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Magnitude of Hepatitis B virus and comorbidity of intestinal parasite and Helicobacter pylori infection in School Children in Ziway, Central Ethiopia

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This is to certify that the thesis prepared by Roza Girma, entitled: ***Magnitude of Hepatitis B virus and comorbidity of intestinal parasite and Helicobacter pylori infection in Children in Ziway Central Ethiopia*** and submitted in partial fulfillment of the requirements for Master of Science degree 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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Abbreviations

AIDS	Acquired Immunodeficiency syndrome
CHB	Chronic hepatitis B
DNA	Deoxyribonucleic acid
ELISA	Enzyme linked immunosorbent assay
HB e Ag	Hepatitis B envelope antigen
HB c	Hepatitis B core antigen
HBs Ab	Hepatitis B surface antibody
HBV	Hepatitis B virus
HBsAg	Hepatitis B surface antigen
H. pylori	Helicobacter pylori
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HRP	Horseradish peroxidase
IgG	Immunoglobulin G
TMB	Tetramethylbenzidine

Abstract

Background: Hepatitis B virus (HBV) infection is globally recognized as a major risk factor for the development of liver cirrhosis and hepatocellular carcinoma in hyper endemic areas. Though some evidences shows that the association of HBV with other infections like *H pylori* and intestinal parasite is described in some places such information is lacking in Ethiopia.

Objective: To determine magnitude of Hepatitis B Surface Antigen positivity rate and it's comorbidity with intestinal parasites and *Helicobacter pylori* infections among School Children in Ziway, central Ethiopia

Methods: Both retrospective and prospective cross-sectional study was conducted from October 2018 to January 2019 on samples collected from Ziway children aged 2-14 years. Socio-demographic and data related to intestinal parasites and *Helicobacter pylori* infection were collected from a data base which has been established from a previous project. HBV surface antigen tests were performed on 348 sera using the Enzyme Linked Immunosorbent Assay (ELISA).Some socio demographic data, *H.pylori* status and intestinal parasites burden were taken from previous data base .Data was analyzed using SPSS version 20. Descriptive statistics was employed to determine proportions. Chi square test was employed to see any relation between HBV and demographic variables. Result was considered statistically significant at $p < 0.05$.

Results: Out of 348 children, 179 (51.4%) were females. Most were in the age group between 5-9 years and accounting 49.6% of the total school children. About 31.9 % (111/348) mothers had non-formal education and 152 mothers (44.0 %) were housewives. The overall magnitude of HBsAg among school children was 3.74 % (13/348). The magnitude was higher in children aged 10-14 years (5.5%) and in males (4.2 % vs 3.4% in females), though not statistically significant. Among 324 serum samples analyzed, 235(72.5%) were positive for *H. pylori* antibody, 25% had protozoa and 9 % had helminthes. Neither *H pylori* antibody nor intestinal parasites were detected in the 13 HBsAg sero positive school children.

Conclusion: Though we used a single marker for HBV infection, it calls for further studies to assess the real burden of HBV infection in the study sites. Health education should be given for the school communities and families of students to increase awareness and take preventive measures towards HBV infection.

Key words: HBsAg, School children, *H. pylori*, Intestinal parasites,

Introduction

1.1 Background

Hepatitis B viral infection is a major global health problem with predilection for the liver and is known to commonly lead to chronic infections after the acute infection. The chronic infection increases risk of death from childhood hepatic failure, cirrhosis of the liver and liver cancer (1).HBV is highly contagious and relatively easy to transmit from one infected person to another by blood contact, during childbirth, unprotected sexual intercourse, intravenous drug abuse, ear piercing, tattooing, and barbers razor and by sharing needles (2).

More than 300 million people have chronic liver infections globally and about 600,000 people die annually from acute or chronic complications of hepatitis B infection. The highest prevalence of hepatitis B infection is in sub-Saharan Africa and East Asia. (3-5).

