Seroprevalence and Risk Factors of Hepatitis E Virus Infection among Pregnant Women in Selected Health Facilities of Addis Ababa, Ethiopia

By

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This is to certify that the thesis prepared by Meseret Abebe Buketie, entitled "**Seroprevalence and risk factors of Hepatitis E virus among pregnant women in selected health facilities Addis Ababa Ethiopia"** submitted in partial fulfillment of the requirements of the Degree of Masters of Sciences in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Dedication

This M.Sc. thesis is dedicated to my beloved mother, W/ro Asamina Adane.

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Acronyms and Abbreviation

A	Absorbance
Ab	Antibody
AHRI	Armauer Hansen Research Institute
ALT	Alanine aminotransferase
ANC	Antenatal Care
ART	Anti Retroviral Treatment
AVH	Acute Viral hepatitis
C.O	Cut-off Value
CFR	Case-fatality rate
CMV	Cytomegalovirus
DNA	Deoxyribonucleic Acid
EIA	Enzyme ImmunoAssay
ELISA	Enzyme Linked Immuno Sorbent Assay
ELISPOT	Enzyme-Linked Immunosorbent Spot
EMA	Ethiopian Medical Association
EMLA	Ethiopian Medical Laboratory Association
EPHA	Ethiopian Public Health Association
FHF	Fulminant Hepatic Failure
HCC	Hepatocellular Carcinoma
HEV	Hepatitis E Virus
HIV	Human Immunodeficiency Virus
HRP	Horse Radish Peroxidase
IFN	Interferon
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin
IP-10	Inducible protein-10
IRB	Institutional Review Board
IUD	Intrauterine death
NIPH	Norwegian Institute of Public Health
NK	Natural Killer
OPD	Outpatient Department

ORFs	Open Reading Frames
PCR	Polymerase Chain Reaction
RNA	Ribonucleic Acid
SOPs	Standard Operating Procedures
SPSS	Statistical Package for the Social Sciences
Th	T helper cell
TNF	Tumor Necrosis Factor
VCT	Voluntary Counseling and Testing
WHO	World Health Organization

Abstract

Background: Hepatitis E virus (HEV) is highly endemic in several African countries with high mortality rate among pregnant women. The prevalence of antibodies to HEV in Ethiopian pregnant women is not known. This study aimed to assess the prevalence of anti-HEV IgG and anti-HEV IgM among pregnant women seen between April 2014 to January 2015 in Gandhi Memorial Hospital and four selected Health centers in Addis Ababa, Ethiopia.

Material and methods: A total of 386 serum samples were collected from pregnant women. All pregnant women socio demographic characteristics were collected using a structured questionnaire form. Serum samples were examined for anti-HEV IgG and anti- HEV IgM using ELISA. The association of anti-HEV status with risk factors was assessed. Factors demonstrating significant association in bivariate analysis were included in multivariate logistic regression models. Analyses were performed using SPSS version 21.

Results: From 386 women, 122 (31.6%) cases were positive for anti- HEV IgG and two women (0.5%) were anti-HEV IgM positive. Age and educational had statistically significant association with HEV infection. There was no significant association between anti-HEV antibody seroprevalence rate with trimester, parity, HIV status and other risk factors.

Conclusion: Conclusion: This study found a high seroprevalence rate of anti-HEV IgG among pregnant women in Addis Ababa Ethiopia. Preventive measures like improvement of education in personal and public hygiene may reduce the risk in pregnant women. Moreover nationwide surveillance of HEV especially in rural setting should be conducted to establish a national estimate and validate our findings.

Keywords: Seroprevalence, Hepatitis E virus, pregnant women, Addis Ababa Ethiopia

1. Introduction

1.1. Back ground

Hepatitis E virus (HEV) is a small non-enveloped, positive-sense single-stranded RNA virus. It has been classified as the single member of the genus Hepevirus and has a similar structure to the viruses of the Caliciviridae and Tombusviridae families. This virus was first discovered during an outbreak in New Delhi, India, in 1955 (1-3) .Although hepatitis E virus (HEV) is sometimes referred to as an emerging infectious agent, it is well-established as a major cause of acute viral hepatitis (AVH) worldwide. An estimated one-third of the world's population has been infected with HEV (4). Of the more than 20 million infections estimated to occur globally each year ~70,000 infections result in death. The vast majority of these deaths occur in resource-poor countries in Asia, Africa, and Latin America (5).

The clinical symptoms are typical of acute viral hepatitis and include jaundice, malaise, anorexia, nausea, abdominal pain, fever and hepatomegaly; anicteric hepatitis is also observed (6). HEV infection is usually self-limiting, but may develop into fulminant hepatitis with a case-fatality rate (CFR) between 1 and 2% in the general population , which can rise to over 40% in pregnant women, especially during the third trimester of pregnancy (4).HEV infection is one of the predominant causes of pregnancy related complications in developing countries (7). HEV infection during pregnancy leads to severe complications which may result in fetal and/or maternal mortality, abortion, premature delivery, or death of a live-born baby soon after birth (7, 8).

HEV is transmitted primarily by the fecal–oral route, and fecally contaminated drinking water is the most frequent cause of transmission. Transmission may occur vertically. Preterm delivery in mothers with hepatitis E is common and associated with poorer neonatal survival (9, 10).Other less common routes are blood transfusion and organ transplantation.

HEV replicates in the cytoplasm, with a subgenomic RNA producing the ORF2 and ORF3 proteins and the full genomic RNA encoding nonstructural proteins and serving as a template for replication (11). It replicates in hepatocytes but also in the small intestine, colon, and lymph nodes (12).The incubation period between infection and clinical signs is variable, usually between 3 and 8 weeks with an average of 40 days.

HEV infection elicits both immunoglobulin M (IgM) and IgG antibodies against HEV. The IgM anti HEV response is rapid, occurring about a month after infection and peaking at the time of onset of biochemical abnormalities and/or symptoms (13). Anti-HEV IgG follows shortly after detection of IgM; however, anti-HEV IgG peaks several weeks later, and can be detected many months and years after infection (14).

HEV infection can be diagnosed either indirectly by detecting serum anti-HEV antibodies or directly by detecting the HEV genome in blood or other bodily fluids. The presence of anti-HEV IgM is a marker of acute infection (15). Increased titers of anti-HEV IgG can indicate recent HEV infection (16, 17). Most primary serological testing uses an EIA format. These assays use recombinant antigens derived from different strains of HEV. This diversity of strains should not affect the accuracy of the assays because it seems that HEV viruses of different genotypes constitute a single serotype (16, 18). HEV RNA was usually detected in both serum and stool in late latent period and early acute infection of patients with HEV. In some HEV cases with both negative results of anti-HEV IgM and IgG, HEV RNA was positive. It is believed that HEV RNA detection is of great help to make a diagnosis in HEV early infection (17).

Diagnostic criteria for acute HEV infection among patients with acute hepatitis would be as follows: (1) IgM negative, RNA positive (window period); (2) IgM positive, RNA positive (early seroconversion stage); (3) IgM positive, RNA negative (postseroconversion stage); and (4) seroconversion to IgG antibody on follow-up (19).

In immunocompetent individuals, acute hepatitis E does not usually require antiviral therapy. The disease typically resolves within 4-6 week of the onset of symptoms, usually without any long-term consequences .Some patients might require treatment of symptoms. A patient infected with HEV genotype 3 who developed severe acute hepatitis E and impaired liver function was treated with ribavirin monotherapy (13, 17).

Treatment options for patients with chronic hepatitis E include reduction of immunosuppression and administration of pegylated interferon alfa or ribavirin. Protective immunity can also be induced by vaccination. Two candidate HEV vaccines have been developed and have been found to be safe and immunogenic. HEV infection can be prevented by providing clean drinking water and improving the sanitary infrastructure in developing countries (15, 17).

Screening and follow up studies of pregnant women and their newborns for HEV infection are important for improving knowledge about the epidemiology (transmission and circulation) and transmission pathway of this virus (20).In Ethiopia, there is no recent study conducted on HEV among pregnant women. However ,A study conducted in 1993 by Tsega E and his colleagues on 32 pregnant and 34 non-pregnant Ethiopian women revealed that Nineteen (59%) pregnant women have hepatitis E virus (HEV) infection as compared to 7 (22%) in the non-pregnant group (21). Since the result shows high prevalence and there is no recent study on seropevalence of HEV among pregnant women. It is important to have up to date data of HEV infection among pregnant women.

1.2. Statement of the Problem

Hepatitis E virus (HEV) is a major public health problem in developing countries. HEV infection in pregnant women is more common and more often fatal in the third trimester. The mortality rate due to HEV-induced hepatitis is as high as 15-20 % (20). Moreover, HEV in pregnancy is associated with high rates of preterm labour and mortality. Plus, death of a live born baby soon after birth is common. Transplacental transmission of HEV in the third trimester of pregnancy has been described; Vertically transmitted HEV infection is known to cause acute hepatitis in newborn babies; it is associated with a high perinatal mortality of the affected newborns.HEV can also cause premature births Thus, HEV infection in pregnancy leads to poor maternal and fetal outcome(22).

A study was conducted in 1993 by Tsega E and his colleagues on 32 pregnant and 34 non-pregnant Ethiopian women with sporadic acute viral hepatitis revealed that Nineteen (59%) pregnant women have hepatitis E virus (HEV) infection as compared to 7 (22%) in the non-pregnant group(21) .

This study, on its part, took a small sample size that compromises the validity of the findings, the samples are drawn purposively from women with sporadic acute viral hepatitis and far from being a prevalence study as well as it has been longtime since the study was carried out and cannot indicate us the current condition of the problem. Thus, it can be safely said that there is no prior study conducted on the prevalence of HEV among pregnant women except the effort that was mentioned above despite the major fetal and maternal health problems caused by Hepatitis E virus reported elsewhere .The present study, therefore, is provoked by this state of knowledge in the country and aim to be conducted taking an empirical research setting at Gandhi Memorial Hospital and four health centers located in Addis Ababa Ethiopia.

1.3. Significance of the study

On a practical level, this study may help to develop strategies that aimed at reducing new HEV maternal infection. In addition, it also supports in the process of designing protective and control measures such as the implementation of health education on hygiene based interventions or treatments for mothers as well as their infants that reduce the spread of the disease in the community. It also opens a good opportunity to increase awareness on its magnitude in the community.

Moreover, the result of this study may be used by program managers, health planners or policy makers, and other concerned bodies to initiate the relevant intervention measures and screening packages in the antenatal care clinics. It is important to have clear data about the prevalence rate of HEV in a country in order to facilitate the possible introduction of large scale national surveillance program and delivery of vaccination in the future and in consequence prevent the maternal and congenital mortality.

By and large, this study will help to provide better insight to concerned stake holders and health planers to design an informed strategy to reduce such life threatening but preventable viral infection and made future initiatives formulated in a way that could protect pregnant women and their new born.

1.4. Literature Review

1.4.1. Hepatitis E Virus

1.4.1.1. Virus Biology

HEV is a small and structurally simple RNA animal virus. The virion is nonenveloped with anicosahedral capsid and a size of 27 to 34 nm is composed entirely of viral protein and RNA (18). It is the sole member of the genus Hepevirus within the family Hepeviridae and has a 7.2-kb positive-sense RNA genome.

