** | 430(93.5) | --- | --- | 3.00(0.30,29.94) | 0.349 |
| **Flush toilet** | 24 (5.2) | --- | --- | 1.22(0.14,10.53) | 0.86 |
| **Animals kept in the house** | 63(13.7) | 3.72(1.06,13.1)* | 0.041 | 1.26(0.67,2.37) | 0.469 |
| **Smoking in the residential** | 434(95.6) | --- | | 1.37(0.48,3.87) | 0.554 |
| **History of vaccination** | 451(97.8) | --- | | 0.43(0.05,3.42) | 0.428 |
| **Charcoal fuel use** | | | | | |
| **Never** | 95(20.6) | 1 | | 1 | |
| **Sometimes** | 50(10.8) | 1.23 (0.21,7.90) | 0.793 | 0.43 (0.18,1.03) | 0.061 |
| **everyday** | 316(68.5) | 0.59 (0.14,2.39) | 0.459 | 0.50(0.30,0.84)* | 0.009 |
| **Insecticide use** | 316(68.5) | 1.26 (0.36,4.39) | 0.713 | 1.47 (0.92,2.36) | 0.11 |
| **Helminthic infection** | 391(84.8) | 0.52 (0.11,2.50) | 0.443 | 1.56 (0.71,3.44) | 0.268 |

Statistically significant (P, 0.05)

**Table 8: Multivariate analysis for confounding variables with allergic symptoms, Ziway, Ethiopia, 2016**

| Variables | N % | Adjusted OR | CI of Adjusted OR | P-value |
|---|---|---|---|---|
| **Maternal history of allergy** | 38(8.3) | 1.97 | 0.89,4.38 | 0.094 |
| **Paternal history of allergy** | 39(8.5) | 2.89 | 1.34,6.24 | 0.007 |
| **Charcoal fuel use** | | | | |
| **Never** | 95(20.6) | 1 | | |
| **Sometimes** | 50(10.8) | --- | --- | --- |
| **Everyday** | 316(68.5) | --- | --- | --- |
| **Insecticide use** | 316(68.5) | --- | --- | --- |

ORs adjusted for maternal and paternal history of allergy, charcoal use and insecticide use with P-value less than 0.2

# 7. Discussion

Very few studies are conducted on association of *H.pylori* infection with atopy and allergy among paediatric groups. In this cross-sectional study we tried to asses if there is association between past and current *H.pylori* infection and atopic sensitization and allergic diseases, even though it was not statistically significant.

## 7.1. Prevalence of *H.pylori* infection

The prevalence of past *H. Pylori* infection obtained in this study (70.3 %) is lower than the reported *H.pylori* prevalence by Bacciogu *et.al* from Turkey [38] which was 83%. In contrast, our result was greater than other study findings done by Holster *et.al* on Dutch children [29] which is 9%. The difference might be due to difference in socio-economic characteristics, location of study setting and study design used. The prevalence of the current *H.pylori* infection was 5.2% which is lower than earlier findings from Ethiopia using stool antigen test kit 25%, 41%, respectively [31, 40]. The difference might be due to the longitudinal cohort study designs and large sample size in those studies. The prevalence of *H. pylori* infection in childhood could be influenced by environmental factors both inside and outside the home even within the same geographic area. In addition, the differences could be due to age difference in the study group where children in the current study were older than those in the earlier studies [39].

## 7.2 Prevalence of atopy and allergic conditions

The prevalence of allergic conditions was 3.7 %, wheeze, 2.2. %, asthma, 13.2 %, eczema 13.2%, and 6.9 % hay fever which was defined by using ISAAC questionnaire was greater compared to other study done by Amberber *et.al* except our report on wheezing which is lower compared to that reported by Amberbir *et al* which was 3.7%. Allergen skin sensitizationto either *D. pteronyssinus*or cockroach allergen was 2.4% which was in line with finding from other studies by Amberber *et.al* which reported 2.0%. On the contrary, 8.7% of the children in the study by Amberbir *et al* showed sensitization to any allergen which is greater than our study results [31, 40]. The difference might be due to study design and in the first few years of life when the development of the immune system is taking place, the child could be susceptible for atopic pathway due to different environmental and genetic factors [41]. In addition, study group age difference and seasonal difference of data collection period could also be the possible reasons [42, 43].

### 7.3. Association between *H.pylori* infection, atopy and allergic conditions

Our result showed no statistically significant association between past and current *H.pylori* infection and the outcomes any sensitivity (positive to either *D. pteronyssinus* or cockroach allergen), any allergic conditions (a positive response to one or more of asthma, hay fever and eczema in the past 12 months). Baccioglu *et.al* [38] and Holster *et.al* [29] supported our finding by reporting the absence of significant association between *H.pylori* infection and atopy or allergic conditions among the study group. In contrast to these findings, a study by Amberber *et.al* [40] which was done in Ethiopia showed the protective effect of *H.pylori* infection from allergic conditions; another study by Amberber *et.al* [31] specifically showed the protective effect of past *H.pylori* infection among the participants which agrees with our result which indicated decreased risk of atopic sensitization with past infection of *H.pylori*. The conflicting results could be due to differences in the study designs, geographical location of the study groups and the results can also be affected with ethnicity since the possibility to be atopic could be inherited. From US Kumar *et.al* [44] reported Puerto Rican ethnicity and mixed origin were associated with degree of atopy even within the Latino groups but being Africans was not associated with degree of atopy. Socio economic conditions could be another potential factor for such differences.

### 7.4. Association between atopy and allergic conditions

A statistically significance association was noted between positive SPT results for either of the two allergens and self-reported allergic conditions. Our result revealed 3.32 times greater risk of developing one of the three allergic conditions. The fining of Scrivener *et al* [45] from Gondar, Ethiopia agree with the current study; on the other hand Gold *et.al* [41] argue not all atopic children will have development of allergic symptoms after exposure to allergens. Both innate and environmental influences also define the development of atopic disease.

### 7.5. Distribution of Total serum IgE and Eosinophil count among the different groups

Childhood allergies are more serious because they may lead to lifetime chronic disease. Atopy, the genetic predisposition to develop IgE antibodies in response to allergen exposure, is an established risk factor [46]. In our study the IgE levels were elevated in current *H.pylori* infected atopic participants that is in line with the findings from Jagadeeshwa *et.al* in India [47], Ching *et.al* in Philippines [48], Kim *et.al* in Seoul, Korea [46] and disagrees with our hypothesis which states about the protective effect of *H.pylori* infection. Total IgE levels are

known to be affected by various factors, such as age, sex, ethnicity, and geographic area. Eosinophilia is also an indicator of atopy [49]. Interaction between an elevated eosinophil level and atopic cases were very strong in children but absent in the oldest adults, which suggests the possible difference could be due to age difference among participants. This finding is in agreement with our study with elevated peripheral eosinophil count.

Finally our result showed no significant association between outcome variables (allergic conditions and any sensitivity) and gender or status of maternal educational level among the study group. A statistically significant association was seen between atopy and animal kept in the house and another significant association was seen between allergic conditions and both maternal and paternal allergic history and charcoal fuel use. The finding of association between family history of allergy and the presence of allergic conditions in the children agrees with several studies. For example, Majeed *et.al* [50], Baççıoğlu *et.al* [51], Amberber *et.al* [40] and Civelek *et.al* [52] reported significant association between family histories of allergy, which agrees with our finding, while Yung *et.al* [53] reported association only with maternal history of allergy. Surprisingly, using charcoal everyday as a source of fuel and keeping animas in the house were negatively associated with the risk of developing allergic symptoms in our study. Even though our result could be conflicting with the general knowledge or biological facts, the possible explanations could be the study design though we used larger sample size than calculated. Cross sectional study results can introduce biases like the chicken or egg dilemma. We could not be sure which one comes first using charcoal or allergy because cross sectional study measures disease and exposure at the same exact time [54].In addition, use of charcoal in the house is not a common practice; thus, the answers given by the participants could be biased even though the questionnaire we used was standard one it has limitation on indicating the exact point of exposure. Although the other exposure variables were not significantly related either to atopy or allergic conditions in our study, there is more and more evidence suggesting that not only one but many factors including physical, chemical, biological and psychosocial environment can influence the development of allergy[55].

# 8. Strength and Limitations of the study

## 8.1 Strength

- The study tried to determine the prevalence of *H.pylori* using both stool antigen test and serum antibody
- The study presented recent figures on Atopic and allergic status in the study area

## 8.2 Limitations of the Study

- Self-reported Allergic outcomes were not confirmed by physicians.
- In this study only two allergens were used.
- *H.pylori* Cag A and VacA seroposetivity status was not detected.
- Atopy specific IgE was not used, so the exact cause for the increment of the serum total IgE level was not known.

# 9. Conclusion and Recommendations

## 9.1 Conclusions

The prevalence of any sensitization (determined by house dust mites (*Dermatophagoides pteronyssinus)* and German cockroach (*Blattella germanica*) were much less than the prevalence of allergic condition (defined by self-reported questionnaire) among the study group. In the study group past and current *H.pylori* infection did not show statistically significant association with atopic sensitization and allergic conditions which disagree with our hypothesis. Significant median difference was seen with total IgE and absolute eosinophil count among the current *H.pylori* positive groups. Our result agree with our hypothesis which says there is association between different factors and atopy and allergic symptoms among young children, by showing significant relation of maternal allergic history ,paternal allergic history and use of charcoal for fuel with allergic conditions in the study group.

## 9.2 Recommendations

- Since very few studies have been done in Ethiopia on the prevalence of *H.pylori* among young children, further study is recommended to supplement the results of this study to be used for future guideline in the development of national policy for *H.pylori* treatment.
- Since our result showed no association with *H.pylori* infection and atopic diseases the eradication of the bacteria may not be assumed to have an effect on allergic inflammation. We recommended advance studies with larger populations in order to come to conclusion.
- As our study was a cross-sectional study which limits our ability to follow disease, we recommended further longitudinal and prospective studies.

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# Annexes

## Annex I: Information Sheet (For Participants, English Version)

**Title of the Research Project:** Association of *Helicobacter pylori* infection with atopy and allergic disorders in central Ethiopia, Ziway

**Name of Investigator:** Miheret Tesfaye (BSc, Msc candidate)

**Name of the Organization:** Addis Ababa University, College of Health Science, Department of Medical Laboratory Science.