The prevalence of HBV varies between 2 % in developed countries and in some developing countries it reaches to 8 %. Sex, age and socio-economic status are important risk factors for infection (6). Africa has the second largest number of chronic HBV carriers after Asia and is considered as a region of high endemicity(.2)

In Ethiopia and neighboring Kenya more than 60 % of chronic liver disease and up to 80 % of hepatocellular carcinoma (HCC) are due to chronic HBV and HCV infections (7).

The outcomes of chronic HBV infection are affected by a range of factors, including viral genotype, the presence of co infections with bacteria and parasites and the impact of other causes of liver disease. Co infection of HBV and bacteria or parasite may worsen the condition of patients. *H. pylori* infection is widespread with a sero prevalence of about 50 % in the general population in many countries. Epidemiological based studies have shown that *H. pylori* prevalence is higher in patients with hepatitis B (8).

Helicobacter pylori infection has been investigated extensively in immune compromised hosts, such as those with acquired immunodeficiency syndrome (AIDS) and organ transplant recipients. However, few reports on *H. pylori* prevalence among individuals with HBV infection are

available. *Helicobacter pylori* mainly cause disease in the stomach and duodenum, where it can induce chronic infection and ulcers (9).

H. pylori is a bacterium that colonizes the gastric mucosa and is responsible for gastritis, peptic ulcer disease, as well as gastric adenocarcinoma and malt lymphoma. Recent studies showed that *H. pylori* infection is associated with the progression of diseases other than gastrointestinal diseases. This includes hematological, cardiovascular or autoimmune diseases, chronic bronchitis and coronary sclerosis, but also liver's diseases. *H. pylori* DNA could be detected in liver samples from patients with chronic liver disease, suggesting that coexistence with *H. pylori* could worsen a patient's condition (10).

Schistosomiasis, hepatitis B virus (HBV), and hepatitis C virus (HCV) co-infections are common in countries where Schistosomiasis is endemic (endemic areas). Chronic Schistosomiasis and HBV co-infection may end with liver cirrhosis to Hepatocellular carcinoma in advanced degrees as the co-infection exaggerated the liver pathology more than mono-infection with HBV. Chronic Schistosomiasis is a considerable risk factor for HBV infection and it can facilitate the HBV entrance through change immune response and liver pathology because some Schistosomiasis patients may need blood transfusion (11).

This study aimed at assessing the magnitude HBV and co infection of intestinal parasite and *H. pylori* infection.

1.2 Statement of the problem

In a country where financial constraint is enormous, it is difficult to provide an all rounded health care services .Even in situations where international grant is available we are not still successful in preventing many communicable diseases including AIDS. The clinical and public health burdens are significant when it comes to HBV. Infections with hepatitis B virus (HBV) and *Helicobacter pylori* (*H. pylori*) are two major public health issues in the Africa in general and in Ethiopia in particular. However, Ethiopia has given priority to depict strategy for surveillance, prevention and control of viral hepatitis, since the country was classified under the geographical regions with intermediate to hyper endemic viral hepatitis B infections (10).

The clinical and public health burdens due to viral hepatitis in general are still given no emphasis in the country's health system. For instance, a recent report showed the presence of very limited knowledge, minimal awareness and underestimation of the viral hepatitis prevalence and disease burden in the country, which have resulted in insufficient budgetary and organizational focus. (7).

HBV can be transmitted either vertically (mother-to-child transmission during pregnancy) or horizontally (through contact with other patients during preschool years) to brood. HBV infection develops about 90% for neonates and 25%-50 % for one-five-year old children acute HBV infections develop CHB. However, this rate is much lower in adults and teenagers with HBV infections, i.e. less than 5% in symptomatic and 5% - 10% in asymptomatic cases. Although most new HBV infections occur among infants and young children, HBV-related morbidity and mortality is not immediately apparent. Acute symptomatic hepatitis B is infrequent among infected infants and children, but the likelihood of progression to chronic infection, which accounts for most HBV-related morbidity and mortality, is highest in these age groups (7).