The HEV genome contains three open reading frames (ORFs). ORF1 is involved in viral replication and protein processing through RNA-dependent RNA polymerase. ORF2 encodes the viral capsid protein, which is involved in attachment to host cells and induction of neutralizing antibodies. Finally, ORF3 encodes for a small immunogenic phosphorylated protein (pORF3) involved in virion morphogenesis and release (15, 23)

1.4.1.2. Mode of transmission

The main route of human HEV transmission is fecal-oral. In epidemic conditions, HEV is transmitted mainly by drinking fecally contaminated water. (Table 1) Recent evidence suggests significant person-to-person transmission in outbreak situations although it is not clear whether this mode of transmission is of comparable magnitude to the person-to-person transmission of HAV infection (3).There have been reports of transfusion related transmission of HEV infection(24). Subsequent studies have found a high prevalence of vertically transmitted HEV infection (3, 7).Preterm delivery in mothers with hepatitis E is common and associated with poorer neonatal survival (25).To date, there has been no evidence for sexual transmission of HEV (26).

1.4.1.3. Risk Factors

The risk factors for HEV infection are related to resistance of HEV to environmental conditions, poor sanitation in large areas of the world, and HEV shedding in feces (18) .

Poor hygienic conditions as a result of poor environmental sanitation facilities have been implicated in the wide distribution of HEV infections in Africa and Asia. This condition has made some developing countries of Asia and Africa and Mexico endemic for the virus and its infections (3, 13, 27).

The spread of HEV to the industrialized countries had been made possible via travelers to and from endemic regions. Sporadic cases of acute hepatitis E without an implicated travel history have also been reported in Europe and the United States (28).Consumption of raw or undercooked pork products is commonly thought as a relevant risk factor for acquisition of HEV in Europe.

Among risk factors associated with hepatitis E, contact with swine seems to increase HEV prevalence. In the United States and Sweden, studies on HEV prevalence among swine handlers and veterinarian workers have shown higher prevalence in these populations (13% versus 9.3% for control subjects in Sweden) (29) .

1.4.1.4. Immunology

The presence of infiltrating lymphocytes of a cytotoxic/suppressor immunophenotype suggests a cell-mediated immune mechanism for hepatocytes damage during HEV infection. Wu et al, studied HEV specific T-cell response together with IgM anti-HEV antibodies in acute hepatitis E patients by enzyme-linked immunosorbent assay and enzyme-linked immunosorbent spot (ELISPOT) assay respectively .Strong HEV specific cellular immune responses occurred against capsid protein, HEV 239 that decreased along with the decreasing IgM anti-HEV antibody titer and normalization of liver function (30).

The decrease in HEV specific T-cell responses with convalescence suggests the involvement of T cell responses in the pathogenesis of acute hepatitis E and recovery Prabhu et al , more

specifically demonstrated the involvement of activated CD8+ T-cells containing granzymes in acute liver failure cases of HEV infection (31). The absence of Treg cells in HEV infected liver tissues suggests that no regulation of immune mediated killing during HEV infection might be associated with severity of the disease (32).

Tripathy et al studied the frequency of peripheral Treg cells (CD4+CD25+Foxp3+ and CD4+CD25Foxp3+) in acute hepatitis E patients by flow cytometry and HEV specific cytokines/chemokines quantitation and compared it with recovered individuals and healthy controls. The median percentage of CD4+CD25+Foxp3+ (True Treg) and CD4+CD25Foxp3+T (Effector Treg) cells in acute hepatitis E patients were significantly higher compared to controls and recovered individuals. The level of IL-10, a signature cytokine of Tregs, was also elevated in the similar manner. The elevation in frequencies of Treg cells and rise in IL-10 cytokine suggests the role of these cells in infection and recovery of hepatitis E (33).

Cells from patients and controls exposed to HEV ORF 2 proteins did not differ in production of IFN, TN alpha, or IL-4; however, IFN gamma was elevated in the supernatants of cultures from ORF2-stimulated PBMCs from patients compared with controls, and up regulation of IFN gamma mRNA transcription was detected only among hepatitis E patients (34). Natural killer (NK) cells may play an important role in mediating the immune response to HEV infection (35).

1.4.1.5. Pathogenesis and clinical presentation

The HEV target population is young to middle aged adults, 15 to 40 years of age. The clinical symptoms are typical of acute viral hepatitis and include jaundice, malaise, anorexia, nausea, abdominal pain, fever and hepatomegaly; anicteric hepatitis is also observed (6).

The pathogenesis of hepatitis E is poorly understood. Entry of the virus into the host is believed to be primarily by the oral route via contaminated water or food. The primary site of HEV replication has not been identified and is believed that virus first replicate in the intestinal tract from where it reaches to the liver presumably via the portal vein. It then replicates in the cytoplasm of hepatocytes and released into the bile and the blood by an unknown mechanism (32).

1.4.1.6. Epidemiology

HEV is the leading cause of acute viral hepatitis in the world .Every year an estimated 20 million HEV infections occur globally resulting in more than 3 million cases and 70,000 deaths. Most cases occur in developing countries where occasional large scale outbreaks also occur(13, 23, 36).Mortality of this infection in general population is about 1-2% that dramatically increasing to 20- 30% in pregnant women (37).The disease is endemic in large parts of Asia, Africa and Latin America from where epidemic and sporadic disease has been reported (Figure 1A) (6) .

On genomic sequence analysis, human and swine HEV isolates group into four genotypes (Table 1), namely genotypes 1, 2, 3 and 4, each with several subtypes. However, HEV has only one serotype(38) .

Table 1 clinical and epidemiological characteristics of HEV infection according to genotypes (Adapted from Hoofnagle J *et al*.,2012)

Characteristics	**Genotype 1 and 2 (Epidemic)**	**Genotypes 3 and 4(Autochthonous)**
Geographic distribution	Developing countries only	Both developing and developed countries
Pattern of spread	Epidemic and sporadic	Sporadic
Species specificity	Human	Swine, human (humans are accidental host)
Major mode of spread	Fecal-oral, waterborne	Food born
Secondary spread	Uncommon	Extremely rare
Rate of icteric illness	High	Low
Age distribution	Disease rates highest among adolescents and young adults	Disease rates highest among older adults
Sex distribution	Similar disease rates among men and women	Highest disease rates among men
Mortality	High among pregnant women	High among older adults
Extra-hepatic features	Few	Neurological complications
Chronic infection	None	Common in Immunosuppressed persons

The phylogenetic analysis divided HEV genotype I into five subtypes, genotype II into 2 subtypes, whereas genotypes III and IV were divided into 10 and 7 subtypes (39).

Each HEV genotype appears to have a specific geographical distribution (Figure 1B).Genotype 1 is the most frequent cause of epidemic and sporadic hepatitis E in the developing world. HEV genotype 2 was first identified from the 1986 epidemic in Mexico and subsequently from Chad and Nigeria (28). Genotype 3 was first identified in human cases of locally acquired hepatitis E in the United States (US); Since then, genotype 3 isolates have been reported from human cases in several industrialized countries in Europe (United Kingdom [UK], France, Netherlands, Spain, Austria, Greece, Italy), Japan, Australia and New Zealand; some genotype 3 sequences have also been reported from Korea and Argentina (38) .

HEV genotype 4 was first described in Taiwan and subsequently found in China, Japan Vietnam and India. Genotypes 3 and 4 also have been isolated from swine in the United States, Africa and Asia (28).

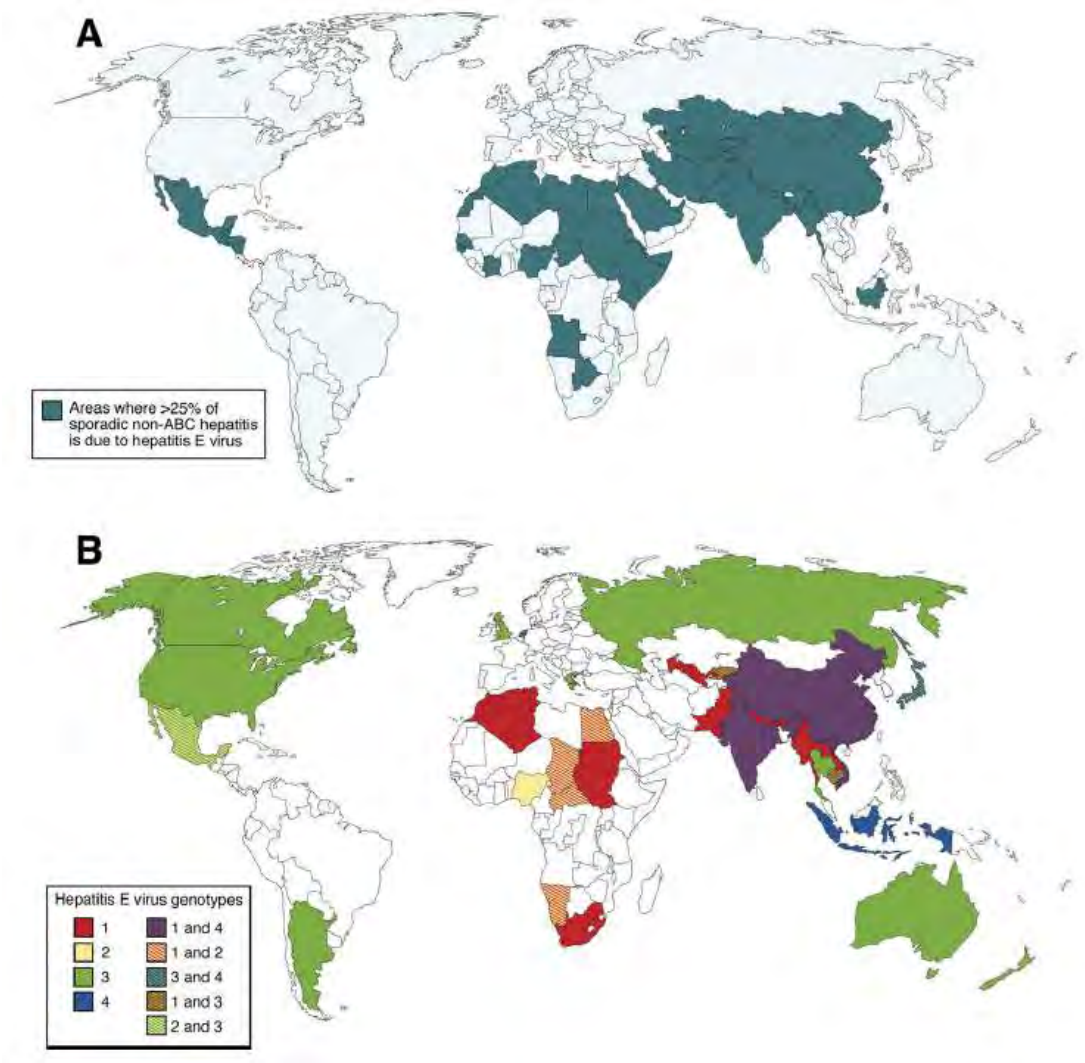


Figure 1 (A) Worldwide prevalence of HEV and (B) the geographic distribution of different HEV genotypes (Adapted from Wedemeyer H *et al*.,2012)

1.4.1.7. Laboratory Diagnosis

Although HEV particles have been visualized by electron microscopy in the stools of infected human beings and there has been recent progress with cell culture, the routine laboratory diagnosis of hepatitis E depends on serology and nucleic acid amplification techniques. Most primary serological testing uses an EIA format, although rapid immunochromatographic assays have been develop (17, 26).Commercial enzyme immunoassays and rapid immunochromatographic kits based on ORF2/ORF3 peptides or recombinant antigens from

HEV1 can detect the presence of IgM or IgG antibodies induced by the four major genotypes of HEV, representing a single serotype. There is no genotype-specific serologic testing. No cross-reactivity of HEV antigen with another pathogen has been reported (40) .