**Introduction**

You are invited to participate in a study to be conducted by MSc student at Addis Ababa University, College of health sciences, School of Allied Health Science, Department of Medical Laboratory Sciences. It is aimed at studying the association of *Helicobacter pylori* infection with atopy and allergic disorders in central Ethiopia.The result will be useful to narrow the contradictory results found in researches done on this topic. It will also be an input for researches that hope to use *Helicobacter pylori*as therapeutic options for atopy. Please read the following statements and ask any unclear point before you agree to participate.Participation in the study is exclusively voluntary. If you are not willing to participate in the study or if you want to withdraw even after deciding to participate, there will be no consequences. If you decide to participate, you have to sign an informed consent form and you can get a copy of this information sheet.

**What is expected from you as a participant of the study?**

As a participant of this study your child is expected to give 3-4 ml blood and stool specimen and have skin prick test for two allergins. In addition you are expected to give answers for some questions about your family and child‟s health, atopic history in the family and socio demographic conditions. You need to know that the results might be discussed with appropriate individuals. But your name or your child‟s name, address and phone number will not be disclosed to anyone and to be more precise, identification code will be used in such conditions.

**How long participation will take you?**

You will spend 20-40 minutes until the consent form is signed, the specimen is collected, skin prick test is done, and the questionnaire is filled.

**What are the risks of participating in this study?**

There are no anticipated risks to your child‟s participation except minor discomfort during skin prick test and venipuncture because well experienced professionals will collect blood samples and perform the test.

**How the information is to be kept confidential?**

All information that you give and the results from your child‟s specimen will be used for this study only. Only limited number of professionals will have access to the information. All the information will be encoded in a computer and will be password protected.

**What are the benefits from participation?**

Since this study is MSc student research, there will not be payment for participants. But your participation is important for studying the association of *Helicobacter pylori* infection with atopy and allergic disordersamong young children and it will be useful in narrowing the conflicting results found in studies carried out on this topic.

**What are your rights as a participant of this study?**

You can ask any questions for further explanation. The principal investigator and the data collectors are responsible to clear any doubt you may have during participation. You have the right to get the results of the analysis for free.

**What can I do if I have a problem or a question?**

Please forward any question or problems you may encounter during this study to

**Miheret Tesfaye**

Mob: +251-989-98-69-83

Email: mercytesfu@yahoo.com

Department of Medical Laboratory Sciences, CHS, AAU

Tel: 0112 75 51 70

**Agree to participate?**

- ☐ Yes
- ☐ No

## Annex II- Subject information sheet (for participants, Amharic version)

አዲስ አበባ ዩኒቨርሲቲ, የጤና ሣይንስ ኮሌጅ ,የአላይድ ጤና ሣይንስ ት/ቤት ,የሕክምና ላቦራቶሪ ሣይንስ ክፍል እድሜያቸው ከ 14 አመት በታች ከሆኑ ህጻናት ላይ የደምና የሠገራ ናሙና ተወስዶ ለሚሰራው በጨጓራ በሽታ አምጪ ህዋስ እና በአላርጅክ መካከል ያለውን ግንኙነት ለማጥናት ታስቦ ለተሳታፊዎች የተዘጋጀ መረጃ ሲሆን ልጅዎም በአዲስአበባ ዩኒቨርሲቲ፡ ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል የማስተርስ ድግሪ ተማሪ የመመረቂያ ጥናት ላይ እንድትሳተፍ/እዲሳተፍ ተጋብዘዋል፡፡እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞናያንብቡና ግልጽ ያልሆነዉን /ኑትን ማንኛዉም ሃሳብ ይጠይቁ፡፡

***መግቢያ***

**የጥናቱ ርዕስ፡** “በጨጓራ በሽታ አምጪ ህዋስ እና በአላርጅክ መካከል ያለውን ግንኙነት በልጅነት የዕድሜ ክልል በሚገኙ ህጻናት ” የሚል ሲሆን እርስዎ በዚህ ጥናት ላይ የሚኖሮት ተሳትፎ ሙሉ በሙሉ በበጎፈቃደኝነት ላይ የተመሰረተ ነዉ፡፡በዚህ ጥናት ዉስጥ ላለመሳተፍ ወይም ለመሳተፍ ከወሰኑ በኋላ ለማቋረጥ የሚወስኑ ቢሆንም እንኩዋ ምንም የሚደርስቦት ችግር የለም፡፡በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጹሁፍ ወይም በጣት ፊርማ ማስቀመጥ ይጠበቅዎታል ፡፡ከፈለጉ ይህንን  መረጃ አንድ ቅጅ ለራስዎ ሊያስቀሩ ይችላሉ፡፡

**የጥናቱ ተሳታፊ በመሆኖ የሚጠበቅቦት ምንድን ነው?**

በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ ከልጅዎ የደምና የሠገራ ናሙና ለመስጠትና ቆዳው ላይ በመብሳት የአለርጂ ምርመራእንዲደረግለት መስማማት ይጠበቅብዎታል፡፡ ይሁን እንጅ ይህ አይነቱ መረጃ የርስዎንም ሆነ የልጅዎን ማንነት የሚገልጡ መረጃዎችን ማለትም ስም፤ አድራሻና የስልክ ቁጥር  የመሳሰሉትን መረጃዎችን አይጨምርም፡፡ ይልቁንም ለዚህ አገልግሎት ብቻ የሚዉልና ለማወቅ የሚያስችል መለያ ቁጥር ጥቅም ላይ እንዲዉል ይደረጋል፡፡ በተጨማሪም ስለልጅዎና ቤተሰቦት አጠቃላይ የጤና ሁኔታ ለሚቀርቡ አንዳንድ ተጨማሪ ጥያቄዎች መልስ መስጠት ይጠበቅብዎታል፡፡

**በዚህ ጥናት መሳተፍ ምን ያህል ጊዜ ይፈጃል?**

የተዘጋጀዉን መጠይቅ ለመሙላት፤ የስምምነት ቅጹ ላይ ለመፈረምና ናሙና ለመስጠት ከ20-40 ደቂቃ ያስፈልጋል፡፡

**በዚህ ጥናት መሳተፍ የሚያስከትላቸዉ ችግሮች ምንድን ናቸዉ?**

የደም ናሙና በሚሰበሰብበት ወቅትና የአለርጂ ምርመራ ለማድረግ ቆዳው ሲበሳ መጠነኛ የሆነ አለመመቸት ሊሰማው፡ሆኖም ልጅዎ ምንም አይነት የከፋ ችግር አያጋጥምዎት ምክንያቱም ናሙናው የሚወሰደውና የአለርጂ ምርመራው ቆዳው ላይ የሚከናወነው ልምድ ባላቸው የጤና ባለሙያዎች በመሆኑ ነው፡፡

**የእኔ የህክምና መረጃ በሚስጥር ተጠብቆ መቆየት የሚችለዉ እንዴት ነዉ?**

የሰጡት ማንኛውም መረጃና ከተወሰደው ናሙና ላይ የተገኘው የላቦራቶሪ ውጤት የሚውለው ለጥናቱ አላማ ብቻ ነው፡፡ ይህንን ማህደር ሊያገኙ የሚችሉት የተወሰኑ የጥናቱ ተባባሪ ሰራተኞች ብቻ ናቸው፡፡ ከዚህም በላይ ስለእርሶ ያለውን ማንኛውም መረጃ የተለየ የይለፍ ቃል ባላው የኮምፒውተር የመረጃ ማህደር ውስጥ እንዲቀመጥ ይደረገል፡፡

**በዚህ ጥናት መሳተፍ የሚያስገኛቸው ጥቅሞች ምንድን ናቸው ?**

ይህ ጥናት የማስተርስ ዲግሪ መመረቂያ ፅሁፍ እንደመሆኑ መጠን ለተሳታፊዎች ገንዘብ አይሰጥም፡፡ ሆኖም ከጥናቱ የሚገኘው ውጤት በጨጓራ በሽታ አምጪ ህዋስ እና በአላርጅክ መካከል ያለውን ግንኙነት በማሳየት በተለያየ ድምዳሜ ላይ ላለው ሳይንሳዊ ዕውቀት ክፍተት ለማጥበብ ተጨማሪ መረጃ ይሰጣል፡፡

**የዚህ ጥናት ተሳታፊ መብቱ ምንድን ነው ?**

ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛውንም አይነት ጥያቄ የመጠየቅና ገለጻ የማግኘት መብት አለዎት፡፡የላብራቶሪ ምርመራ ውጤቱንም በነጻ ማግኘት ይችላሉ፡፡

**ጥያቄ ካለኝ ወይም ችግር ቢያጋጥመኝ ምን ማድረግ ይገባል?**

ይህንን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካለዎት በሚመለከተው አድራሻ ይጠቀሙ፡፡

**ምህረት ተስፋዬ**

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ኢሜይል: mercytesfu@yahoo.com

የህክምና ላባራቶሪ ሳይንስ ት/ክፍል ,የአላይድ ጤና ሳይንስት/ቤት , የጤና ሳይንስ ኮሌጅ

አዲስ አበባ ዩኒቨርሲቲ

ስልክ: 0112 75 51 70

ለመሳተፍ ይስማማሉ?

እስማማለሁ☐ አልስማማም☐

## Annex-III- Consent (assent as needed) Form (for participants, English version)

Code number----------

Name of the participant----------

I have been informed about the study which is aimed at studying the association of *Helicobacter pylori* infection with atopy and allergic disorders in central Ethiopia, Ziway for this study blood and stool samples and skin prick test are required from a participant. The aims of the study and possible risks were explained to me as well.

I am also informed that all the information contained within the questionnaire is to be kept confidential. Moreover I have been well informed of my right to keep hold of information, decline to cooperate and make withdrawal from the study.

It is therefore with full understanding of the situation that I gave the informed consent voluntarily to the researcher to use my child‟s blood and stool sample for the investigation. In addition, I have had the opportunity to ask questions about it and received clarification to my satisfaction. I have also been informed that the benefit of participation is to get the results of analysis from my child‟s sample measured for free via the health personnel.