Younger age at acquisition of infection continues to be the most important predictor of chronic carriage and those who develop chronic hepatitis B have a 15 - 40 % risk of developing the complications and this chronicity being due to immature immune system. More than 95 % of adults spontaneously recover from acute HBV infection as defined by clearance of the HBsAg from the blood, an effect that reflects the host's degree of immune response (10).These age

group populations have strong attachment with each other, which means they could share different sharp material. It is well known that children have a particular risk of developing chronic hepatitis B (CHB) after viral exposure (12).

The magnitude of HBV in Ethiopia is available in different segments of the population . However, data is lacking among school children along with comorbidity with Intestinal parasitic and *H. pylori* infection. Hence we attempted to determine the magnitude of HBsAg along with Intestinal Parasitic infection and *H. pylori* infection.

1.3 Significance of the study

Determining the magnitude of HBV infection and co morbid conditions in school children is important for planning and policy making and intervention strategy to control this infection . It can also help us to create awareness among all categories of healthcare workers about the magnitude of the infection and generate baseline data to the health sector about the magnitude of HBV in school children.

2 Literature review

2.1 HBV Burden

Ikobah J *et al*., (2016) had conducted study on 749 children aged between 11 years to 19 years. Nine of the participants were positive for HBsAg giving a sero prevalence of 1.2 %. Of these, 477 (63.7 %) were females and 272 (36.3 %) were males (1).Eke C *et al.*,(2015) had conducted study on 420 students and the mean age was 14.26 years while the median age was 14.0 (range 10–18) years. Thirteen subjects tested positive for HBsAg, giving an overall sero prevalence of 3.1%. Eight (61.5) children of the 13 positive HBsAg cases were males (13).

A total of 1,217 children and adolescents attending daycare centers and schools from Rio de Janeiro, Brazil were included in the study by Villar L *et al*. The mean age was10.39±4.11years, ranging from 0 to18 years and 51.6 % were females. Active HBV infection (HBsAg positive) was observed in 22 individuals (1.8%) (14).

The same kind of study in brazil (an epidemiological survey) for HBV and HCV infection was conducted among individuals aged 10 to 69 years living in the five geographic regions of Brazil by Villar L *et al*, and this survey reported the overall HBsAg, anti-HBc, and anti-HCV sero prevalence rates of 0.37%, 7.4%, and 1.38%, respectively, Among individuals aged 10 to 19 years, the prevalence of anti-HCV was 0.75% (11). A study was conducted in USA by Wasley A *et al* showed the prevalence of anti-HBc decreased among persons 6–19 years of age (from 1.9% to 0.6 %). Prevalence of chronic HBV infection was 0.28% (95% CI, 0.21–0.36%), which represents∼730,000 infected persons (95% CI, 550,000–940,000). Prevalence of markers of vaccine-induced immunity was 22.2 % (95 % CI, 21.3 %–23.1 %) (15).

In a study by Bukbuk DN *et al* the overall sero-positivity of Hepatitis B surface antigen (HBsAg) in the pupils was 44.7 % (95 % C.I: 36.6 53.0). The prevalence of HBsAg was found to increase with age, rising from 40.6 % in children aged 10-11 years to 75 % in children aged above 13

years. The sero-prevalence was 47.2 %, (95% C.I: 37.5 57.1) among males while among the females it was slightly lower 38.1 % (95 C.I: 23.6 54.4%) (16).

The gradual fall in HBsAg prevalence among children as a result of HBV immunization was reported by a number of authors in Nigeria and this calls for strengthening of routine immunization and sustained efforts to reduce significantly the hyperendemicity of HBV (17).

The frequency of *H. pylori* infection among patients with chronic hepatitis B Virus is around 30–80 % . *H. pylori* infection is confirmed in 79 % of patients with post inflammatory liver cirrhosis connected with HBV infection. Favorable effect of *H. pylori* eradication on the course of the disease, including increased platelet count, has been demonstrated in the studies on patients chronically infected with HBV, with compensated liver cirrhosis and thrombocytopenia (18).