Following an incubation period of 2 to 6 weeks, an initial short lived IgM response is followed by longer-lasting IgG antibodies. At clinical presentation, anti-HEV IgM levels had already reached the peak level, but it remained at relatively high levels for 8 weeks. IgM antibodies declined rapidly thereafter, falling below the cutoff level among most patients after 32 weeks. The presence of anti-HEV IgM is a marker of acute infection. HEV IgG levels were rising when patients presented. The antibody reached peak levels about 4 weeks after the onset of symptoms and was maintained at high levels for more than 1 year. Increased titers of anti-HEV IgG can indicate recent HEV infection (15) .

HEV RNA was usually detected in both serum and stool in late latent period and early acute infection of patients with HEV. In some HEV cases with both negative results of anti-HEV IgM and IgG, HEV RNA was positive. It is believed that HEV RNA detection is of great help to make a diagnosis in HEV early infection (17).

1.4.1.8. Treatment

HEV infection is usually self-limiting and so only supportive treatment is needed. There is no established therapy for hepatitis E virus (HEV) infection. The successes with ribavirin have led to its use for severe, acute hepatitis E, with promising results (3), but ribavirin is contraindicated in pregnancy due to teratogenicity and fetal loss (41). Liver transplantation is the only treatment for patients with fulminate hepatic failure. Treatment options for patients with chronic hepatitis E include reduction of immunosuppression and administration of pegylated interferon alfa or ribavirin Peginterferon, ribavirin, or a combination of the two agents leads to viral clearance in most patients and a sustained response in a high proportion of patients. Ribavirin alone in doses of 600 to 800 mg daily for 12 weeks yields sustained virologic responses in at least two thirds of patients with chronic hepatitis E (17, 42).

1.4.1.9. Prevention and control

The main way to prevent HEV infection in developing countries is by maintaining good hygiene and to supply a safe water source (13) . HEV3 infection may be prevented by avoiding eating undercooked meat, especially pork products. Note that it has been shown that HEV is completely inactivated when heated above 70°C (43)

Following subsequent work, HEV vaccine has been developed. The HEV 239 vaccine (Hecolin®) was shown to induce immediate immunity to HEV following two doses within one month with 100% efficacy against symptomatic HEV, lasting five months until the third dose. This supports its use in limiting an outbreak of HEV. No adverse effects of the vaccine have been observed in pregnancy and its efficacy and safety have been confirmed in a phase III trial. The HEV 239 vaccine is currently licensed for use in China (41).

1.4.2. HEV and Pregnancy

1.4.2.1. Clinical presentation of HEV in pregnancy

HEV infection can lead to more severe, acute liver disease in pregnant women and sometimes progress to fulminant hepatic failure (FHF). Fulminant HEV infection in pregnancy contributes to highest mortality rate of the fetus and mother. The mortality in the second trimester is around 20% and reaches up to 45% in the third trimester (44).It has been reported that a significant proportion of pregnant women with acute hepatitis E (up to 70%) progress to acute liver failure with a short pre-encephalopathy period, rapid development of cerebral edema and high occurrence of disseminated intravascular coagulation (6). In a large-scale prospective study from Northern India on maternal and fetal outcomes of HEV, approximately 60% of viral hepatitis in pregnant women was due to HEV infection. Moreover, FHF was more likely among HEV-infected women (55%), who were at 2.7 times greater risk than non-HEV infected women (20%).Maternal mortality secondary to FHF was 6 times higher in the HEV infected group (41%) compared to the non-HEV group (7%) (9, 45).

Vertical transmission of HEV from a pregnant woman to her unborn fetus is very well documented. Khuroo *et al* investigated fetal outcomes of HEV infection in pregnant women and

found in utero transmission with fetal outcomes ranging from intrauterine fetal death to symptomatic and asymptomatic neonatal liver infection (46).

1.4.2.2. Immunological changes during pregnancy and HEV infection

Pregnancy in women is associated with an altered status of sex steroid hormones and immunity. Steroid hormones directly influence viral replication through their effects on viral regulatory elements .Extrapolation from serial measurements of IFN gamma and IL-6 (as biomarkers of Th1- and Th2-type responses, respectively) in 35 healthy pregnant women in Quebec suggested that Th1-type responses prevail until the mid-second trimester, with IFN-gamma decreasing and IL-6 increasing from the 10th to 40th weeks of gestation(47, 48) .Serial serum samples from a cohort of 50 healthy pregnant and postpartum women in New York City showed elevated levels of TNF-alpha throughout pregnancy compared with 6 months postpartum(49) .These women also experienced increases in levels of IP10 and decreases in IFN-gamma during gestation(49, 50) .

There is a direct correlation between decreased cell-mediated immunity and a reduction in specific T cell populations, which is significant in the third trimester of pregnancy. During pregnancy, a shift from T helper cell type 1 (Th1) - dominated to T helper cell type 2 (Th2)-dominated immune responses, or "Th2 bias," has been hypothesized to help protect the fetus by suppressing macrophage activation (51). Th1-type responses are marked by increases in IFN-gamma synthesis, and this type of response appears to help protect against parasitic infections (5). Th2 responses, on the other hand, are tipped toward anti-inflammatory cytokines, such as IL-4, IL-6, and IL-10, and increases in antibody production (48, 52).

Malnutrition and reduced immune response have also been postulated to be responsible for the increased severity of viral hepatitis in pregnant women (53).

The central epidemiological question in hepatitis E is its increased severity in pregnancy. There are still no concrete answers. Pregnant monkeys experimentally infected with HEV do not show this differential effect and studies in human patients have not adequately addressed this issue. Much of known about this is the potential role of HEV proteins in pathogenesis is based on their

over-expression in cultured cells, which itself can be misleading. There has been an increased understanding of the role of pORF3 in HEV pathogenesis, but almost all the information is derived from over-expression studies. The importance of this protein in HEV pathogenesis is however reinforced by the lack of experimental infection in monkeys by an ORF3-null virus (6).

Patients with fulminate hepatic failure (FHF) show Th 1 biasness in terms of higher IL-12/IL-10 ratio. Thus, this shift of Th2 biasness, which is a characteristic of normal pregnancy, in the HEV infected pregnant women, is suggestive of the role of immunological shift during hepatitis E related FHF in pregnancy. This immune alteration in turn may lead to reduced fetal protection which is probably due to higher activity of NK cells leading to fetal death. Viral load is comparatively higher in FHF than AVH and also higher in patients with fetal morality in both AVH and FHF, suggesting its role with the disease severity. High viral load and Th1 immunological state together may attribute to the poor pregnancy outcome in hepatitis E (1) .

Jilani *et al* found that HEV infected pregnant women with fulminant hepatic failure had lower CD4 count and higher CD8 counts. They also observed that the levels of estrogens, progesterone and beta-HCG were significantly higher in the above-mentioned group when compared to HEV negative patients or control healthy pregnant females (6, 53) Although the levels of hormones were physiologically high in the normal control population; patients with HEV infection seem to have higher levels than controls, which probably explain the direct interaction of HEV with the immune system.

In another interesting study, Pal *et al* studied the cellular immune response in both pregnant and non pregnant women with acute hepatitis E and the control population they found that pregnant women with HEV had generalized immune suppression characterized by decrease in lymphocyte response to phytohemagglutinin (PHA) with a predominant Th2 bias as compared to non pregnant women with hepatitis E and normal healthy controls.

This study was important from a number of perspectives. The thought that normal pregnancy is an immunosuppressed state is challenged because normal healthy pregnant women did not demonstrate decreased response to PHA. Also nonpregnant patients with HEV did not show any defective PHA response either highlighting that HEV by itself does not produce the

immunological changes and needs a pregnancy as a physiological state to produce the above mentioned changes (53, 54).

1.4.2.3. Seroprevalence of HEV antibodies

Rates of anti-HEV antibody in the general population are lower in Europe and the United States than in Asia and Africa (3). In a study from Switzerland, prevalence rates of IgG anti-HEV antibody in pregnant women was 2.1% (2 of 94)(55) .

In a study that included 245 Turkish pregnant women (average age 26.3 ±7.6 years) and 76 age-matched controls, 31 (12.6%) pregnant women and nine (11.8%) controls were found to be anti-HEV IgG positive (56). The positivity rates were higher in illiterate women, those from lower income group and those with rural residence. In another study of 386 pregnant Turkish women (257 urban and 129 rural; mean age 24.28±4.56 years), anti-HEV IgG was detected in 27 (7.0%) (57) .Women with higher educational level were less likely to be seropositive, but there was no difference between the seropositive and the seronegative women in age, source of water supply and residence.

In a study of HEV seroprevalence in Saudi Arabia among 469 pregnant women, 93 (20%) were anti-HEV positive and 28 (30%) of these 93 were HEV-RNA positive and symptomatic with on-going infection. Four women developed FHF (58) .

Hepatitis E virus (HEV) is highly endemic in several African countries with high mortality rate among pregnant women(59).Seroprevalence of HEV varies by country from 84.3% among pregnant Egyptian women to 0% among village residents in Gabon (4).

In one study, 157 Ghanaian pregnant women were screened for the presence of anti-HEV IgG and anti-HEV IgM antibodies.The HEV sero-prevalence rate among pregnant women was 28.66% (45 out of 157). Of the HEV IgG seropositive pregnant women, 64.40% (29 out of 45) tested positive for anti-HEV IgM. The overall prevalence rate of antibodies to HEV was highest (46.15%) among pregnant women 21–25 years of age, followed by 42.82% in ≤ 20 year group, then 36.84% in ≥ 36 year group. There was no correlation between increasing age and HEV sero-positivity. HEV seroprevalence detected in women in their third trimester of pregnancy (30.25%;

36 out of 119) was higher, than in women in their second trimester of pregnancy (25.0%, 9 out of 36). There were only two women in their first trimester of pregnancy and they were negative for both IgG and IgM anti- HEV antibodies (60).

In a seroprevalence study of 404 Tunisian women, the prevalence of anti-HEV IgG and anti-HEV IgM, was 12.1 % and 0 % respectively. In multivariate analysis age (>30 years) and the number of persons per room (>2) in the house were independent factors predicting HEV infection. History of agricultural work, kind of water, sewage treatment, and use detergent to wash vegetables, contact with animals and parenteral risk factors were not correlated with the presence of anti-HEV IgG (61).

In another prevalence study of anti-HEV IgG in 840 samples collected from pregnant women living in the five main cities of Gabon, central Africa. They found that 14.1% (119/840) of pregnant women had anti-HEV IgG. The prevalence differed between regions and between age groups. From their finding of a higher risk HEV infection in urban than in rural areas, They also found a highest seroprevalence in the younger (14–20 years; 14.8%) and older (31–44 years; 16.8%) age groups than in the others (59).