Participant‟s signature /finger print ----------

Name of Data collectors ---------- signature---------- Date----------

**Please direct any questions or problems you may encounter during this study to:**

**Miheret Tesfaye**

Mob: +251-989-98-69-83

Email: mercytesfu@yahoo.com

For additional information, please contact Addis Ababa University, College of Health Sciences, and Department of medical laboratory sciences office at:

Tell. +251-12-75 -51-70

P.O.Box. 9086, Addis Ababa, Ethiopia

## Annex-IV – Consent (assent as nedded) form (for participants, Amharic Version)

**<u>የተሳታፊዎች ስምምነት ማረጋገጫ ቅጽ</u>**

የሚስጥርቁጥር ----------

የተሳታፊዉስም ----------

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ በጨጓራ በሽታ አምጪ ህዋስ እና በአላርጅክ መካከል ያለውን ግንኙነት በልጅነት የዕድሜ ክልል በሚገኙ ህጻናት ስለሚደረገው ጥናት በቂ ገለጻ ተደርጎልኛል፡፡ለጥናቱ ም ከልጄ የተወሰደ የደምና የሰገራ ናሙና እንደሚያስፈልግ እና የአለርጂ ምርመራ ቆዳውን በመብሳት እንደሚከናወን ተገልጾልኛል፡፡የጥናቱንም አላማዎች በሚገባ ተረድቻለሁ፡፡

በመጠይቁ ላይ የገለጽኳቸው መረጃዎች በሙሉ በሚስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል፡፡ በጥናቱ ላይ ያለ መሳተፍና ማንኛውንም መረጃያ ለመስጠት እንዲሁም በማንኛውም ጊዜ ከጥናቱ ልጄን የማግለል መብቴ የተጠበቀ እንደሆነ ተገልጾልኛል፡፡

ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳት ና በፍጹም ፍቃደኝነት ነው፡፡የምሰጠውም ናሙና ለምርምር ብቻ እንደሚውልም ተረድቻለው፡፡ በተጨማሪም ጥያቄ ለመጠየቅ ተፈቅዶልኝ ለማወቅ የፈለኩትን ያህል ማብራሪ ያአግኝቻለሁ፡፡ የዚህ ጥናት ተሳታፊ በመሆኔ የማገኘው ጥቅም የሁሉን ምምርመራ ውጤት በነጻ ማግኘት እንደሆነ ተረድቻለሁ፡፡

የተሳታፊዉ ፊርማ /የጣት አሻራ ----------

| የምስክር ሙሉ ስም | ፊርማ |
|---|---|
| 1. ---------- | ---------- |
| 2. ---------- | ---------- |
| 3. ---------- | ---------- |

(የስምምነት ቅጹን ማንበብ ለማይችሉ ተሳታፊዎች)

የመረጃሰብሳቢው ስም ---------- ፊርማ ----------ቀን----------

**<u>ጥናቱን የሚያካሂደዉ ሰዉ ማረጋገጫ</u>**

ይህን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካሎዎት በሚከተለው አድራሻ ይጠቁሙን፡፡

**ምህረት ተስፋዬ**

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ለተጨማሪ መረጃዎች የህክምና ላባራቶሪ ሳይንስት/ክፍል ,የአላይድ ጤና ሳይንስት/ቤት , የጤና ሳይንስ ኮሌጅ

አዲስ አበባ ዩኒቨርሲቲ

ስልክ: 0112 75 51 70

## ANNEX V: QUESTIONNAIRE ENGLISH VERSION

**Introduction**

Identification code_____

Date of data collection _____

I_____ am working as a data collector in this study to assess the association of *Helicobacter pylori* infection with atopy and allergic disorders in central Ethiopia, Ziway.The name of the participant is not going to be written but the demographic and clinical data. All the extracted data will be kept entirely confidential.

Name of the data collector_____

Supervisor_____

**Instruction**: fill the information, either thick in the appropriate boxes by using "X" or a word or phrases where required.

**Socio-demographic and clinical characteristic of the study participants**

1. Sex

   Male □ Female □

2. Place of residences Age

   Urban □ Rural □

3. Maternal educational status

   Formal □ Illiterate □

4. Maternal occupation status

   Housewife □

   Farming and related □

   Trading and related □

   Horticulture □

   Others □

5. History of vaccination

   Vaccinated □

   Not vaccinated □

6. Breast feeding till 3 years old

   Yes □

   No □

7. Maternal history of allergy

   Yes □

   No □

8. Paternal history of allergy

   Yes □

   No □

# QUESTIONNAIRE ENGLISH VERSION

**Section 1_ Child Characteristics**

| | | | | | |
|---|---|---|---|---|---|
| G01 | Has your child ever had wheezing or whistling in their chest? | Yes | 1 | | WHZL6A |
| | | No | 2 | | |
| G02 | In the last 2 years, has your child had wheezing or whistling in their chest? | Yes | 1 | | WHZT6A |
| | | No | 2 | | |
| G03 | In the last 1 year, has your child had wheezing or whistling in their chest? | Yes | 1 | → G04<br>→ G05 | WHZ6A |
| | | No | 2 | | |
| G04 | How many times in the last year has your child had an attack of wheezing? | 0 | 1 | | WHZFRQ6A |
| | | 1-3 | 2 | | |
| | | 4-12 | 3 | | |
| | | >12 | 4 | | |
| G05 | Has your child ever had Asthma? | Yes | 1 | | ASTL6A |
| | | No | 2 | | |
| G06 | In the last 2 years, has your child had Asthma? | Yes | 1 | | ASTT6A |
| | | No | 2 | | |
| G07 | Has your child had Asthma in the last year? | Yes | 1 | → G08<br>→ G09 | AST6A |
| | | No | 2 | | |

| | | | | | |
|---|---|---|---|---|---|
| G08 | Has this been confirmed by a doctor? | Yes | 1 | | ASTHDR6A |
| | | No | 2 | | |
| G09 | Has your child ever had an itchy skin rash which has affected the skin creases (eg, the folds of the elbow or behind the knees)? | Yes | 1 | | RASHL6A |
| | | No | 2 | | |
| G10 | In the last 2 years, has your child had an itchy skin condition affecting the skin creases (front of the elbow, behind the knees, the front of the ankles, around the neck, or around the eyes? | Yes | 1 | | RASHT6A |
| | | No | 2 | | |

| | | | | | |
|---|---|---|---|---|---|
| G11 | In the last 1 year, has your child had an itchy skin condition affecting the skin creases (front of the elbow, behind the knees, the front of the ankles, around the neck, or around the eyes? | Yes | 1 | →G11A | RASH6A |
| | | No | 2 | → G12 | |
| G11A | IF YES, has this rash affected any of the following places? **(Multiple Answers possible)** | The elbow folds | | 1 | RASHL6AA |
| | | | | 2 | |
| | | Behind the knees | | 1 | RASHL6AB |
| | | | | 2 | |
| | | In front of the ankles | | 1 | RASHL6AC |
| | | | | 2 | |
| | | Under the buttocks | | 1 | RASHL6AD |
| | | | | 2 | |
| | | Around the neck | | 1 | RASHL6AE |
| | | | | 2 | |
| | | Around the eyes/ears | | 1 | RASHL6AF |
| | | | | 2 | |

| | | | | | |
|---|---|---|---|---|---|
| G12 | Has your child ever had hay fever or persistent sneezing attacks? | Yes | 1 | | HAYFL6A |
| | | No | 2 | | |
| G13 | In the last 2 years, has your child had hay fever or persistent sneezing with sneezing or running nose (excluding colds or flu), or problems with itchy watery eyes? | Yes | 1 | | HAYFT6A |
| | | No | 2 | | |
| G14 | In the last year, has your child had hay fever or persistent sneezing with sneezing or running nose (excluding colds or flu), or problems with itchy watery eyes? | Yes | 1 | | HAYF6A |
| | | No | 2 | | |
| G15 | How many people are living in your home now? [ ][ ] | [ ] [ ] | | | PEOP6A |
| G16 | How many older brothers/sisters does your child have who are alive now? | [ ] [ ] | | | SIBS6A |

| | | | | | |
|---|---|---|---|---|---|
| G17 | Is paracetamol the same as aspirin? | Yes | 1 | → G18 | PARASP6A |
| | | No | 2 | → G19 | |
| G18 | Can you tell me which one of these is paracetamol and which aspirin? **(show medication strip)** | Correct identification | 1 | | PARADIF6A |
| | | Incorrect identification | 2 | | |
| G19 | Has your child taken any paracetamol/panadol in the last year? | Yes | 1 | → G20 | PARA6A |
| | | No | 2 | → G21 | |
| G20 | How many tablets of paracetamol/panadol has your child taken in the last month? | [ ][ ] | | | PARAFR6A |

| | | | | | |
|---|---|---|---|---|---|
| G21 | Can you name any symptoms for which you have given your child paracetamol?<br><br>**(Multiple answers possible)** | Headache | Yes | 1 | PAHED6A |
| | | | No | 2 | |
| | | Fever | Yes | 1 | PAFEV6A |
| | | | No | 2 | |
| | | Malaria | Yes | 1 | PAMAL6A |
| | | | No | 2 | |
| | | Common cold | Yes | 1 | PACOLD6A |
| | | | No | 2 | |
| | | *Birrd* | Yes | 1 | PABIRD6A |
| | | | No | 2 | |
| | | Wheeze | Yes | 1 | PAWHEZ6A |
| | | | No | 2 | |
| | | Cough | Yes | 1 | PACOU6A |
| | | | No | 2 | |
| | | Shortness of breath | Yes | 1 | PASOB6A |
| | | | No | 2 | |
| | | Sneezing/running nose/itchy eyes | Yes | 1 | PASNEZ6A |
| | | | No | 2 | |
| | | Skin rash in the creases | Yes | 1 | PARASH6A |
| | | | No | 2 | |
| | | Other (specify) | | | PAOTHE6A |
| G22 | Is paracetamol available close to where you live? | Yes | | | PAVAIL6A |
| | | No | | | |
| G23 | Is paracetamol affordable to you? | Yes | | | PAFORD6A |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | No | | | | |
| G24 | Do you avoid giving your child aspirin? | Yes | | | | ASAVOD6A |
| | | No | | | | |
| G25 | Should any people **NOT** take aspirin? **(PW – people with....)** | Children | Yes | 1 | | ASCHIL6A |
| | | | No | 2 | | |
| | | PW gastritis | Yes | 1 | | ASGAS6A |
| | | | No | 2 | | |
| | | PW asthma | Yes | 1 | | ASASTH6A |
| | | | No | 2 | | |
| | | PW hay fever | Yes | 1 | | ASHAY6A |
| | | | No | 2 | | |
| | | Don‟t know | | 9 | | ASAVDK6A |
| G26 | Do you prefer to give aspirin or paracetamol for your child? | Aspirin | 1 | | | ASPREF6A |
| | | Paracetamol | 2 | | | |
| | | Depends | 3 | | | |
| | | Don‟t mind | 4 | | | |
| G27 | Has your child taken any drug prescribed by the health institution for any illnesses currently? **(Other than paracetamol/panadol/aspirin)** | Yes | 1 | → G27A | | ANTIB6A |
| | | No | 2 | → G28 | | |
| G27A | If Yes, please observe the drug and write the name and type the child currently taking. | ---------- | | | | ANTIBM6A |
| G28 | Has your child taken any de-worming medication in the last 6 months? **(De-worming refers to antihelmintics treatment given by the heath office free of charge without stool examination)** | Yes | 1 | | | DEWOR6A |
| | | No | | | | |