Among people chronically infected with HBV with primary liver cancer, *H. pylori* infection is found in 69 % of patients. In the group of patients with primary liver cancer, but without HBV infection, *H. pylori* infection is much less frequent, as it is found in 33 % of patients .These observations consistently point to unfavorable effect of *H. pylori* infection among HBV infected patients with liver cirrhosis onto the risk of occurrence of primary liver cancer. Frequency of *H. pylori* infection among patients with chronic hepatitis B correlates with the incidence of hepatocellular cancer, both in men and women. Among this type of patients,fast progression of inflammatory changes in the liver is observed, as well as intensified fibrosis,which promotes occurrence of primary neoplastic lesions (19).

As james A et al reported that out of 360 samples analyzed, thirty-five (35) representing 9.7% of the study population was positive for HBsAg. The children aged 7-9 years had a prevalence of 14(3.9 %). However there exists no statistical evidence at 95% significant level to show that age of the subjects had effect on their HBV status. With regards to sex distribution of the children screened, out of the 35(9.7%) positive subjects screened, 22(6.1%) were males (20).

The overall seroprevalence of HBsAg among the pupils was 23.3%. The distribution of HBsAg by age and genderamong subjects studied. The seroprevalence was highest in children aged 7-

8years; and it was also higher in males (15.8%) than females (7.5%). The difference in age and gender were statistically significant (21).

Out of 200 students participated in a study 63(31.5%) were positive for HBV, while 137(68.5%) were not reactive to HBV. Of the 61 males and 139 females participants, 26(43%) and 37(27%) respectively were infected (6).

Of the 70 respondents, the study revealed that children aged 1 year or less had 5.9% positivity for HBsAg, Children aged 2 years had 9.1% positivity, aged 3 years had 13.3 % positivity, aged 4 years had 18.2 % positivity, while aged 5 years had no positivity (22).

The overall seroprevalence among school children in Ohaukwu area in Nigeria was 6.5%. Children living in rural area had higher (9.8%) prevalence than their counterpart in urban areas (4.6 %) (23).
Among 295 children participated 3 had a positive HBsAg serology giving an overall prevalence of HBsAg of 1.1% (24) and a prevalence of 1.5 % (3/200) had been reported (25).

The prevalence of HBsAg was 0.3% (4/1,200; 95% CI: 0.1–0.9%) among children and adolescents aged 1–17 years. The four children tested positive for HBsAg were all Chinese, and one child was a girl in the agegroup of 7–12 years (26).

Of 450 samples selected in South Africa, 47% were females and 53% were males, with a median age of 5 years. HBsAg was positive in two children, 0.4% (2/450) (27).
Four hundred and six subjects were recorded in the gastroenterology clinic during the 6-year period under review.Ninety-two of these had HBV infection giving a prevalence of 22.7%. Sixty-three (68.5%) were males and 29 (31.5%) females giving a male to female ratio of 2.2:1. The age of the subjects ranged from 1 to 14 years with mean value of 8.0 ± 3.0 years (28).

Being smokers and having sexual contact were a risk factors among students in (29).

H-pylori infection is a major global health issue. Since the infection with *H. pylori* is common, about two-thirds of the world's population have it in their bodies and acquired the infection through early in life, especially among poverty stricken groups. *H. pylori* is noninvasive, for most people, it doesn't cause ulcers or any other symptoms. However, *H. pylori* infection is linked with the development of certain upper gastrointestinal diseases. This bacterium cause of chronic gastritis; peptic ulcer disease, gastric cancer and mucosa-associated lymphoid tissue lymphoma.Treatment and eradication of *H. pylori* infection cure duodenal or gastric ulcers in over 80% of patients (30).

H. pylori prevalence in chronic Hepatitis B virus (CHB) patients was 63.9% and it was higher than in the healthy donors (43.3%) ($P<0.01$, $\chi2=66.064$, OR 2.313, 95% CI 1.889-2.832) (31).