Another study in Burkina Faso reports a 11.6% seroprevalence anti-HEV IgG among 819 pregnant women. The anti-HEV seroprevalence of pregnant women from Burkina Faso was comparable to those found in Tunisia (12.1%) and Turkey (12.6%).However in a study of sera comprising 453 healthy Moroccan pregnant women, IgG anti- HEV antibodies were detected in only 3.96% of subjects (62).

In a seroprevalence study that included 2,428 Egyptian pregnant women, the prevalence of anti-HEV was 84.3%. In this study, the seroprevalence rate of anti HEV varied with the place of residence, and increased with increasing age from 70% in those aged 20 years or below to over 90% in those aged 30 years or more (63).

In a study done by Edemariam Tsega and his colleagues on 32 pregnant and 34 non pregnant Ethiopian women between 15 and 45 years of age with sporadic acute viral hepatitis 19 (59%) pregnant women had the hepatitis E virus (HEV) infection as compared to 7 (22%) in the non

pregnant group. Of a total of 10 maternal deaths, 8 occurred in association with HEV infection. In addition to 6 foetal losses as a result of maternal death, there were 2 foetal deaths and 7 premature deliveries as a direct result of acute viral hepatitis, all but 2 associated with HEV infection. Comparison of socioeconomic and nutritional status, clinical features, mean aminotransferase and bilirubin levels did not show differences in the two groups and they conclude that pregnant women are more at risk to acquire HEV infection than nonpregnant women and HEV infection in this group of Ethiopian pregnant women is associated with high maternal mortality and neonatal complications (21).

An outbreak of acute hepatitis E virus (HEV) infection occurred from October 1988 to March 1989 in military camps in northern Ethiopia. The epidemic was waterborne and entirely confined to military men, of whom 423 hospitalized, icteric patients were studied. The clinical course was mild and short, without any fulminant hepatitis or death (64). Another outbreak from April 2014 to January 2015 was reported among refugees residing in the Gambella region. Among total 1,117 suspected cases of HEV, there were 21 (1.9%) deaths, eighteen (1.6%) cases occurred among pregnant or postpartum women, two of whom died (case fatality rate = 11%)(65).

2. Objectives

2.1. General Objective

To determine the seroprevalence and risk factors of HEV infection among pregnant women attending antenatal clinic (ANC) in selected Health facilities of Addis Ababa, Ethiopia from April 2014- January 2015.

2.2. Specific Objectives

1. To determine the seroprevalence of HEV IgG among pregnant women attending ANC in selected Health facilities of Addis Ababa, Ethiopia.
2. To determine the seroprevalence of HEV IgM among pregnant women attending ANC in selected Health facilities of Addis Ababa,Ethiopia.
3. To identify risk factors for HEV infection among pregnant women attending ANC in selected Health facilities of Addis Ababa, Ethiopia.

3. Material and Methods

3.1. Study Design and period

Institution based Cross sectional study was conducted from April 2014 to January 2015.

3.2. Study Area

The study was carried out in Gandhi memorial Hospital and Bole 17, Woreda 23(coca),Woreda 3 (Gebreal) and Arada health centers of Addis Ababa Ethiopia. Gandhi memorial Hospital is a governmental Hospital which is specialized in maternity. This Hospital is under the Addis Ababa city government health bureau and is located in the central part of Addis Ababa, Kirkos sub city. The hospital gives 10-15 delivery services per day. Beside maternity service it provides ART and VCT and there is also a rape treatment clinic in the hospital. Bole 17, Woreda 23(coca),Woreda 3 (Gebreal) and Arada health centers are under Addis Ababa city government health bureau and they are found in Bole, Addis Ketema, N/lafto and Arada sub cities in Addis Ababa. They give OPD, family planning, ART, laboratory, pharmacy, ANC and delivery services to the community. At the time of the study, 8-12 new pregnant women attended ANC daily, and the health centers gave 2-5 delivery services per day.

3.3. Populations

- **Source population:** All pregnant women who were attending antenatal care during the study time.
- **Study population:** All consented pregnant women who have given a blood sample until the required sample size is reached.

3.4. Study Variables

Dependent Variables	Independent Variables
Seroprevalence of HEV IgG and IgM among pregnant women	Age, residence, educational level, marital status, ethnicity occupation, monthly income Parity, trimester,HIV status, source of drinking water, hygiene, pet owner ship, having blood transfusion,

3.5. Inclusion and exclusion criteria

- Inclusion criteria: All pregnant women who have given an informed consent for participation.
- Exclusion criteria: Those pregnant women who were unable to communicate due to mental problem, coma, ambulatory.

3.6. Sample size determination and Sampling

Sample size determination: The sample size was calculated based on single sample size estimation as shown below. The value of p taken as 50% (0.5) because there was no previous prevalence study on HEV among pregnant women in Ethiopia.

$$n = \frac{(Z\alpha/2)^2 P (1 - P)}{d^2}$$

Where n = sample size,

Z = Z statistic for a level of confidence,

P = expected prevalence or proportion (P = 0.5),

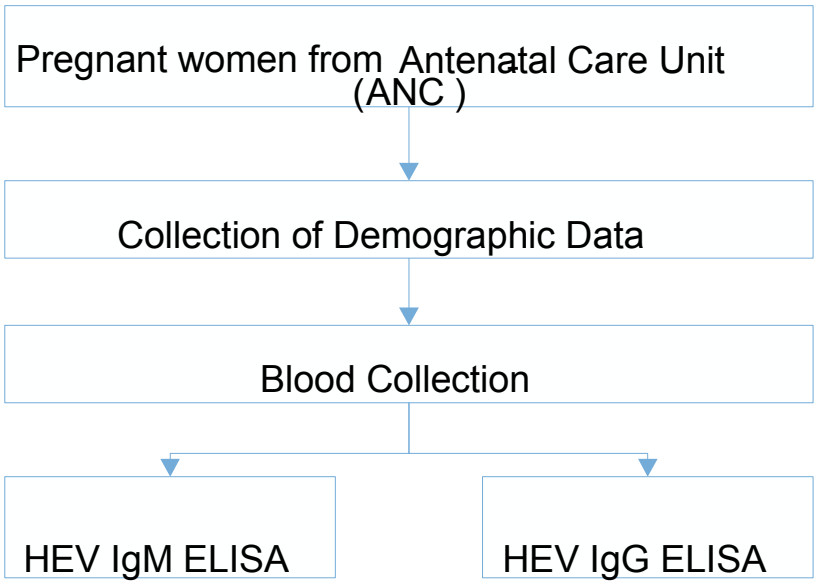
and d = precision (d = 0.05; Z = Z statistic: For the level of confidence of 95%, which is conventional, Z value is 1.96.

$$\frac{(1.96)^2 \ 0.5(1-0.5)}{(0.05)^2} = 384$$

Therefore the sample size was: 384

Sampling technique: Gandhi memorial Hospital and those four Health centers were selected using a non-probability convenient method from a total public Hospitals and Health centers. Sample size was assigned to each selected health facility proportional to the number of pregnant women who had follow up in the facility.70 pregnant women were enrolled from each health centers and 106 pregnant women were recruited from Gandhi memorial Hospital. A total of 386 study participants were enrolled by using consecutive sampling techniques. Pregnant women attending the ANC were consecutively enrolled until the desired sample size was reached. They were asked to participate in the study by the data collector. Following their consent, each was interviewed individually and blood sample was taken.

3.7. Work flow



3.8. Data collection methods

Socio demographic data collection: Mothers who consented to participate were subjected to a face-to-face interview with the data collector whereby pre-test counseling was done, and then the questionnaire was filled in to obtain information on relevant medical, obstetrical and socio-demographic characteristics.

Blood sample collection: Five ml of blood sample was collected in a vacutainer tube and sera were separated by centrifugation and kept at -20oC until used.

3.9. Anti HEV IgG and Anti HEV IgM ELISA

HEV ELISA was performed at the Immunology Laboratory of Armauer Hansen Research Institute. All of the sera were screened in duplicate for IgG and IgM to HEV using ELISA Kit (WANTAI HEV Ab ELISA China) which shows high sensitivity and specificity compared to other assays including molecular methods (4).

3.9.1. Anti-HEV IgG Assay

3.9.1.1. Principle of the test

WANTAI HEV-IgG ELISA employs solid phase, indirect ELISA method for detection of IgG-class antibodies to HEV (anti-HEV) in two-step incubation procedure. Polystyrene microwell strips are pre-coated with HEV recombinant antigen. During the first incubation step (at 37°C for 30 minutes), HEV specific antibodies, if present,will be bound to the solid-phase pre-coated HEV antigens. The wells are washed to remove unbound serum proteins and then, anti-human IgG antibodies (anti-IgG) conjugated to horseradish peroxidase (HRP-conjugate) is added. During the second incubation step (at 37°C for 30 minutes), these HRP-conjugated antibodies will be bound to the antigen-antibody (IgG) complexes previously formed and the unbound HRP-conjugate is then removed by washing. Chromogen solutions containing Tetramethylbenzidine (TMB) and urea peroxidase are added into the wells and the plate was incubated in a dark place at 37°C for 15 minutes and in presence of the antigen-antibody-anti-IgG (HRP) immunocomplex, the colorless chromogens are hydrolyzed by the bound HRP-

conjugate to a blue-colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity can be measured and is proportional to the amount of antibody captured in the wells,and to the sample respectively .Wells containing samples negative for HEV-IgG remain colorless.

3.9.1.2. Calculation and Interpretation of results

The presence or absence of anti-HEV IgG antibody was determined by relating the absorbance of the specimen to the cut-off value, which is the mean absorbance of the negative control plus 0.16. The presence or absence of HEV IgG in the sample was determined by the amount of color intensity. The amount of color intensity is proportional to the amount of antibody captured in the wells, and to the amount of antibody in the sample respectively. Wells containing samples negative for HEV IgG were colorless.

Specimens giving a value less than the Cut-off value were negative for this assay, which indicates that no anti-HEV IgG antibodies have been detected with WANTAI HEV-IgG ELISA; therefore there are no serological indications for infection with HEV. (A / C.O. < 1)

Specimens giving a value equal to or greater than the Cut-off value were considered initially reactive, (A / C.O. ≥ 1) which indicates that anti-HEV IgG antibodies have probably been detected with WANTAI HEV-IgG ELISA.

Specimens with A value to Cut-off ratio between 0.9 and 1.1 were considered borderline and retested to confirm the initial test result.

3.9.2. Anti-HEV IgM Assay

3.9.2.1. Principle of the test

WANTAI HEV-IgM ELISA is two-step incubation, solid-phase antibody capture ELISA assay in which polystyrene microwell strips are pre-coated with antibodies directed to human immunoglobulin M proteins (anti-μ chain). The patient's serum/plasma sample is added, and during the first incubation step (at 37°C for 30 minutes) , any IgM-class antibodies will be captured in the wells. After washing out all the other substances of the sample and in particular IgG-class antibodies, the specific HEV IgM captured on the solid phase is detected by the addition of recombinant HEV ORF2 antigen conjugated to the enzyme horseradish peroxidase (HRP-conjugate). During the second incubation (at 37°C for 30 minutes), the HRP-conjugated antigens will specifically react only with anti-HEV IgM antibodies. After washing to remove the unbound HRP-conjugate, chromogen solutions are added into the wells and the plate was incubated in a dark place at 37°C for 15 minutes . In presence of (anti-μ) - (anti-HEV-IgM) - (HEV Ag-HRP) immunocomplex, the colorless chromogens are hydrolyzed by the bound HRP-conjugate to a blue-colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity can be measured and is proportional to the amount of antibody captured in the wells, and to the amount of antibody in the sample respectively.