| | | | | | |
|---|---|---|---|---|---|
| G29 | Is there anyone who smokes cigarettes in your home? | Yes | 1 | → G29A | HCIGR6A |
| | | No | 2 | → G30 | |
| G29A | If yes, please write the total number of people who smoke cigarettes in the home where the child living? | [ ] | | | HCIGRN6A |
| G30 | What does your child sleep on? | Bed | | 1 | CHSLP6A |
| | | Medeb | | 2 | |
| | | Floor | | 3 | |
| | | „Jibba“ | | 4 | |
| | | „Sigaja“ | | 5 | |
| | | Other (Specify) | | 9 | |
| G31 | What is your child“s bed made of? | Iron metal | | 1 | CHBED6A |
| | | Wood | | 2 | |
| | | Flat metal | | 3 | |
| | | Rope | | 4 | |
| | | leather | | 5 | |
| | | No bed | | 6 | |
| | | Other (Specify) | | 9 | |
| G32 | What is your child“s mattress made of? | Cotton | | 1 | CHMAT6A |
| | | Sponge | | 2 | |
| | | Greass | | 3 | |
| | | Kapoak | | 4 | |
| | | No matress | | 5 | |
| | | Other (Specify) | | 9 | |
| G33 | What is your child“s pillow | Cotton | | 1 | CHPIL6A |

| | | | | |
|---|---|---|---|---|
| | made of? | Sponge | 2 | |
| | | Grass | 3 | |
| | | kapoak | 4 | |
| | | Cloth | 5 | |
| | | No pillow | 6 | |
| | | Other (Specify) | 9 | |

**Section 2_Maternal Characteristics**

| | | | | | |
|---|---|---|---|---|---|
| G34 | Have you had wheezing or whistling in your chest in the last 1 year? | Yes | 1 | → G35 | MOWHZ6A |
| | | No | 2 | → G36 | |
| G35 | How many times in the last year have you had an attack of wheezing? | 0 | 1 | | MOWHFR6A |
| | | 1-3 | 2 | | |
| | | 4-12 | 3 | | |
| | | >12 | 4 | | |
| G36 | Have you had asthma in the last 1 year? | Yes | 1 | → G37 | MOAS6A |
| | | No | 2 | → G38 | |
| G37 | Was this confirmed by a doctor? | Yes | 1 | | MOASSDR6A |
| | | No | 2 | | |
| G38 | Has the baby"s father had wheezing or whistling in the chest in the last 1 year? | Yes | 1 | | FAWHEZ6A |
| | | No | 2 | | |
| G39 | Has the baby"s father had asthma in the last 1 year? | Yes | 1 | → G40 | FAAS6A |
| | | No | 2 | → G41 | |
| | | NA | 9 | | |
| G40 | Was this confirmed by a doctor? | Yes | 1 | | FAASDR6A |
| | | No | 2 | | |
| G41 | In the last 1 year have you had hay fever? | Yes | 1 | | MOHAY6A |
| | | No | 2 | | |
| G42 | In the last 1 year has the baby"s father had hay fever? | Yes | 1 | | FAHAY6A |
| | | No | 2 | | |
| | | NA | 9 | | |
| G43 | Have you had eczema in the last 1 year? | Yes | 1 | | MOEZC6A |
| | | No | 2 | | |
| G44 | Has the baby"s father had eczema in the last 1 year? | Yes | 1 | | FAEZC6A |
| | | No | 2 | | |
| | | NA | 9 | | |
| | | Yes | 1 | | |

| | | | | |
|---|---|---|---|---|
| | in the last year? | No | 2 | |
| G46 | How many tablets of paracetamol/Panadol have you taken in the last month? | [ ]<br>[ ] | 1 | MOPAF6A |
| G47 | Have you taken any drug prescribed by the health institution for any problem currently? (Other than Paracetamol/Panadol) | Yes<br>No | 1<br>2 | GANTIB6A |
| G48 | If Yes, please observe the drug and write the name and type the mother currently taking. | ---------- | | GANBTY6A |

**Section 3_Housing characteristics**

| | | | | |
|---|---|---|---|---|
| G49 | What type of roof does your house have? | Thatched | 1 | GROOF6A |
| | | Corrugated iron | 2 | |
| | | Other (specify) | 9 | |
| G50 | What are the walls of your house made of? | Wood | 1 | GWALL6A |
| | | Wood and grass | 2 | |
| | | Cement | 3 | |
| | | Brocket | 4 | |
| | | Bricks | 5 | |
| | | Corrugated iron | 6 | |
| | | Other (specify)_____ | 9 | |
| G51 | How many rooms does your house have? **(observe and fill the no of rooms)** | [ ] | | GROOM6A |
| G52 | What type of floor does your house have? | Cement | 1 | GFLOOR6A |
| | | Wood | 2 | |
| | | Bricks | 3 | |
| | | Mud | 4 | |
| | | Other (specify)_____ | 9 | |
| G53 | Is the floor covered by any material? | Yes | 1 | GCOVER6A |
| | | No | 2 | |

| | | | | |
|---|---|---|---|---|
| G54 | Where do you do most of your cooking? (tick one that applies) | Inside the house in the main living area | 1 | GCOOK6A |
| | | Inside the house in a room other than the main living area | 2 | |
| | | Outside the house in a separate building | 3 | |
| | | Outside the house in the open air | 4 | |
| G55 | How often do you use the following for cooking? | | | |

| | | |
|---|---|---|
| | | GFUEL6A |

| Fuel | Never | Sometimes | Every day |
|---|---|---|---|
| 1. Charcoal | 1 | 2 | 3 |
| 2. Wood | 1 | 2 | 3 |
| 3. Leaves | 1 | 2 | 3 |
| 4. Dung | 1 | 2 | 3 |
| 5. Nafta/Lanba | 1 | 2 | 3 |
| 6. Gas | 1 | 2 | 3 |
| 7. Electricity | 1 | 2 | 3 |
| 9. Other | 1 | 2 | 3 |

| | | |
|---|---|---|
| G56 | How often do you use the following inside the house for purposes other than ing (eg heating, lighting)? | GFUELA6A |

| Fuel | Never | Sometimes | Every day |
|---|---|---|---|
| 1. Charcoal | 1 | 2 | 3 |
| 2. Wood | 1 | 2 | 3 |
| 3. Leaves | 1 | 2 | 3 |
| 4. Dung | 1 | 2 | 3 |
| 5. Nafta/Lanba | 1 | 2 | 3 |
| 6. Gas | 1 | 2 | 3 |
| 7. Electricity | 1 | 2 | 3 |
| 8. A locally made battery | 1 | 2 | 3 |
| 9. Other | 1 | 2 | 3 |

| | | |
|---|---|---|
| G57 | Which of the following animals do you or your household keep? (Multiple answers possible) | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | Animal | Not available | Inside | Outside | GANIM6A |
| | | 1. Cat | 1 | 2 | 3 | |
| | | 2. Dog | 1 | 2 | 3 | |
| | | 3. Hen | 1 | 2 | 3 | |
| | | 4. Cow/ox | 1 | 2 | 3 | |
| | | 5. Sheep | 1 | 2 | 3 | |
| | | 6. Horse | 1 | 2 | 3 | |
| | | 7. Pig | 1 | 2 | 3 | |
| | | 8. Goat | 1 | 2 | 3 | |
| | | 10. mule/donkey | 1 | 2 | 3 | |
| | | 9 Other | 1 | 2 | 3 | |

| | | | | |
|---|---|---|---|---|
| G58 | What is your main source of drinking water? **(Tick one which applies)** | Piped into compound | 1 | GWATER6A |
| | | Piped outside compound | 2 | |
| | | Open well or spring | 3 | |
| | | Covered well or spring | 4 | |
| | | River, pond or dam | 5 | |
| | | Rainwater | 6 | |
| G59 | What type of toilet facility do you use? **(Tick one which applies)** | Flush toilet | 1 | GTOILET6A |
| | | Ventilated improved pit | 2 | |
| | | Traditionnel pit toilet | 3 | |
| | | None/bush/field | 4 | |

| | | | | | |
|---|---|---|---|---|---|
| G60 | How do you dispose your waste? | Pit | | 1 | GSAND6A |
| | | Open field | | 2 | |
| | | Burning | | 3 | |
| | | Garbage bin | | 4 | |
| | | Other(Specify)_____ | | 9 | |
| G61 | Do you use any of the following insecticides in your house? **(Multiple answers possible)** | DDT | Yes | 1 | GINSE6AA |
| | | | No | 2 | |
| | | Malathion | Yes | 1 | GINSE6AB |
| | | | No | 2 | |
| | | Flit | Yes | 1 | GINSE6AC |
| | | | No | 2 | |
| | | Application of dung | Yes | 1 | GINSE6AD |
| | | | No | 2 | |
| | | Other(specify) | Yes | 1 | GINSE6AE |
| | | | No | 2 | |
| G61 | Where do you place insecticides in your house? (observe) | Out of reach of children | | 1 | PROT6A |
| | | Within reach of children | | 2 | |