70 patients with the co-infection, 49 (70%) were male compared to 21 (30%) who were female. Gender was not significantly related to *H. pylori* infection in HBV carriers ($p = 1.00$).Furthermore, there was no association between the patient age and *H. pylori*-HBV co-infection ($p = 0.18$) (23).

In a meta-analysis of a Chinese population, the prevalence of *H. pylori* infection among patients with HBV-related liver diseases increased as the disease severity increased. Namely, the *H. pylori*-positive rate in patients with chronic hepatitis B patients but not cirrhosis or HCC was 2.44-fold higher than that in healthy controls (pooled OR: 2.44, 95%CI: 1.85-3.24; $P < 0.01$). Furthermore, the *H. pylori*-positive rate in patients with HBV-induced cirrhosis was 4.28-fold higher (pooled OR: 4.28, 95%CI: 2.99-6.13, $P < 0.01$) than that in healthy controls, while it was 6.02-fold higher (pooled OR: 6.02, 95%CI: 4.33-8.37, $P = 0.821$) in patients with HBV-related HCC. Therefore, the presence of *H. pylori* may accelerate the progression of HBV-related liver pathogenesis, but the precise pathogenicity in the liver remains to be elucidated (32).

2.2 Morphology of hepatitis B virus

Hepatitis B virus (HBV) is the prototype member of the Hepadnaviridae member family having a strong preference for infecting the liver cells. HBV virions are double-shelled particles, 40-42nm

in diameter, with an outer lipoprotein envelope that contains three related envelope glycoprotein or surface antigen. Within the envelope is the viral nucleocapsid, or core that contains the viral genome, a relaxed circular, partially duplex DNA of 3.2kb, and a polymerase that is responsible for the synthesis of viral DNA in infected cells. In addition to virions, HBV-infected cells produce two distinct subviral lipoprotein particles: 20 nm spheres and filamentous form of similar diameter (33).

2.3 The hepatitis B life cycle

The HBV virion binds to a receptor at the surface of the hepatocyte . A number of candidate receptors have been identified, including transferrin receptor, the asialoglyco protein receptor molecule, fibronectin, interlukin-6, apolipoprotein and liver endonexin. The mechanism of HbsAg binding to a specific receptor to enter the cells has not been established yet. After the viral membrane fuses with the cell membrane of the host the viral genome is released into the cells. The viral genome is then taken to the nucleus where viral polymerase converts the partial double stranded DNA .As a result of this conversion in the nucleus, second strand DNA synthesis is completed and the gaps in both strands are repaired to yield a covalently closed circular (ccc) supercoiled DNA molecule that serves as a template for the transcription of four viral RNAs that are 3.5, 2.4, and 0.7 kb long .The pre core polypeptide is transported into the endoplasmic reticulum lumen, where it's amino and carboxy termini are trimmed and the resultant protein is secreted pre core antigen (eAg). The X protein contributes to the efficiency of HBV replication by interacting with different transcription factor, and capable of stimulating both cell proliferation and cell death. The HBV polymerase is multifunctional enzyme. The product of the gene are involved in the multiple functions of the viral life cycle, including a priming activity to initiate minus strand DNA synthesis, a polymerase activity which degrades the RNA strand of the RNA-DNA hybrids, and the packaging of the RNA pre genome into nucleocapsids. Nuclear localization signals on the polymerase mediate the transport of covalently linked viral genome through the nuclear pore (34-35).