3.9.2.2. Calculation and Interpretation of results

The presence or absence of anti-HEV IgM antibody was determined by relating the absorbance of the specimen to the cut-off value, which is the mean absorbance of the negative control plus 0.26. The amount of color intensity is proportional to the amount of antibody captured in the wells, and to the amount of antibody in the sample respectively. Wells containing samples negative for HEV IgM were colorless.

Specimens giving a value less than the Cut-off value were negative for this assay, which indicates that no anti-HEV IgM antibodies have been detected with WANTAI HEV-IgM ELISA; therefore there are no serological indications for infection with HEV. (A / C.O. < 1)

Specimens giving a value equal to or greater than the Cut-off value were considered initially reactive, (A / C.O. ≥ 1) which indicates that anti-HEV IgM antibodies have probably been detected with WANTAI HEV-IgM ELISA..

Specimens with A value to Cut-off ratio between 0.9 and 1.1 were considered borderline and retested to confirm the initial test result.

3.10. Quality Assurance

Pre-analytical Consideration: Protocol for sample collection, transportation and processing was strictly followed. The quality of the questionnaire preparation has been checked and pre-tested before the detailed work is started. Data collectors were trained and informed prior to data collection time. In addition, there was a daily follow up by the principal investigator and supervisors.

Analytical Consideration: Like that of the pre analytical stage of quality assurance, the manufacturers' instructions were followed strictly while performing the above mentioned tests by complying with the SOPs. The following procedure was the quality control procedure that was done for the tests that we have performed.

Quality control for HEV IgG/IgM ELISA: In each plate, three negative controls and two positive controls were included. Each microplate was considered separately when calculating and interpreting the results of the assay, regardless of the number of plates concurrently processed. The results were calculated by relating each specimen absorbance value to the Cut-off value (C.O.) of the plate. The Cut-off reading is based on single filter plate reader and the results were calculated by subtracting the Blank well absorbance value from the print report values of specimens and controls.

The test results were valid if the A value of the Blank well, which contains only Chromogen and Stop solution, is < 0.080 at 450 nm. The absorbance values of the Positive control must be ≥ 0.800 at 450/630nm or at 450nm after blanking. The absorbance values of the negative control must be ≤ 0.100 at 450/630nm or at 450nm after blanking. When one of the negative control absorbance values did not meet the Quality Control criteria, it was discarded, and the mean value

should be calculated by using the remaining two values.When more than one Negative control absorbance values did not meet the Quality Control range specifications, the test was regarded as invalid and repeated.

Post-analytical Consideration: The result obtained was collected on data collection sheet. In relation to data entry and analysis, the data were double entered and cleaning was done accordingly. Before dispatching of all the laboratory tests, the laboratory results were checked by senior personnel working in AHRI laboratory.

3.11. Statistical analysis & interpretation

Double entry of data was done using Microsoft Excel 2007 and analysis of the data is performed by SPSS version 21.The descriptive statistics such as frequency, median and percentage were calculated. Statistical significance association between dependent and independent variables were evaluated using Chi-square test (Pearson or Fisher exact test). P-value < 0.05 was considered as indicator for statistical significance. Variables that showed significant association were selected for further analysis and binary logistic regression was performed (with a p-value < 0.05 regarded as statistically significant). Strength and direction of association between dependent and independent variables was interpreted using an adgusted odds ratio and the statistical significance of odds ratio evaluated by 95% confidence interval. Finally, the results were presented on graphs and tables.

3.12. Ethical Considerations

The proposal was approved by " Research and Ethical Review Committee" of the Department of Medical Laboratory Science Collage of Health Sciences Addis Ababa University, Armauer Hansen Research Institute (AHRI) Ethical Review Committee" and Addis Ababa city government health bureau. Permission was also obtained from the Hospital and Health centers administrators. Written informed consent was sought from all study participants. Positive laboratory results were communicated to Physicians.

3.13. Dissemination of results

The results of the study will be submitted to the department of Medical Laboratory Sciences, School of Allied Sciences, College of Health Science, and Addis Ababa University and Armauer Hansen Research Institute (AHRI). The principal investigator will submit the study abstract to local associations (like EMA, EPHA and EMLA) and other international associations to present the results of the project during continuous medical education events or conferences organized by these associations. The summary of the thesis will be submitted to the international or national peer reviewed journal for publication.

3.14. Operational Definitions

Seroprevalence: The proportion of individuals with Hepatitis E Virus positive status.

Seropositive: The presence of HEV antibody in the participants' serum.

Seronegative: The absence HEV antibody in the participants' serum.

Sensitivity: is the proportion of people who have the disease who test positive for it. Sensitivity relates to the test's ability to identify positive results.

Specificity: is the proportion of patients who do not have the disease who will test negative for it. Specificity relates to the ability of the test to identify negative results.

Fulminant hepatitis: Severe and rapidly progressive loss of hepatic function due to hepatitis E virus infection with associated coagulopathy and encephalopathy.

4. Result

4.1 Socio demographic Characteristics

A total of 386 pregnant women were screened for the presence of anti-HEV IgG and anti-HEV IgM antibodies. Their ages ranged from 16 to 40 years, with a mean age ± SD of 28.9 ± 5.76 years. Among the study subjects 90.4% were married and 59.8 % of the study subjects belonged to 26-35 years of age.The predominant religion was Christian (81.6%), and the majority (48.7%) belongs to Amhara ethnic -group. Only 32(8%) pregnant women were illiterates. Sociodemographic characteristics of the participants are presented in Table 2

Socio-demographic Characteristics	Frequency
Age	
<=25	141(36.5)
26-35	231(59.8)
>=36	14 (3.6)
Religion	
Christian	315 (81.6)
Muslim	61 (15.8)
Don't response	10 (2.6)
Marital status	
Married	349 (90.4)
Single	28 (7.3)
Divorced	5 (1.3)
Cohabiting	4 (1)
Educational status	
No formal education	32 (8.3)
Primary	113 (29.3)
Secondary	137 (35.5)
College and above	104 (26.9)
Occupation	
Government	55 (14.2)
Self employed	129 (33.4)
House wives	180 (46.6)
Student	22 (5.7)
Ethnicity	
Amhara	188 (48.7)
Oromo	75 (19.4)
Tigray	26 (6.7)
SNNP	83 (21.5)
Others	14 (3.6)

Pregnant women from all trimesters were included in the study (Figure 2). Of the 386 pregnant women, only 17 (4.4%) of them had received blood from a donor previously. In relation to HIV status, 368 (95.3 %) of the participants were seronegative.

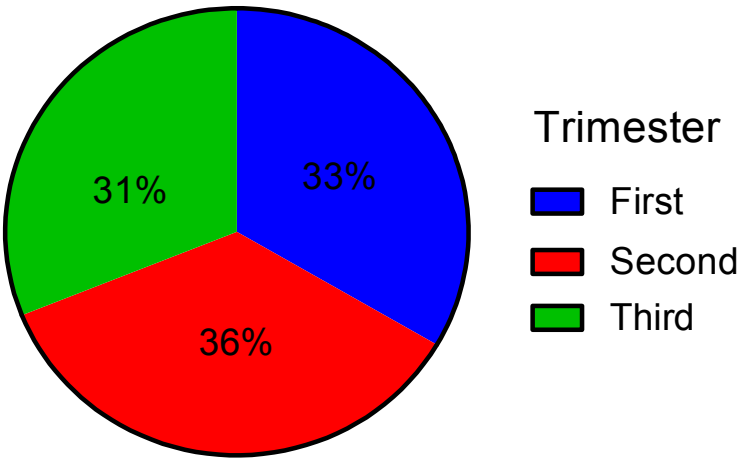


Figure 2 Study participants stratified by trimester

4.2. Magnitude of HEV Infection.

Overall, the HEV sero-prevalence rate among pregnant women recruited in to the study over the 6 month period was 31.9 %.Of the total study participants , 0.5 % (2 out of 386) tested positive for anti-HEV IgM whereas 31.9 % (122 out of 386) tested positive for anti-HEV IgG.

4.3. Risk factors associated with HEV Infection

Anti-HEV seroprevalence had a strong association with age of the participant ($p< 0.05$). As shown in figure 3 the overall prevalence rate of antibodies to HEV was highest (`78.5%) among pregnant women ≥ 36 year group, followed by (33.7 %) in 26-30 years of age, then (23 %) in ≤ 25 year group. The odds of pregnant women whose age ≤ 25 and 26-35 being infected by HEV is 10 and 2 times lower than the odds of pregnant women whose age is ≥36, respectively (Table 3).

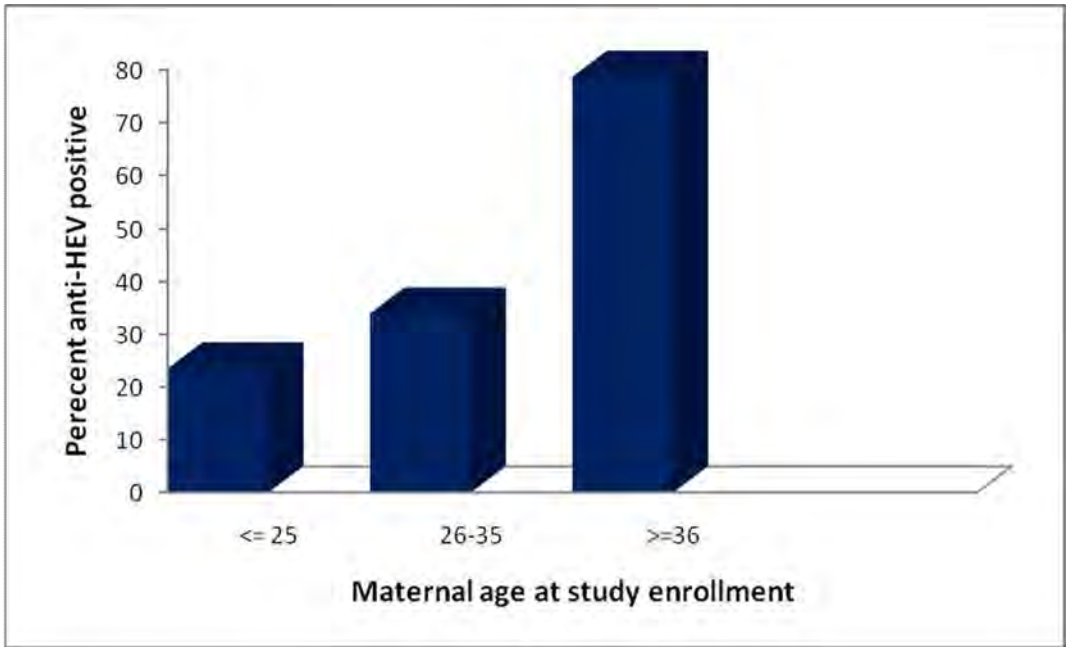


Figure 3 Anti-HEV seroprevalence stratified by age

There was a strong statistical association ($p<0.05$) between educational level and HEV seroprevalence. Anti-HEV reactivity among pregnant women with primary education (36%; 41/113), was higher than that of their counterparts with secondary (35 %, 49/137), and college and above (22 %; 23/ 104) level of education.