## ANNEX VI. QUESTIONNAIRE – AMHARIC VERSION

አሁን ልጅዎ ስላጋጠመው የደረት ህመምና ተመሳሳይነት ያላቸውን ጉዳዮች □□□ቅ□ታ ስሁ።ባክዎን ባለፉትጊ□ያቶች ውስጥ ያጋጠሙዋን ከዚህ ጋር ተያያጅነት ያላቸው ችግሮች በማስታወስ ይንገሩኝ።

## 1.1 ህፃኑን/ኗን የተመለከተ

| | | | | | |
|---|---|---|---|---|---|
| **G01** | ህፃኑ/ኗ ከተወለደ/ች ጀምሮ በየትኛውም ጊ□ ቢሆን በደረቱ/ትዋ ውስዓ ሲዓ ሲዓ □ሚል ወይም የፉጨት ድምፅ ኖሮት ጸዉቃል/ታዉቃለች? | አዎን | 1 | | WHZL6A |
| | | □ለም | 0 | | |
| **G02** | ባለፉት ሁለት አመ□ት በየትኛውም ጊዜ ቢሆን በ□ረቱ/ትዋ ውስዓ ሲዓ ሲዓ □ሚል ወጀም የፉጨት ድምፅ ኖሮት ያውቃል/ታዉቃለች? | አዎን | 1 | | WHZT6A |
| | | □ለም | 0 | | |
| **G03** | ባለፉት 12 ወራት ዉስጥ በሕፃኑ/ኗ ደረት ውስጥ ሲጥ ሲጥ የሚል ወይም የፉጨት ድምፅ ተሰምቶ ያውቃል/ታዉቃለች? | አዎን | 1 | →G04<br>→G05 | WHZ6A |
| | | □ለም | 0 | | |
| **G04** | ባለፉት 12 ወራት ህፃኑ/ኗ ደረት ውስጥ ሲጥ ሲዓ □ሚል ወይም የፉጨት ድምፅ ተሰምቶ የነበረዉ ስንት ጊዜ ነበር? | 0 | 0 | | WHZFRQ6A |
| | | 1-3 | 1 | | |
| | | 4-12 | 2 | | |
| | | ከ13 በላይ | 3 | | |
| **G05** | ህፃኑ/ኗ ከተወለደ/ች ጀምሮ በየትኛውም ጊ□ ቢሆን አስም ኖሮት ያውቃል/ታዉቃለች? | አዎን | 1 | | ASTL6A |
| | | □ለም | 2 | | |
| **G06** | ባለፉት ሁለት አመ□ት በየትኛውም ጊዜ ቢሆን ህፃኑ/ኗ አስም ኖሮት/ሯት ያውቃል/ታዉቃለች? | አዎን | 1 | | ASTT6A |
| | | □ለም | 0 | | |
| **G07** | ባለፉት 12 ወራት ዉስጥ ህፃኑ/ኗ አስም ኖሮት ጸዉቃል/□ታዉቃለች? | አዎን | 1 | →G08 | AST6A |
| | | □ለም | 0 | | |
| **G08** | ህፃኑ/ኗ አስም □እንዳለበት/ባት በሐኪም ተረፋፅ□ል? | አዎን | 1 | | ASTHDR6A |
| | | □ለም | 0 | | |
| **G09** | ህፃኑ/ኗ ከተወለደ/ች ጀምሮ በየትኛውም ጊ□ ቢሆን በአጥንት መታ፟ኝጸ ቦታዎቹ(ጀ) (በክርን መታጠፍያ፤ከጉልበቱ ኋላ ባለዉ መታጠፍያ፤በቁርጭምጭሚት ፊት ለፊት፤ በአንገት ዙሪያ፤እና በአይን አካባቢ) የሚያሳክክ ሽአታ ወጥቶበት (ወጥቶባት) ነበር? | አዎን | 1 | | RASHL6A |
| | | □ለም | 0 | | |
| **G10** | ባለፉት ሁለት አመ□ት በየትኛውም ጊዜ ቢሆን ህፃኑ/ኗ በአጥንት መታ፟ኝጸ ቦታዎቹ(ጀ) (በክርን መታጠፍያ፤ከጉልበቱ ኋላ ባለዉ መታጠፍያ፤በቁርጭምጭሚት ፊት ለፊት፤ በአንገት ዙሪያ፤እና በአይን አካባቢ) የሚያሳክክ ሽአታ ወጥቶበት (ወጥቶባት) ነበር? | አዎን | 1 | | RASHT6A |
| | | □ለም | 0 | | |
| **G11** | ባለፉት 12 ወራት ዉስጥ ልጁ(ልጅቷ) በአጥንት መታ፟ኝጸ ቦታዎቹ(□) (በክርን መታጠፍያ፤ከጉልበቱ ኋላ ባለዉ መታታጠፍያ፤በቁርጭምጭሚት ፊት ለፊት፤ በአንገት ዙሪያ፤ እና በአይን አካባቢ) የሚያሳክክ ሽአታ ወጥቶበት (ወጥቶባት) ነበር? | አዎን | 1 | →G11A | RASH36 |
| | | □ለም | 0 | | |
| **G11A** | **መልሱ አዎን ከሆነ፡** ሽአታዉ የነበረዉ በየትኛዎቹ ቦታዎች ላይ ነዉ? (መልሱ ይነበብ፤ ከአንድ በላይ መልስ መስጠት ይቻላል) | በክርን መታ፟ኝጸ | 1 | | RASH6AA |
| | | | 0 | | |
| | | ከጉልበትዎ ኋላ | 1 | | RASH6AB |
| | | | 0 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | በቁሮ□ ም□ ሚት ፊትለፊት | 1<br>0 | | | RASH6AC |
| | | ከመቀመጫ በታች | 1<br>0 | | | RASH6AD |
| | | በአንገትዎ ዙሪያ | 1<br>0 | | | RASH6AE |
| | | በአይንና በጆሮዎች ዙሪያ | 1<br>0 | | | RASH6AF |
| **G12** | ህፃኑ/ኗ ከተወለደ/ች ጀምሮ በየትኛውም ቂ□ ቢሆን ንፍጥ የሚያበዛ ጉንፋን፤የማያቋርጥ ማስነጠስ፤አፍንጫ ወይም ዐይንን የሚያቃጥል ሕመም ነበረበት(ባት)?**(እነኚህ ምልክቶች የታዩት ህፃኑ በጉንፋን ሳይያዝ መሆን አለበት)::** | አዎን<br>□ለም | 1<br>0 | | | HAYFL6A |
| **G13** | ባለፉት ሁለት አመ□ት በየትኛውም ጊዜ ቢሆን ህፃኑ/ኗ ንፍጥ የሚያበዛ ጉንፋን፤የማያቋርጥ ማስነጠስ፤አፍንጫ ወይም ዐይንን የሚያቃጥል ሕመም ነበረበት(ባት)?<br>**(እነኚህ ምልክቶች የታዩት ህፃኑ በጉንፋን ሳይያዝ መሆን አለበት)::** | አዎን<br>□ለም | 1<br>0 | | | HAYFT6A |
| **G14** | ባለፉት 12 ወራት ዉስጥ ልጅዎ ንፍጥ የሚያበዛ ጉንፋን፤የማያቋርጥ ማስነጠስ፤አፍንጫ ወይም ዐይንን የሚያቃጥል ሕመም ነበረበት(ባት)?**(እነኚህ ምልክቶች የታዩት ህፃኑ በጉንፋን ሳይያዝ መሆን አለበት)::** | አዎን<br>□ለም | 1<br>0 | | | HAYF6A |
| **አሁንህፃኑን/ኗ በህመሙ□ □ራሴ□ሞልናየመሳሰሉትንየህመም ማስታ□ሻ □እንደወሰዐ □ቷጁቅ- ታለሁ::** | | | | | | |
| G15 | □ራሴ□ሞል ከአ□ፕሪን ጋር አንድ ነው? | አዎን<br>□ለም | 1<br>0 | <br>→G17 | | PADIF6A |
| G16 | ከነዚህ ከሁለቱ መካከል ፓራሴታሞልና አስፕሪንን ልትለይልን ትችያለሽ ? | ትክክለኛ መለጸ<br>□ተሳሳተ መለጸ | 1<br>0 | | | PADFA6A |
| **G17** | ሕጻኑ(ኗ) ባለፈዉ ዓመት ፓራሴታሞል/ፓናዶል ወስ□(□) ጸ□ ቃል(ታዉቃለች)? | አዎን<br>□ለም | 1<br>0 | →G18<br>→G20 | | PARA6A |
| **G18** | ህፃኑ/ኗ በላፈው ወር ስንት የፓራሴታሞል ወጁም<br>ፓናዶል ኪኒኖች ወሰዶዋል? **(በቁዓር ጁ□ለን)** | [ ][ ]ወስደዋል/ስች:: | | | | PARAFR6A |
| **G19** | □ራሴታሞል ለሕጻኑ(ኗ) የሰጡበት □ሕመም ምልክቶች/በሽታዎችከሚከተሉት የትኞቹ ናቸው?<br>**(መልሱ ይነበብ፤ ከአንድ በላይ መልስ መስጠት ይቻላል)** | ራስ ምታት | አዎን<br>□ለም | 1<br>0 | | PAHED6A |
| | | ትኩሳት | አዎን<br>□ለም | 1<br>0 | | PAFEV6A |
| | | ወባ | አዎን<br>□ለም | 1<br>0 | | PAMAL6A |
| | | ጉንፋን | አዎን<br>□ለም | 1<br>0 | | PACOLD6A |
| | | ብርት | አዎን<br>□ለም | 1<br>0 | | PABIRD6A |
| | | ሳል | አዎን<br>□ለም | 1<br>0 | | PACOU6A |
| | | ሲዓ ሲዓ ሰ□ልበት/ባት | አዎን<br>□ለም | 1<br>0 | | PAWHEZ6A |
| | | የትንፋሽ ማጠር | አዎን | 1 | | PASOB6A |

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| | | | □ለም | 0 | |
| | | ማስነጠስ/ □እንደንፍጥያለ በአፍንጫ ሲወርት/የአይን ማሳከክ ሲኖር | አዎን | 1 | PASNEZ6A |
| | | | □ለም | 0 | |
| | | □ቆ□ ኸአ□ በመታ፟ኝጸ አካባቢዎች ሲኖር | አዎን | 1 | PARASH6A |
| | | | □ለም | 0 | |
| | | ሌላ (ጁ፞ሀሸን) _____ | | | PAOTHE6A |
| G20 | በሚኖሩበት አካባቢ □ራሴታሞልን በቅርበት ያገኘ□ል? | አዎን | 1 | | PARAV6A |
| | | □ለም | 0 | | |
| G21 | □እርስ- □ራሴታሞልን ለመግዛት ዋጋውን ይችሉ□ል? | አዎን | 1 | | PAFORD6A |
| | | □ለም | 0 | | |
| G22 | አስፕሪን ለልጅዎ ላለመስጠት ይሞክራሉ? | አዎን | 1 | | ASAVOD6A |
| | | □ለም | 0 | | |
| G23 | አስፕሪን መውሰድ የሌለባቸው ሰዎች አሉ? **(መልሱ ይነበብ፤ ከአንድ በላይ መልስ መስጠት ይቻላል)** | ሕፃናት | አዎን | 1 | ASCHIL6A |
| | | | □ለም | 0 | |
| | | ጨጓራ ያለባቸው | አዎን | 1 | ASGAS6A |
| | | | □ለም | 0 | |
| | | አስም ያለባቸው | አዎን | 1 | ASASTH6A |
| | | | □ለም | 0 | |
| | | የአፍንጫ አስም ያለባቸው | አዎን | 1 | ASHAY6A |
| | | | □ለም | 0 | |
| | | አላውቅም | አዎን | 1 | ASAVDK6A |
| | | | □ለም | 0 | |
| | | ሌላ (ጁ፞ሀሸን)_____ | | | |
| G24 | □እርስዎ ለልጅዎ ለመስጠት የሚመርጡት አስፕሪንን ነው ወይንስ □ራሴታሞልን? | አስፕሪን | 1 | | ASPREF6A |
| | | □ራሴታሞል | 2 | | |
| | | □እንደ ሁኔ□ ው፡ | 3 | | |
| | | ምንም ምርጫ የለኝም | 4 | | |
| G25 | ህፃኑ/ኗ ለማንኛውም አይነት መትኃኒት ለየትኛውም አይነት በሽታ□ በሕክምና በቅርብ ቂ□ □ታዘለት/ላት ያውቃል(ለች)? **(ይህን ጥያቄ ለማኘኛውም አይነት በሽታ□ □ታዘዘለትን/ላትን መድሃኒት ያካትታል ነገር ግን □ራሴታሞል/ፓናዶልን ወይም አስፕሪንን አይጨምርም።)** | አዎን | 1 | →G25A | ANTIB6A |
| | | □ለም | 0 | →G26 | |
| G25A | መልስዎ አዎ ከሆነ የሚወስዱትን መድሃኒት | 1.---------- | | | BANTA6A |

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| | **አይነቱንና ስሙን በማየት ይሞላ።** | **2.----------**<br>**3.----------** | | | BANTB6A<br>BANTC6A |
| **G26** | **ህፃኑ/ኗ ባለፉት ስድስት ወራት ለሆድ ትላትል መከላከያ መድሃኒት ወስዶአል?**<br>**(የሆድ ትላትል መከላከያ ሲባል በዋነኛነት በጤና ባለሙጸ በአመት ሁለት ጊዜ ቤት ለቤት በነፃ □ሚታደል ማለት ነው።)** | **አዎን** | **1** | | DEWOR6A |
| | | **□ለም** | **0** | | |
| G27 | **በቤት ውስጥ ምንያህል ሰዎች ይኖራሉ?** | [ ][ ] | | | PEOP6A |
| G28 | **ህፃኑን/ኗ ስንት □ታላቅ ወንድምና□እህቶች በሕይወት አሉት/አሏት?** | [ ][ ] | | | SIBS6A |
| **G29** | **ህፃኑ/ኗ በሚኖርበት/በምትኖርበት ቤት ውስጥ ሲ.ጋራ/ትምባሆ የሚያጨስ ሰው አለ?** | **አዎን** | **1** | **→G29A** | HCIGR6A |
| | | **□ለም** | **0** | **→G30** | |
| **G29A** | **መልሱ አዎ ከሆነ የሚጸፊ ስ ሰው፡**<br>**ብዛት ጠይቀሽ መዝግቢ** | [ ][ ] | | | HCIGRN6A |
| **G30** | **ህፃኑ/ኗ በምን ላይ ነዉ □ሚተኛ**<br>**ዉ(የምትተኛ□ )?** | **አልጋ** | | **1** | CHSLP6A |
| | | **መ□ብ** | | **2** | |
| | | **ወለል** | | **3** | |
| | | **ፀ ባ** | | **4** | |
| | | **ስፉጽ** | | **5** | |
| | | **ሌላ (ጁፅለ□)** | | **9** | |
| **G31** | **ህፃኑ/ኗን ለመኘ□**<br>**የሚጠቀሙዉ/የምትጠቀመዉ አልጋ**<br>**ከሆነ የተሠራው ከምንድ ነው?** | **ከሸቦ** | | **1** | CHBED6A |
| | | **ከእንጨት** | | **2** | |
| | | **ከቦንዳ** | | **3** | |
| | | **ከገመድ** | | **4** | |
| | | **ከቁርበት** | | **5** | |
| | | **አልጋ የለኝም** | | **6** | |
| | | **ሌላ (ጁፅለ□)** | | **9** | |
| **G32** | **ህፃኑ/ኗን ለመኘታ□**<br>**የሚጠቀሙዉ/የምትጠቀመ □ አራሽ** | **ከጥጥ** | | **1** | CHMAT6A |
| | | **ከስፖንጅ** | | **2** | |
| | | **ከሳር** | | **3** | |

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| | ከሆነ የተሠራው ከምንድ ነው? | ከአበባ የሚገኝ ጥጥ መሰል ነገር | 4 | |
| | | ፍራሽ የለኝም | 5 | |
| | | ሌላ (ጁፅለ□ | 9 | |
| G33 | ህፃኑ/ኗን ለመኝታ□<br>የሚጠቀሙዉ/የምትጠቀመዉ ትራስ<br>ከሆነ የተሠራው ከምንድ ነው? | ከጥጥ | 1 | CHPIL6A |
| | | ከስፖንጅ | 2 | |
| | | ከሳር | 3 | |
| | | ከአበባ የሚገኝ ጥጥ መሰል ነገር | 4 | |
| | | ፊ ርቅ ወጁም ልብስ | 5 | |
| | | ከጨርቅ የተሰራ ትራስ | 6 | |
| | | ትራስ የለውም/ላትም | 7 | |
| | | ሌላ (ጁፅለ□ | 9 | |

## 1.2 የህፃኑ/ኗ □ናትን/አባትንየተመለከተ

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| G34 | ባለፉት 12 ወራት በደራትሥ ዉስጥ ሲጥ ሲጥ የሚል ወይም የፉጨት ድምፅ ነበረብሥት? | አሥ | 1 | →G35 | MWHZ6A |
| | | □ለም | 0 | →G36 | |
| G35 | ባለፉት 12 ወራት በደራትሥ ዉስጥ ሲጥ ሲዓ □ሚል ወይም የፉጨት ድምፅ ተሰምቶሥት የነበረዉ ስንት ጊዜ ነበር? | 0 | 0 | | MWHFR6A |
| | | 1-3 | 1 | | |
| | | 4-12 | 2 | | |
| | | ከ13 በላይ | 3 | | |
| G36 | ባለፉት 12 ወራት አስም ነበረብሥት? | አሥ | 1 | →G37 | MOAS6A |
| | | □ለም | 0 | →G38 | |
| G37 | □እርስሥ አስም እንዳለብሥት በሐኪም ተረፉ□ል? | አሥ | 1 | | MASDR6A |
| | | □ለም | 0 | | |
| G38 | ባለፉት 12 ወራት □ልፃ (ልፀ ታ) አባት በ□ረታቸው □ስዓ ሲዓ ሲዓ □ሚል ወጁም የፉጨት ድምፅ ነበረባቸው? | አሥ | 1 | | FWHZ6A |
| | | □ለም | 0 | | |
| | | አይመለከትም | 9 | | |
| G39 | ባለፉት 12 ወራት የልጁ(ልጅታ) አባት አስም ነበረባቸዉ? | አሥ | 1 | →G40 | FAAS6A |
| | | □ለም | 0 | →G41 | |
| | | አይመለከትም | 9 | | |