In multivariate analysis, parity was not significantly associated with HEV infection, but showed an association in bivarate analysis (95 % CI (1.385-9.143) $p<0.05$). Highest rate of infection (55%) was seen in pregnant women who have two children and above compared with those who have one child and pregnant women with first pregnancy experience.

The number of positive cases varied in different periods of pregnancy. HEV seroprevalence detected in women in second trimester of pregnancy (40%; 49/137) was the highest, followed by women in their first trimester of pregnancy (32.7%, 40/129) then women in their third trimester (27%, 33/120). However, anti-HEV reactivity was not significantly associated with the stage of pregnancy ($p>0.05$).

The risk of infection with HEV through blood transfusion was assessed. Subjects with a history of blood transfusion had higher seropositivity (41%) than those with no history of transfusion (31%), but the result was not statically significant. ($p > 0.05$).

When potential risk factors were adjusted using multivariate analysis age and educational level remained the only significant factors. Our data did not show any stastical significant association between HEV infection with marital status, HIV status, washing hand after defecation and pet ownership (Table 3).

Table 3 Risk factors associated with maternal anti-HEV: bivariate and multivariate analysis

Variable	Total	HEV IgG sero status		COR (95%CI)	p -value[a]	AOR(95 % CI)	p- value[b]
		Pos n(%)	Neg n (%)				
Age							
<=25	141	33(23.4)	108(76.6)	1			
26-35	231	78(33.8)	153(66.2)	1.688 (1.04-2.69)	0.035*	1.689(1.07-2.823)	0.047*
>36	14	11(78.6)	3(21.4)	12 (3.16-45.6)	0.000*	9.88 (2.436-40.12)	0.001*
Trimester							
First	129	40(31)	89(69)	1			
Second	137	49(35.8)	88(64.2)	1.185(0.69-2.05)	0.544		
Third	120	33(27.5)	87(72.5)	1.468(0.86-2.45)	0.157		
Marital status							
Married	349	113 (32.4)	236(67.6)	1			
Single	28	6(21.4)	22(78.6)	0.48(0.067-3.44)	0.464		
Cohabited	4	2(50)	2(50)	0.273(0.032-2.36)	0.238		
Divorced	5	1(20)	4(80)	0.25(0.013-4.739)	0.355		
Education level							
Illiterates	32	9(28.1)	23(71.9)	1.37(0.561-3.386)	0.484	1.234(0.536-3.362)	0.530
Primary	113	41(36.3)	72(63.7)	2.0 (1.099-3.659)	0.023*	2.257(1.215-4.193)	0.010*
Secondary	137	49(35.8)	88(64.2)	1.96(1.098-3.503)	0.023*	1.824(1.003-3.317)	0.049*
College and above	104	23(22.1)	81(77.9)	1			
Parity							
1st pregnancy	176	45(25.6)	131(74.4)	1			
1-2	185	64(34.6)	121(65.4)	1.54(0.978-2.425)	0.063	1.224(0.788-2.001)	0.421
2-3	20	11(55)	9(45)	3.55(1.385-9.143)	0.008*	2.236(0.810-6.170)	0.120
Morethan3	5	2(40)	3(60)	1.941(0.314-11.9)	0.475	0.794(1.08-5.823)	0.821
HIV status							
Positive	18	6(33.3)	12(66.7)	1.086(0.398-2.96)	0.872		
Negative	368	116(31.5)	252(68.5)	1			
Blood Transfusion							
Yes	17	7(41.2)	10(58.8)	1.55(0.574-4.164)	0.389		
No	369	115(31.2)	254(68.8)	1			
Wash hand							
Yes	372	115(30.9)	257(69.1)	1			
No	14	7(50)	7(50)	2.235(0.76-6.518)	0.141		
Pet ownership							
Yes	104	36(34.7)	68(65.3)	1.16(0.717-1.874)	0.548		
No	282	88(31.3)	194(68.7)	1			

*significant at p<0.05, COR-crude odds ratio,ADR-Adjusted odds ratio, p -value[a] for COR, p – value[b]– for AOR, 1-logical reference

5. Discussion

Hepatitis E virus is a major cause of liver disease worldwide, and although long-term sequelae are rare, the disease carries appreciable mortality in pregnant women (27).

Our study showed that the overall seroprevalence of HEV infection among study population in selected health facilities, Addis Ababa Ethiopia was (31.6 %) which is consistent with a study done in Darfur, Western-Sudan where the seroprevalence of hepatitis E virus in pregnant women was 31.1 % (66). Our finding was higher than the result of similar studies done in BurkinaFaso (11.6%) Gabon (14.1%) and Ghana (28.6%) (59, 60, 62), but lower than the sero-prevalence of HEV infection among pregnant women in Egypt (84.3%), Sudan (41%) and India (33.6%)(40, 63, 67).

In the developed world the rate is significantly low. A study in spain by Lindemann et al on 1040 pregnant women reported the rate of anti-HEV IgG was 3.6%. Prevalence of HEV IgG was found to be 7.7% and 10 % in pregnant women in France and China, respectively which is much lower than our finding (68-70). Regarding anti-HEV IgM antibodies, 0.5% (2 /386) of pregnant women were positive. Other have reported prevalence rate of 0% in France, 0.64 % in Spain and 10% in Ghana (60, 69, 70).

Reason for these differences could be due to difference in level of hygiene, educational status, social status, endemicity of virus, different lifetime exposures of the participants to HEV and use of different test systems with varying sensitivity.For exampl in Egypt a study found higher HEV seroprvalence among pregnant women (84.3 %).Their result confirmed that Egypt's high HEV endemicity and show that almost all women of child bearing age in the community had prior HEV exposures without a history of liver disease. They suggested that reasons for high HEV sero prevalence and lack of clinical hepatitis could be the result of early childhood HEV exposures, producing long-lasting immunity and/or modify subsequent responses to exposure.(63)

Mansuy et al. recently reported 53% prevalence of HEV antibodies in blood donors in southwestern France (71), a figure considerably higher than the 17% prevalence reported earlier for the same geographic region, when a different test system was used (72).

High rates of HEV infection are usually seen in areas with low standard of living where major contamination of water supply is likely to occur. These conditions include, low standard of hygiene, lack of proper disposal system plus unsafe water supply (73).

We found a significant association between age and higher anti-HEV positive values ($p<0.05$) which was consistent with a study done by Stoszek *et al*. (63).Our findings showed that the seroprevalence of hepatitis E virus in the age range of >=36 years was much higher (78.5%) than in <=25 years old (23.4%). The strong association between age and HEV seroprevalence in our study most likely reflects cumulative lifetime exposure to the virus.

Most studies demonstrated role of education in decline of HEV seroprevalence, and prevalence of hepatitis E in educated women is significantly low (37, 40) which was in agreement with our study, where women with college and above educational level have the lowest seropositivity from all study participants (22% 23/104) and education level has significant association with HEV infection ($p<0.05$).

HEV infections are spread mainly by the faecal-oral route (39), but we found that washing hands with soap after defecation has a border line significant association (95 % CI 0.08-1,$p=0.07$) with seropositivity after adjustment. This could be due to the fact that the questionnaire used for the case-control study may have been susceptible to responder bias. For example, as is common in many settings, proper hygiene and sanitation practices may have been over reported by respondents.

In our study most of the respondents, 97.4% (376/ 386) used piped water, 1.8% (7 /386) used bottled water,0.5 % (2/386) used river water where as only one of the participant used well water and we did not find any significant association of source of water with HEV positivity , but our result was not in agreement with other studies which reported that the main source of HEV infection was drinking of contaminated water especially slowly moving water (74).This could be

due to the fact that since most of the participants were sourced from urban population of the capital city Addis Ababa, it was difficult to compare water source and HEV positivity on this insufficient data and further study should be conducted on rural setting of Ethiopia to prove this assumption.

Interesting area of HEV infection is its relation with pregnancy and consequence of disease. Pregnant women, particularly in second and third trimesters are more affected with high rate of morbidity and mortality (37). A study conducted in Ghana has revealed a result that anti-HEV antibodies detected in women in their third trimester of pregnancy (30.2%) was significantly higher, $p < 0.05$, than in women in their second trimester of pregnancy (25.0%) whereas a study conducted in Gorgan, Iran demonstrated higher seropositivity of HEV in pregnant women in second trimester significantly (37, 60) but unlike other studies in the present study no significant association has been found between HEV positivity and trimester. In addition with immunological differences of the participants and endemicity of the virus in different countries ,the reason for the difference could be due to the fact that in some studies the researchers didn't take equal proportions of study participants from all trimesters and this will cause a big impact on data analysis.For example a study conducted on 157 Ghanian pregnant women, 119 (75.8%) were in their third trimester of pregnancy while 36 (22.9%) were in their second trimester and only 2 pregnant women were in their first trimester of pregnancy which was not proportional.

There is evidence that HEV/HIV co-infected pregnant women have higher HIV RNA load(70) although the data from Africa in this regard is very scarce. Even though it was not statistically significant, in our study HIV infected pregnant women have higher HEV antibodies (38.8%, 7/18) compared to HIV negative pregnant women (31% 115/368).Similarly, a study conducted in southwest England showed no difference in anti-HEV seroprevalence between patients with HIV infection and control subjects [34] ,However our result was not consistent with a study done in the Russian Federation which showed an association between a higher HEV prevalence and more advanced HIV related disease and with a study conducted in central Africa which gives evidence that HIV-1-infected women are at risk for acute or severe infection if they are exposed to HEV during pregnancy (75).This might be because of common drug-induced liver injury among patients receiving antiretroviral therapy (76).

In India, where human HEV is endemic, anti-HEV IgG have been identified in different animal species including dogs and rodents. Of the 44 dogs screened, 10 were positive (22.7%). Among rodents, over 50% serum samples were positive for anti-HEV antibodies (77).The presence of anti-HEV in a cat of an acutely infected patient in Japan suggests cats may be reservoirs for human HEV infections (78) . These findings are different from our study where ownership of pet animals was not significantly associated with HEV infection. This might be due to the fact that in Addis Ababa, dogs and cats spend most of their time usually outdoors and their contact with the house holders is minimal. But since most of the people in Addis Ababa live in densely populated areas where the sanitary conditions are very deplorable and also where rats share their habitat, the potential transmission by rodents should be taken into account.

A study conducted in Northern India has shown that the low socio-economic status of pregnant women appeared to be the only risk factor associated with HEV seropositivity (40), but in our study it was difficult to assess the hypothesis that correlates income with seropositive status., because most of the pregnant women were not volunteer to fill their income on the questionnaires. But the high prevalence of HEV infection in pregnant women in our study might be due to the fact that in the study the samples were collected from governmental health institutions in which medical care is free and therefore women of low socio-economic status frequently attend and our study population may lack a portion of pregnant women with higher socioeconomic and educational level.

Documented direct evidence for transfusion transmitted HEV infection has been reported in several countries (79). But , in our study we found no significant association, this could be due to the fact, in our study only 4% (17/386) of the participants has received blood previously, so it is difficult to determine the association of the variable with HEV seropositivity with this insufficient data.

Regarding to HEV IgM antibody, since only two study participants were HEV IgM positive it was impossible to perform statistical analysis. Both of HEV IgM positive pregnant women had similar age which was 28. They were married and at their first trimester of pregnancy. One of HEV IgM positive study participant was HIV positive.

6. Limitation

This study was limited to a small subset of population in Addis Ababa. Hence the results cannot be generalized for the whole country. Furthermore, the exclusion of pregnant women seen at private health facilities and in rural settings could also affect our findings.

There was also lack of molecular techniques (HEV RNA detection) for confirmation of our results.

All of the risk factors for the acquisition of HEV infection were not be addressed in the questioner. However, the findings of this study have adequately shed light into the problem of Hepatitis E infection among pregnant women in Ethiopia.

7. Conclusion

In conclusion, the seroprevalence of HEV among pregnant women in this study is high. Age and educational level had significant association with HEV infection. Old age and poor education are predicators of HEV infection, indicating continued exposure as well as feco-oral transmission of the virus. There was no difference between the anti-HEV antibody seroprevalence rates among the study participants in terms of trimester, source of water, habit of washing hand after defecation, HIV status, marital status, pet ownership and history of blood transfusion.

8. Recommendation

Based on the findings of our study the following recommendations are forwarded

- Preventive measures to decrease the spread and transmission of HEV are warranted. These measures should include improvement of education in personal and public hygiene and systematic HEV screening of pregnant women in order to counsel them about the risk of contracting and transmission of HEV.
- Since the findings of this study are limited by the small sample size of pregnant women, more extensive studies should be conducted to evaluate the seroprevalence, to characterize the circulating HEV genotypes and to determine the current pathological and risk status in the general population of Ethiopia.
- A further larger-scale survey of HEV infection among pregnant women in urban and rural settings is important to evaluate the cost-effectiveness of antenatal HEV screening in Ethiopia

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Annex

Annex I: Participant information sheet (English Version)

Department of Medical Laboratory Science, Collage of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia

Title: Seroprevalence and risk factors of HEV infection among pregnant women in Addis Ababa. Ethiopia.

First of all we would like to thank you in advance for your cooperation and consent in participation in this study. Please read or listen when it is read for you about the general information of the study. If you have any question regarding the study please ask freely.

Background information

Background

Hepatitis E virus (HEV) is a major public health problem in developing countries. HEV infection in pregnant women is more common and more often fatal in the third trimester. It has been reported that a significant proportion of pregnant women with acute hepatitis E (up to 70%) progress to acute liver failure with a short pre-encephalopathy period, rapid development of cerebral edema and high occurrence of disseminated intravascular coagulation. It disseminates orofecaly. Vertical transmission of HEV infection from mother to infant, although rare, has been reported. The mortality rate due to HEV-induced hepatitis is as high as 15-20 per cent.

However HEV infection might be prevented by simple hygienic precautions, such as frequent and thorough hand washing. If the pregnant women become positive for HEV virus they will be given antiviral treatments.

Aim of the study

The purpose of this study is to determine the seroprevalence of HEV among pregnant women and to identify the potential risk factors of acquiring HEV in Addis Ababa, Ethiopia.

Benefits for participants

The study participants may receive no monetary benefit from this study. However, knowledge gained from this study may help in the management of infectious diseases in the future. Those positives for HEV will be contacted with the physician in the hospital for treatment and follow up and also Psychosocial support will be facilitated to the participants by providing information on care, rehabilitation and support for the mothers in case of confirmed infection by linking the child and mother to child rehabilitation service providers and social workers. Most importantly, this study will contribute to provide base line information or data for the nationwide study and to develop health programmers for health policy makers.

Risks and complication

There is no considerable risk to the study subjects in participating in the study other than the possible minor bleeding from the site of venipuncture when they give sample. Venipuncture is a routine clinical practice for blood sample collection and has minimal risk, and the amount of blood collected will be 5 ml (1 to 2 tea spoon) blood only.

Right of withdrawal

You have a right to refuse information, decline to cooperate and drop out of the study if you want and none of your action will have any bearing at all on your overall health care. Your blood sample will be discarded if you are not interested by the laboratory investigation.

Confidentiality

Confidentiality will be respected and no information that discloses participant's identity will be released without consent. The study participants will be identified by a participant identification number. Only interested participants can retrieve their own laboratory result using their code

number, and the information can only be accessed through the physician in charge of the mother to child department. The physician will be responsible for the interpretation of the results and providing treatment.

Assurance of Principal Investigator

I put my signature below to confirm you that I take over the responsibility for the scientific ethical and technical conduct of the research project and for provision of progress reports for all stakeholders of the research project.

Meseret Abebe (PI)

Signature: _____ Date: _____

Note: If you have any questions about this study, you should feel free to ask now or anytime throughout the study by contacting:

PI Address: Meseret Abebe: Department of Medical Laboratory Sciences, Collage of health sciences, Addis Ababa University, Addis Ababa, Ethiopia

E-mail: Mesinia90@gmail.com; Tel.: +251913081982

Department of Medical Laboratory Sciences Tel.: +251-0112755170

Annex II: Participant Informed consent

I have been informed about the study which plans to determine the seroprevalence of HEV and the risk factors for acquiring HEV on pregnant women in Addis Ababa, Ethiopia. The objective and the application of the study were briefly explained to me. I am also informed that all information contained within the questionnaire is to be kept confidential. Moreover, I have been well informed of my right to refuse information, decline to cooperate and drop out of the study if I want and none of my actions will have any bearing at all on my overall health care.

It is therefore with full understanding of the situation that I agreed to give the informed consent voluntarily to the researcher to give my blood for the mentioned study. I agreed that the specimen would be tested for HEV detection. I have had the opportunity to ask questions about the project and received clarification to my satisfaction in a language I understand. I was also told that results for the analysis of blood for HEV will be given to the health facility and that I may ask the information if I want.

I _____ hereby give my consent for giving of the requested information and specimen for this study.

Participant code: _____ Signature: _____

Date: _____

Annex III: Questionnaire

Addis Ababa University Collage of Health Sciences Department of Medical Laboratory Science questionnaire for the demographic characteristics and assessment of risk factors of HEV among pregnant women in Addis Ababa, Ethiopia.

Facility name _____ Year _____ Participant code _____Participants address (Sub city) _____ Telephone _____ signature_____

Data collector name_____date_____signature_____.

I. Socio- Demographic Characteristics of the Study participants.

1. Registration no ----------
2. Age _____
3. Address
   a) Urban ☐
   b) Rural ☐
   c) Region ----------
   d) Zone ----------
   e) Woreda----------
   f) Kebele----------
   g) Telephone no----------
4. Marital status (a) Married ☐ (b) Unmarried ☐ (c) Divorced ☐ (d) Widowed ☐ e) Cohabiting ☐
5. Education level attained. (a) No formal education ☐ (b) Primary education ☐ (c) Secondary education ☐ (d) Collage and above ☐

6. Occupation.

(a) Government employee ☐ (b) Self employee ☐ (c) House wife ☐ (d) Student ☐

7. Monthly income (in Ethiopian birr) _____

8. Ethnicity. (a) Amhara ☐ (b) Oromo ☐ (c) Tigray ☐ (d) SNNP ☐ (e) Others (specify) _____

9. Trimester (a) First ☐ (b) Second ☐ (c) Third ☐

10. Parity a) first pregnancy ☐ b) 1-2 ☐ c) 2-3 ☐ (d) More than 3

11. HIV status (a) Positive (b) Negative

II. Assumed potential risk factors for acquiring HEV in the study subjects

12. What kind of water source that you use for drinking.

(a) Piped water ☐ (b) River water (c) Well water (d) Other specify----------

13. Did you wash hands with soap and water after returning from toilet?

(a) Yes ☐ (b) No ☐

14. Have you Pet in the house hold?

(a)Yes ☐ (b) No ☐

15. Have you ever had a blood transfusion?

(a) Yes (b) No

Annex IV: Participant information sheet (Amharic Version)

የተሳታፊወች መረጃ ቅጽ

አዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የህክምና ላቦራቶሪ ሳይንስ ዲፓርትመንት

አርዕስት፡ በአዲስ አባባ ከተማ፣ የሔፓታይተስ ኢ ቫይረስ በሽታ በእርጉዝ ሴቶች ላይ ያለውን ስርጭትና ለቫይረሱ ስርጭት ምክንያት ሊሆኑ የሚችሉ ነገሮችን

አጠቃላይ መረጃ

በጥናቱ በመሳተፍዎ ከልብ እያመሰገንን ከመወሰንዎ በፊት፡- ይህንን ቅጽ በትክክል አንብቡ ወይም ሲነበብልዎ በትክክል ያድምጡ፤ እንዲሁም ግልፅ ያልሆነልዎትን ነገር በነፃነት ይጠይቁ ::

መግቢያ

ሔፓታይቲስ ኢ ቫይረስ በማደግ ላይ ባሉ ሀገሮች ከፍተኛ የሆነ የጤና ችግር ያመጣል፡፡ቫይረሱ በእርግዝና ወቅት የተለመደ ሲሆን ይበልጥ ገዳይ የሆነው ከ6 እስከ 9 ወር ባለው የእርግዝና ወቅት ነው፡፡በ ሔፓታይቲስ ኢ ቫይረስ ከተጠቁት እርጉዞች 70% የሚሆኑት ለከፋ የጉበት በሽታ ከአንጎል ማበጥ ጋር እንዲሁም ለሰረሰር እብጠት እና ለደም መርጋት ይጋለጣሉ፡፡ቫይረሱ በቆሻሻ/ሰገራ/ የተበከለ እጅን ወደአፍ በማስገባት /በንጽህና ጉድለት/ የሚተላላፍ ሲሆን ከእናት ወደ ልጅም በተወሰነ መልኩ ይተላለፋል፡፡ከ 15-20% የሚሆኑት እርጉዝ ሴቶች በዚሁ ቫይረስ ምክንያት ይሞታሉ፡፡

የጥናቱ አላማ

የጥናቱ አላማ የሔፓታይተስ ኢ ቫይረስ በሽታ በእርጉዝ ሴቶች ላይ ያለውን ስርጭት ማዎቅ እና ለ ቫይረሱ ስርጭት ምክንያት ሊሆኑ የሚችሉ ነገሮችን መለየት

ለጥናቱ ተሳታፊዎች ያለው ልዩ ጥቅም

በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለውም:: ነገር ግን የጥናቱ ውጤት ለሆስፒታሉ ስለሚሰጥ በሽታው የተገኘባቸው ተሳታፊዎች ከሚመለከታቸው የጤና ተቋሙ ባለሙያዎች ጋር በመነጋገር አስፈላጊ የሆነ ህክምና፣ እና ክትትል ማድረግ ይችላሉ በተጨማሪም በሽታው የተገኘባቸው ተሳታፊዎች በእናቶች እና ህጻናት ላይ ከሚሰሩ ድርጅቶች እና ከማህረሰብ ሰረተኞች ጋር በማገናኘት ስለበሽታው መረጃ፡ ስነልቦናዊና ማህበራዊ እርዳታ እንዲያገኙ ይመቻቻል፡፡