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| **G40** | □ልዓ (ልፀታ) አባት አስም □እንዳለባቸው በሐኪም ተረጋጧል? | አዎ | 1 | | FASDR6A |
| | | □ስም | 0 | | |
| **G41** | ባለፉት 12 ወራት ዉስጥ ንፍጥ የበዛበት ጉንፋን፤ የማያቋርጥ ማስነጠስ፤ አፍንጫ ወይም ዓይን ማቃጠል ነበረብዎት? | አዎ | 1 | | MOHAY6A |
| | | □ስም | 0 | | |
| **G42** | ባለፉት 12 ወራት ዉስጥ የልጁ(ልጅቷ) አባት፤ ንፍጥ የበዛበት ጉንፋን፤ የማያቋርጥ ማስነጠስ፤ አፍንጫ ወይም ዓይን ማቃጠል ነበረባቸዉ? | አዎ | 1 | | FAHAY6A |
| | | □ስም | 0 | | |
| | | አይመለከትም | 9 | | |
| **G43** | ባለፉት 12 ወራት የሚያሳክክና ፤በተለጁም የአጥንት መታጠፊያአካባቢዎችያሉትንየሰዉነትክፍሎችን/ለምሳሌ የክንድ፤ከጉልበት በሰተኋላ □□ኝ ቆዳዎችን/ □ሚጸዳቃ □ቆ□ ሽአ□ ነበረብዎት? | አዎ | 1 | | MOEZCA6A |
| | | □ስም | 0 | | |
| **G44** | ባለፉት 12 ወራት የልጁ(ልጅቷ) አባት፤በተለይም የአጥንት መታጠፊያአካባቢዎችያሉትንየሰዉነትክፍሎችን/ለምሳሌ የክንድ፤ከጉልበት በሰተኋላ መታ□ኝ ቆዳዎችን/ □ሚጸዳቃ □ቆ□ ሽአታ□ ነበረባቸዉ? | አዎ | 1 | | FAEZC6A |
| | | □ስም | 0 | | |
| | | አይመለከትም | 9 | | |
| **G45** | በላፈው ዓመት ፓራሴታሞል/ፓናዶል ወስ□ዉጸዉቃሉ? | አዎ | 1 | | MOPAR6A |
| | | □ስም | 0 | | |
| **G46** | በላፈው ወር ሰንት የፓራሴታሞል ወጁም ፓናዶል ኪኒኖችዉጠዋል? | [ ] [ ] መስዳስች። | | | MOPAFR6A |
| **G47** | ማንኛውም አይነት መድኃኒት ለየትኛዉም አይነት በሽታ□ በሕክምና በቅርብ ጊዜ □ታዘልዎት<br><br>ጸዉቃል? **(ይህን ጥያቄ ለማኝኛውም አይነት በሽታ የታዘዘልዎትን መድሃኒት ያካትታል ነገር ግን ፓራሴታ□ሞል/ፓናዶልን ወይም አስፕሪንን አይጨምርም።)** | አዎን | 1 | | MANTIB6A |
| | | □ስም | 0 | | |
| **G48** | መልስዎ አዎ ከሆነ የሚወስዱትን መድሃኒት አይነቱንና ስሙን በማየት ይሞላ። | 1.---------- | | | MANTA6A |
| | | 2.---------- | | | MANTB6A |
| | | 3.---------- | | | MANTC6A |

## 1.3 ቤትዎን የተመለከተ

| | | | | |
|---|---|---|---|---|
| G49 | የቤትዎ ጣራ የተሰራው ከምንድን ነው? | ሣር | 1 | GROOF 6A |
| | | ቆርቆሮ | 2 | |
| | | ሌላ (ጁ.ዘለ□) | 9 | |
| G50 | ግድግዳው ከምን የተሠራ ነው? | □እንጨትና ጭቃ | 1 | GWALL6A |
| | | □እንጨት፣ ጭራሮና ሳር | 2 | |
| | | ድንጋይና ሲሚንቶ | 3 | |
| | | ብሎኬት | 4 | |
| | | ጂ.ብ | 5 | |
| | | ቆርቆሮ | 6 | |
| | | ሌላ (ጁ.ዘለ□) | 9 | |
| G51 | የህፃኑ/ኗ መኖሪያ ቤት ወለል የተሰራው ከምንድን ነው? | | | GFLOOR6A |
| | | ከሲሚንቶ | 1 | |
| | | ከጣውላ ወይም □ንጨት | 2 | |
| | | ከሸክላ | 3 | |
| | | ከአፈር | 4 | |
| | | ሌላ(ጁ.ፅ.ለ□) | 9 | |
| G52 | የህፃኑ/ኗ መኖሪያ ቤት ወለል በምንጣፍ ወይም በሌላ ነገር ተሸፍኗል? | አዎን | 1 | GCOVER6A |
| | | □ለም | 0 | |

| | | | | |
|---|---|---|---|---|
| G53 | ቤተሰቡ ምግብ በብዛት የሚያበስሉት የት ነው? (አንዱ ላይ ብቻ ምልክት አድርጊ) | በዋናው ቤት ውስጥ | 1 | GCOOK6A |
| | | □ቤት ውስጥ ሆኖ ከዋናው ቤት ሌላ | 2 | |
| | | ከቤት ውጭ ማዕድ ቤት | 3 | |
| | | ከቤት ውጭ ክፍት ቦ□ | 4 | |

**G54** የህፃኑ/ኗን ቤተሰበ ከሚከተሉት የማገዶ ዓይነቶች ምግብ ለማብሰል ት

ቂ□ው□ንድሚ.ቀሙ ቢቶቂናል?

| ማገዶ/ነዳጅ | አልፎቀ ምም | አንዳን ት ቂ□ | ም □ቀ ኑ |
|---|---|---|---|
| 1. ከሠል | 1 | 2 | 3 |

GFUEL6AA

GFUEL6AB

| | | | | | | |
|---|---|---|---|---|---|---|
| | | 2. □እንጨት | 1 | 2 | 3 | GFUEL6AC |
| | | 3. ቅቷል | 1 | 2 | 3 | GFUEL6AD |
| | | 4. ኩበት | 1 | 2 | 3 | GFUEL6AE |
| | | 5. ናፍጣ/ላንባ | 1 | 2 | 3 | GFUEL6AF |
| | | 6. ቡታፋ□ | 1 | 2 | 3 | GFUEL6AG |
| | | 7. መብራት/ኮርነቲ | 1 | 2 | 3 | GFUEL6AH |
| | | 9. ሌላ (ጁፅለ□) | 1 | 2 | 3 | |
| G55 | የህፃኑ/ኗን ቤተሰበ ከሚከተሉት የማገዶ ዓይነቶች ምግብ ከማብሰል ውጭ ለሌላ ጉዳይ ይጠቀማል (ለምሳሌ ለሙቀትና ለመብራት )? | | | | | |
| | | ማገዶ/ነዳጅ | አልጠቀምም | አንዳንቲ□ | በ□ቀኑ | |
| | | 1. ከሠል | 1 | 2 | 3 | GFUEL6AAA |
| | | 2. □እንጨት | 1 | 2 | 3 | GFUEL6AAB |
| | | 3. ቅቷል | 1 | 2 | 3 | GFUEL6AAC |
| | | 4. ኩበት | 1 | 2 | 3 | GFUEL6AAD |
| | | 5. ናፍጣ/ላንባ | 1 | 2 | 3 | GFUEL6AAE |
| | | 6. ቡታፋ□ | 1 | 2 | 3 | GFUEL6AAF |
| | | 7. ባትሪ ድንጋይ | 1 | 2 | 3 | GFUEL6AAG |
| | | 8. መብራት/ኮርነቲ | 1 | 2 | 3 | GFUEL6AAH |
| | | 9. ሌላ (ጁፅለ□) | 1 | 2 | 3 | GFUEL6AAI |

| | | | |
|---|---|---|---|
| G56 | የህፃኑ/ኗን ብተሰብ ከሚከተሉት □እንሳሳትናከብቶችዓይነቶችየትኞቹአሏችው? | | |

| □እንስሳ | በቤት ውስጣ ጁታበቃሉ/ ጸትራሉ | ከቤት ውቸ ጁታበቃሉ | |
|---|---|---|---|
| 1. ድመት | 1 | 2 | GANIM6AA |
| 2. ውሻ | 1 | 2 | GANIM6AB |
| 3. □ሮ | 1 | 2 | GANIM6AC |
| 4. ላም/በሬ | 1 | 2 | GANIM6AD |
| 5. በፅ | 1 | 2 | GANIM6AE |
| 6. □ረስ | 1 | 2 | GANIM6AF |
| 7. አ□ል | 1 | | GANIM6AG |
| 8. በቅሎ/አህያ | 1 | 2 | GANIM6AH |
| 9. ሌላ | 1 | 2 | GANIM6AI |

| | | | | |
|---|---|---|---|---|
| G57 | የህፃኑ/ኗን ቤተስብ የመጠጥ ውሃ በዋንኝነት የሚያገኙት ከየት ነው? | በግቢው ውስጥ ከሚገኝ ጊንቧ | | WAT6A |
| | | ከግቢ ውጪ ከሚገኝ ጊንቧ | | |
| | | ከጉድጓድ ወይም ምንጭ | | |
| | | ከተጠበቀ ጉድጓድ ወይም ምንጭ | | |
| | | ከወንዝ፣ ከኩሬ፣ከጉድጓድ | | |
| | | ከዝናብ ውሃ | | |
| G58 | የህፃኑ/ኗን ቤተስቡ □ሚገለገልበት መፀዳጀ ቤት ምን አይነት ነው? | በውሃ የሚሰራ ሽንት ቤት | | SANIT6A |
| | | ሽታ□ አልባ መ□□ጽ | | |
| | | የተለመደ ዓይነት የሽንት ቤት ጉድጓድ | | |
| | | ሜ□ ላጁ ወጀም □ ካ | | |
| | | በየቀኑ | 2 | |

| | | | | |
|---|---|---|---|---|
| | | ቢያንስ በሳምንት አንድ ጊዜ | 3 | |
| | | ቢያንስ በ15 ቀን አንድ ጊዜ | 4 | |
| | | በበዓል ቀን ወይንም ለየት ባለ ቀን | 5 | |
| | | አላውቅም | 6 | |
| | | መልስ መስጠት አልፈለኩም | 7 | |
| G59 | የህፃኑ/ኗ ቤተሰብ ቆሻሻ ለመጣያነት የሚገለገሉበት ምንድን ነው? | በቱትጃት ውስጣ | 1 | GSAND6A |
| | | ሜ□ ላጁ | 2 | |
| | | አንድ ላይ ሰብስቦ በማቃጠል | 3 | |
| | | በቆሻሻ መ□ጸ □ቃ | 4 | |
| | | ሌላ (ጁፃለን) | 9 | |
| G60 | የህፃኑ/ኗ ቤተሰብ ከሚከተሉት □ተባጁ ማዓኝጸ መት□ኒቶች በቤቱ<br>ውስጥ የትኛውን ይጠቀማሉ?<br>(ከአንድ በላይ መልሰ መስጠት ይቻላል) (መልሱ ይነበብ) | ዲዲት | 1<br>0 | GINSE6AA |
| | | ማላ□ ይን | 1<br>0 | GINSE6AB |
| | | ፍሊት | 1<br>0 | GINSE6AC |
| | | ሌላ (ጁፅለ□) | 1<br>0 | GINSE6AD |
| G61 | □ንደ ፊሊት የመሳሰሉት ጸረተባይ መድሀኒቶች የት ነው የሚያስቀምጡት?(ስፍራዉን □ንዲያሳዩሽ ጠይቂያቸዉ)<br>አሁን ቤት ውስጥ ጸረተባይ መት□ኒቶች ከሌላቸው ቢኖራቸው ኖሮ የት □ንደሚያስቀምጡ ጠይቂያቸው | ልጆች ሊደርሱበት በሚችሉበት ቦ□ | 1 | PROT6A |
| | | ልጆች ሊደርሱበት በማይችሉበት ቦ□ | 0 | |

## ANNEX VII: LABORATORY PROTOCOLS

# Sandwich ELISA Protocol for total human IgE

### Specimen collection and preparation

1. Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum sample without additives only. Avoid grossly hemolytic (bright red), lipemic (milky), or turbid samples.