በጥናቱ ተሳታፊዎች ላይ ያለው ጉዳት እና ተዛማጅ ችግር

በዚህ ጥናት ላይ በመሳተፍዎ ሊደርስብዎ የሚችል አንድም ጉዳት አይኖርም፡፡ ለዚህ ጥናት የምንጠቀምበት የደም ናሙና ከክንድዎ የሚወሰድ ሲሆን መጠኑም ከ 5 ሚሊ ሊትር (ከ 1 -2 የሻይ ማንኪያ) ነው፡፡ በመወጋትዎም ከትንሽ የደም መፍሰስ በስተቀር በጤናዎ ላይ ምንም አይነት ጉዳት አይደርስም፡፡

ጥናቱን የማቋረጥ መብት

ለጥናቱ መረጃ ያለመስጠት፣ያለመተባባር እና ከጥናቱ በፍላጎትዎ የመውጣት መብት አለዎት፡፡ በዚህም ምክንያት ምንም አይነት መጉላላት አይደረስብዎትም፡፡ በላቦራቶሪ ምርመራው ፈቃደኛ ካልሆኑ የደም ናሙናዎት እንዲወገድ ይደረጋል፡፡

የመረጃ ሚስጥራዊ አጠባበቅ

የሚሰጡት መረጃ በጥናቱ ወቅትም ሆነ ከዛ በኃላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚያዘዉ በስም ሳይሆን በ መለያ ቁጥር ይሆናል፡፡ በጥናቱ ላይ እያሉ በፈለጉት ጊዜ የማቆም ወይም የማቋረጥ መብት አለዎት፡፡ ይህ መረጃ በጥንቃቄ የሚያዝና መረጃውን በፈለጉ ጊዜ ሊያገኙ የሚችሉ ይሆናል፡፡ በመጨረሻም የደም ናሙናው ውጤትም ለ ተቋሙ ተልኮ ተገቢውን ህክምና የሚያደርጉ ና የጥናቱም ውጤት ለሚመለከተው አካል ለጥናቱ አላማ ብቻ የሚገለፅ ይሆናል፡፡

ያስታውሱ: ስለዚህ ጥናት ማንኛውም ጥያቄ ካለዎት በማንኛውም ጊዜ ከዚህ በታች በተጠቀሱት አድራሻዎች መጠየቅ ይችላሉ፡፡

የዋና ተመራማሪዋ አድራሻ

መሰረት አበበ የሕክምና ላቦራቶሪ ቴክኖሎጂ ዲፓርትመንት፣ የጤና ሳይንስ ኮሌጅ፣ አዲስ አበባ ዩኒቨርሲቲ- አዲስ አበባ፣ ኢትዮጵያ

ኢ-ሜይል፣ mesinia@gmail.com ስልክ ፣ +251913081982

የሕክምና ላቦራቶሪ ት/ቤት ስልክ ፣ +251-0112755170

Annex V: Consent Form (Amharic Version)

የሔፓታይተስ ኢ ቫይረስ በሽታ አዲስ አበባ ባሉ ነፍሰ ጡር ሴቶች ላይ ያለው ስርጭት በሚል ርዕስ በሚደረገዉ ጥናት ላይ እንድሳተፍ፤ የጥናቱ ዓላማና ጥቅም ተገልጾልኛል፡፡ በመጠይቁ ላይ የምሰጠዉ ሙሉ መረጃም ሆነ የደም ናሙና ውጤቴ በሚስጥር እንደሚያዝ ተነግሮኛል፡፡ በተጨማሪም በጥናቱ ላይ ያለመሳተፍ መብት እንዳለኝ፤ በማንኛዉም ጊዜ ከጥናቱ በራሴ ዉሳኔ መዉጣት እንደምችልና በዚህም ምክንያት ምንም አይነት መጉላላት እንደማይደርስብኝ በሚገባ ተረድቻለሁ፡፡

ስለሆነም ሁኔታዉን በሚገባ በማጤን በፈቃደኝነት በምርምሩ ላይ ለመሳተፍ ለተመራማሪዉ ፈቃዴን ሰጥቻለሁ፡፡ በተጨማሪም የምሰጠዉ የደም ናሙና ለሔፓታይተስ ኢ ቫይረስ ምርመራ ብቻ እንደሚዉል ተነግሮኝ ተስማምቻለሁ፡፡ ማንኛዉንም ያልገባኝን ነገር የመጠየቅ ዕድል ተሰጥቶኝም በሚገባኝ ቋንቋ መልስ አግኝቻለሁ፡፡ በተጨማሪም የላብራቶሪ ምርመራ ዉጤቴ ለጤና ተቋሙ እንደሚሰጥና ዉጤቴን ማወቅ ከፈለኩ ማግኘት እንደምችል ተነግሮኛል፡፡

እኔ _____ የተባልኩ ግለሰብ ይህን ሁሉ በመረዳት ምርምሩ ላይ መረጃ እና የደም ናሙና ለመስጠት ተስማምቻለሁ፡፡

ፊርማ _____ ቀን _____

መረጃዉን ያስረዳዉ አካል _____ ፊርማ_____

Annex VI: Questionnaire (Amharic Version)

በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ዲፓርትመንት

ይህ የመጠየቅ ቅጽ ስለተሳታፊዎች (ስለ ነፍሰ ጡር ሴቶች) አጠቃላይ መረጃ ና ለበሽው (ለሔፓታይተስ ኢ በሽታ) መንስኤ የሆኑትን ምክንያቶች ለማወቅ የሚደረግ መጠይቅ ነው፡፡ መጠየቁን የሚያስሞላው በጥናቱ ጊዜ የተመረጠው(ችው) የህክምና ባለሙያ ሲሆን(ስትሆን) መጠየቁ የሚሞላው ጥናቱ በሚካሄድበት ቦታ ነው፡፡ እባክዎን ለጥናቱ መሳካት ያግዘን ዘንድ ጥያቄዎችን በጥንቃቄ ይሙሉልን፡፡

የተቋሙ ስም---------- ዓ.ም. ---------- የተሳታፊው መለያ ቁጥር----------

አድራሻ (ክፍለ-ከተማ)----------

የመረጃ ሰብሳቢው ስም---------- ቀን---------- ፊርማ----------

I. የተሳታፊውን አጠቃላይ መረጃ በተመለከተ የሚሞላ

እባክዎን ትክክለኛውን መልስ ይምረጡ

1.መለያ ቁጥር----------

2.እድሜ --------

3.አድራሻ ሀ) ከተማ ☐ ለ) ገጠር ☐ ሐ)ክልል ---------- መ)ዞን ----------

ሠ)ወረዳ---------- ረ) ቀበሌ---------- ሰ) ስልክ ቁጥር----------

4. የጋብቻ ሁኔታ ሀ) ያገባች ☐ ለ) ያላገባች ☐ ሐ) የተፋታች ☐ መ) ባሏ የሞተባት ☐ ሠ) ሳትጋባ አብራ የምትኖር

5. የትምህርት ደረጃ. ሀ). መሰረተ ት/ት ያልተማረች ☐ ለ) 1ኛ ደረጃ ☐ ሐ)2ኛ ደረጃ ☐ መ) የኮሌጅ ት/ት እና ከዛ በላይ ☐

6. የስራ ሁኔታ. ሀ) የመንግስት ስራ☐ ለ) የግል ስራ ☐ ሐ) የቤት እመቤት ☐ መ) ተማሪ ☐

7. ወርሀዊ ገቢ (በብር) ----------

8. ብሔር. ሀ) አማራ ለ) ኦሮሞ ሐ)ትግሬ መ)ደቡብ ሠ) ሌላ ከሆነ ይጠቀስ----------

9. የስንት ወር እርጉዝ ነዎት? ሀ) ከ1-3 ወር ☐ ለ) ከ4-6 ወር ☐ ሐ) ከ7-9 ወር ☐

10. ስንት ልጅ ወልደዋል? ሀ) የመጀመሪያ እርግዝና ☐ ለ) ከ 1-2 ☐ ሐ) ከ2-3 ☐ መ) ከ3 በላይ ☐

11. የኤች አይቪ ኤድስ ምርመራ ውጤትዎት ምንድን ነው? ሀ)ፖዘቲቭ ለ) ኔጋቲቭ

II. በሔፓታይተስ ኢ ቫይረስ በሽታ ለመያዝ መንስኤ የሚሆኑ ነገሮች

12. ለመጠጥ የሚጠቀሙትን ውሃ ከየት ነው የሚያገኙት?

(ሀ) የቧንቧ ውሃ ☐ (ለ) የወንዝ ውሃ (ሐ) የጉድጓድ ውሃ (መ)ሌላ ካለ ይጥቀሱ----------

13. ከሽንት ቤት ሲመለሱ እጅዎትን በሳሙና ይታጠባሉ ?

ሀ) አዎ ☐ ለ) አልታጠብም ☐

14. የቤት ውስጥ እንስሳ አለዎት?

(ሀ)አለኝ ☐ (ለ) የለኝም ☐

15. ደም በልገሳ ተቀብለው ያውቃሉ ? ሀ)አውቃለሁ ለ)አላውቅም

Annex VII Test procedures for HEV IgG and HEV IgM ELISA.

The test procedure for HEV IgG ELISA and HEV IgM ELISA was similar. All reagents and specimens were brought to room temperature (15-30°C) before starting the anti-HEV ELISA.

1. Mark three wells as Negative control, two wells as Positive control **and** one well as Blank.
2. Add **100µl** of Specimen diluent into each well except the Blank.
3. Add **10µl** of Positive control, Negative control, and Specimen into their respective wells except the Blank and mix by tapping the plate gently.
4. Cover the plate with the plate cover and incubate for **30 minutes at 37° C**.
5. At the end of the incubation, remove and discard the plate cover. Wash each well **5 times** with diluted Wash Buffer. Each time allow the microwells to soak for **30-60 seconds**. After the final washing cycle, turn down the plate onto blotting paper or clean towel, and tap it to remove any remainders.
6. Add **100µl** of HRP-Conjugate into each well except the Blank.
7. Cover the plate with the plate cover and incubate for **30 minutes at 37° C**.
8. At the end of the incubation, remove and discard the plate cover. Wash each well **5 times** with diluted Wash Buffer. Each time allow the microwells to soak for **30-60 seconds**. After the final washing cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remainders.
9. Add **50µl** of Chromogen A and **50µl** of Chromogen B solutions into each well including the Blank. Incubate the plate at **37° C for 15 minutes avoiding light**. The enzymatic reaction between the Chromogen solutions and the HRP-Conjugate produces blue color in Positive control and HEV IgM positive sample wells.
10. Using a multichannel pipette or manually, add **50µl** of Stop Solution into each well and mix gently. Intensive yellow color develops in Positive control and HEV IgM positive sample wells.

11. Calibrate the plate reader with the Blank well and read the absorbance at **450nm**. If a dual filter instrument is used, set the reference wavelength at **630nm**. Calculate the Cut-off value and evaluate the results. (**Note:** read the absorbance within **10 minutes** after stopping the reaction).

Annex VIII. Declaration

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

MSc. candidate: Meseret Abebe (BSc.)

Signature: _____ Date of submission: _____

This thesis has been submitted with our approval by Advisors.

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Abraham Aseffa (MD, PhD, Scientific Director; AHRI)

Signature:_____ Date_____ Place: Addis Ababa, Ethiopia.

Adane Mihret (DVM, PhD; AHRI)

Signature: _____ Date_____ Place: Addis Ababa, Ethiopia.