2. Specimens should be capped and may be stored for up to 48 hours at 2-8°C.Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing [56].

### Reagent preparation

1. All reagents should be allowed to reach room temperature (18-25°C) befor use.

2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.

3. Samples with expected values greater than 800 IU/mL should be diluted with Zero Standard prior to assaying. A 1:100 initial dilution is recommended.

### Assay procedure

1. Secure the desired number of coated wells in the holder.

2. Dispense 20µL of standards, samples, and controls into appropriate wells.

3. Dispense 100 µL of Zero Buffer into each well.

4. Thoroughly mix for 10 seconds. It is very important to have complete mixing in this setup.

5. Incubate at room temperature (18-25°C) for 30 minutes.

6. Remove the incubation mixture by flicking plate content into a waste container.

7. Rinse and flick the microtiter plate 5 times with distilled or deionized water. (Please do not use tap water.)

8. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.

9. Dispense 150µL of Enzyme Conjugate Reagent into each well. Gently mix for 10 seconds.

10. Incubate at room temperature (18-25°C) for 30 minutes.

11. Remove the incubation mixture by flicking well contents into a suitable waste container.

12. Rinse the wells 5 times with running distilled or deionized water. (Please do not use tap water.)

13. Strike the wells sharply on absorbent paper to remove residual water droplets.

14. Dispense 100 µL TMB Substrate Reagent into each well. Gently mix for 5 seconds.

15. Incubate at room temperature, in the dark, for 20 minutes.

16. Stop the reaction by adding 100µL of Stop Solution (1N HCl) into each well.

17. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.

18. Read OD at 450nm with a microtiter well reader within 15 minutes.

**Results**

1. Calculate the mean absorbance value (OD450) for each set of reference standards, controls and samples.

2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in IU/mL on graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.

3. using the mean absorbance value for each sample, determine the corresponding concentration of IgE in IU/mL from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.

4. Sample Dilution - If a sample contains more than 800 IU/mL of IgE, make a 1:100 dilution or further dilutions with the zero standard. After assaying the diluted sample, multiply the calculated value by the appropriate dilution factor.

5. Any diluted samples must be further converted by the appropriate dilution factor.

## Laboratory protocol for H.pylori stool Ag Rapid Test

**Specimen Collection and Handling** Consider any materials of human origin as infectious and handle them using standard biosafety procedures [57].

**Procedure A: Solid stool samples**

1. Collect a random stool sample in a clean, dry receptacle.

2. Open the stool collection device by unscrewing the top and use the collection stick to randomly pierce the stool sample in at least five different sites. Do not scoop stool sample as this may lead to an invalid test result.

3. Ensure stool sample is only in the grooves of the collection stick. Excess stool sample may lead to an invalid test result.

4. Replace the collection stick and tighten securely to close the sample extraction tube.

5. Shake the sample extraction tube vigorously.

**Procedure B: Watery stool samples**

1. Collect a random stool sample in a clean, dry receptacle.

2. Open the sample extraction tube by unscrewing the top.

3. Fill the plastic dropper with the sample; dispense 2 drops (70-85 μL) into the sample extraction tube.

4. Replace the collection stick and tighten securely to close the stool collection device.

5. Shake the sample extraction tube vigorously.

Note: Specimens extracted may be stored at 2°C-8°C for up to 3 days. If longer storage is required, freezing at ≤-20°C is recommended.

## Procedure

Step: 1 Bring the specimen and test components to room temperature if refrigerated or frozen.

Step: 2 when ready to test open the pouch at the notch and remove the test device. Place the test device on a clean, flat surface.

Step: 3 shake the sample collection tube vigorously to ensure a homogenous liquid suspension.

Step: 4, Position the stool collection device upright and twist off the dispenser cap. Holding the sample extraction tube vertically, dispense 2 drops of the solution into the sample well of the test device. Do not overload sample.

Step: 5 set up the timer.

Step: 6 Results can be read 15 minutes after adding the specimen. Positive results can be visible in a time period as short as 1 minute.

***Do not read results after 20 minutes. To avoid confusion, discard the test device after interpreting the result.***

**Interpretation of Assay Result**

1. Negative Result: If only the C line is developed, the test indicates that no detectable H.pylori antigen is present in the specimen. The result is negative.

2. Positive Result: If both C and T lines are developed, the test indicates the presence of H.pylori antigen in the specimen. The result is positive.

3. Invalid: If no C line is developed, the assay is invalid regardless of any colour development on the T line as indicated below. Repeat the assay with a new test device. **Excess faecal specimen can lead to invalid test results; if this is the cause, re-sample and re-test (*see instructions for collection of specimen).***

## Laboratory protocol for H.pylori stool Ab Rapid Test

### Specimen Collection and Handling

Consider any materials of human origin as infectious and handle them using standard biosafety procedures [58].

**Plasma**

1. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively in Vacutainer® ) by vein puncture.

2. Separate the plasma by centrifugation.

3. Carefully withdraw the plasma into new pre-labeled tube.

**Serum**

1. Collect blood specimen into a red top collection tube (containing no anticoagulants in Vacutainer®) by veinpuncture.

2. Allow the blood to clot.

3. Separate the serum by centrifugation.

4. Carefully withdraw the serum into a new pre-labelled tube. Test specimens as soon as possible after collecting. If the specimens aren‟t tested immediately store at 2°C-8°C.

### Assay procedure

Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen. Mix the specimen well prior to assay once thawed.

Step 2: When ready to test, open the pouch at the notch and remove the test strip. Place the strip on a clean, flat surface.

Step 3: Fill the plastic dropper with the specimen. Holding the dropper vertically, dispense 1 drop (about 30-45 µL) of specimen into the sample pad making sure that there are no air bubbles. Then add 1 drop (about 35 – 50 µL) of Sample Diluent immediately.

Step 4: Set up timer

Step 5: Results can be read in 15 minutes. Positive results can be visible in as short as 1minute. Don‟t read result after 15 minutes. To avoid confusion, discard the test device after interpreting the result.

**Interpretation of assay result**

1. NEGATIVE RESULT: If only the C band is developed, the test indicates that no detectable antibodies to H. Pylori are present in the specimen. The result is negative.

2. POSITIVE RESULT: If both C and T bands are developed, the test indicates for the presence of antibodies to H. Pylori in the specimen. The result is positive. Samples with positive results should be confirmed with alternative testing method(s) and clinical findings before a positive determination is made.

3. INVALID: If no C band is developed, the assay is invalid regardless of color development on the T band as indicated below. Repeat the assay with a new device.

## Differential WBC count Protocol for absolute eosinophil count

Providing the thin blood film iswell made and correctly stained. Allow the stained film to dry completely before examining it [59, 60].

1. Place a drop of immersion oil on the lower third of the blood film and cover with a clean Cover glass.

2. Examine the film microscopically. Focus the cells using the 10objective with the condenser iris closed sufficiently to see the cells clearly. Check the staining and distribution of cells.

3. Move to a part of the film where the red cells are just beginning to overlap and bring the 40 objective into place. Open the iris diaphragm more.

4. Systematically examine the blood film and count the different white cells seen in each field, preferably using an automatic differential cell counter, or if this is not available, record the count in chart form

5. Calculate the absolute number of each white cell type by multiplying the number of each cell counted (expressed as a decimal fraction) by the total WBC count

6. Report the presence of white cell and red cell abnormalities.

**Differential WBC reference range***

*These values are guideline figures only. They should be checked locally. Neutrophil counts are lower in Africans and Afro-Caribbean people.

**Absolute number Percentage**

ADULTS

Neutrophils . . . . . 1.5–7.5 109/l (40–75%)

Lymphocytes‡ . . . 1.2–4.0 ““(21–40%)

Monocytes . . . . . 0.2–1.0 ““(2–10%)

Eosinophils . . . . . 0.02–0.6 ““(1–6%)

Basophils . . . . . 0.01–0.1 ““(0–1%)

CHILDREN (2–6 y)

Neutrophils. . . . . 1.5–6.5 109/l (20–45%)

Lymphocytes . . . 6.0–8.5 “ “ (45–70%)

Monocytes . . . . . 0.1–1.0 “ “ (2–10%)

Eosinophils . . . . . 0.3–1.0 “ “ (1–6%)

Basophils . . . . . 0.01–0.1 “ “ (0.1–1%)

‡In an adult, lymphocytes are mainly of the small type whereas in a child, large lymphocytes predominate.

## Skin prick test protocol for sensitization test

**Procedure**

1. Seat the study subject as comfortable as possible and ask them to hold their left forearm towards you.

2. Explain that you are going to measure the skin‟s response to some solutions, which this will not hurt but that occasionally it can be itchy.

3. Use a biro and the ruler to draw an 8cm line longways down the middle of the palmar side of the forearm.

4. Label sections from the top: N (saline); Dp (Dermatophagoides); C (Cockroch); P (Histamine)

5. Use the dropper in the bottle to put a tyni drop of each solution at the correct leabl.

6.Place the skin prick lancet in to the drop of solution almost parallel with the skin, press the tip in lightly so it just catches, then lift the skin for 2 seconds and release. Use a skin prick lancet for each drop of solution, or the solution from one may contaminate the next.

7. Wait 15 minutes. You may use this time to start filing in the questionnaire.

8. For each section, use the ruler to measure the biggest diameter where the skin is raised as well as red.Record this as „Dl"on the sheet. Then measure the diameter at right angles to the first. Record this as „D2". Don"t forget to record the participant"s identification number.

## Declaration

I the undersigned, declare that this is my original work and has not been presented for a degree in this or any other university and all sources of materials used for this thesis have been acknowledged.

Name: Mheret Tesfaye (BSc)

Signature _____

Place _____

Date of submission _____

This thesis has been submitted with my approval as advisors.

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