2.4 Diagnosis

Diagnosis is based on clinical, laboratory and epidemiologic findings. HBV infection cannot be differentiated based on clinical symptoms and definitive diagnosis depends on the results of serologic testing. Serologic markers of HBV infection may vary depending on whether the infection is acute or chronic. HBsAg is the most commonly used test for diagnosing acute HBV infection or detecting carriers. HBsAg can be detected as early as one to two weeks and as late as 11 to 12 weeks after exposure to HBV when sensitive assays are used. The presence of HBsAg indicates that a person is from vaccine. Anti-HBc generally persists for life and is not a serologic marker in acute infection. IgM anti-HBc appears in person with acute disease about the time of illness onset and indicates recent infection with HBV. IgM anti-HBc is generally detectable four to six months after the onset of illness and is the best serologic marker of acute infection. A negative test for IgM anti-HBc together with a positive test for HBsAg in a single blood sample identifies a chronic HBV infection. HBV DNA assays are used to monitor the response to treatment, assess the likelihood of maternal to child transmission of HBV and to detect the presence of occult HIV infection. Anti-HBs (surface antibody) is protective, neutralizing antibody indicating recovery and immunity against re infection. It can be acquired as an immune response to Hepatitis B vaccine (36).

2.5 Pathogenesis of Hepatitis B

Approximately 90 % of newborns with the infection through birth become chronic carriers, except when they are vaccinated at birth. The danger of chronic HBV infection decreases to 30 % of children infected within the ages 1 to 4 years. After HBV infection, majority of the patients either develop immunity (87–90 %) and clear the infection or become chronic carriers. A small percentage develops liver disease or develops chronic active hepatitis with a high risk of HCC, liver cirrhosis or both. The mortality of these diseases and their attribution to hepatitis infection is well known. More than 780,000 HBV-related deaths were occurs yearly and 73% of all liver cancer mortalities worldwide are as a result of hepatitis viruses (37).

2.6 Treatment of HBV infection

It is well accepted that antiviral therapy for chronic hepatitis is effective to improve prognosis of patients with HBV by preventing development of hepatitis state and HCC. It is then obvious that the primary goal of treatment is to eliminate or suppress HBV; that is, to decrease pathogenicity and infectivity, and thereby to stop or reduce hepatic necro-inflammation. The need for treatment of hepatitis B depends on the natural development of the disease. The decision to treat chronic HBV generally is based on a combination of clinical, laboratory, and histologic factors. Selection of appropriate patients for antiviral therapy depends on identification of HBV replication and an elevated alanine aminotransferase level or histologic liver injury. The aims of treatment of chronic HBV infection are to achieve sustained suppression of HBV replication and to induce remission of liver disease before cirrhosis and HCC develop. The management of patients with chronic HBV infection should be tailored according to the patient's age, comorbid conditions, and extent of HBV replication and liver disease. Approved drugs and agents in development for the treatment of chronic HBV infection fall into two categories: immune modulators, namely recombinant interferon alfa-2b (Intron A); and direct inhibitors of HBV replication, including lamivudine (Epivir) and Adefo-virdipivoxil .WHO recommends Tenofovir and Entecavir because they suppress the hepatitis B virus and rarely lead to drug resistant compared to other drugs. In most people, however, the treatment does not cure hepatitis B infection, but suppresses the replication of the virus (38).

2.7 Prevention

The hepatitis B vaccine is the mainstay of hepatitis B prevention. WHO recommends that all infants should receive hepatitis B vaccine as soon as possible after birth, within 24 hours. The birth dose should be followed by 2 or 3 doses to complete the primary series. In most cases, 1 of the following 2 options is considered appropriate: A 3-dose schedule of hepatitis B vaccine, with the first dose (mono-valent) being given at birth and the second and third (mono-valent and combined vaccine) given at the same time as the first and third doses of diphtheria, tetanus-(DIP) vaccine or A 4-dose schedule where a mono-valent birth dose is followed by three mono-valent or combined vaccine doses, usually given with other routine infant vaccine. The complete

vaccine series induces protective antibody level in more than 95% of infants, children and young adults. Protection lasts at least 20 years and is probably lifelong (39).

2.8 HBV and Intestinal parasite

Xiao P *et al.,* had a study on 438 participants, out of which 39.3% were males, 65.3% were illiterate and 22.8 % were unmarried. Infection rates of HBV, *A. lumbricoides* and *T. trichiura* were 9.1, 13.5 and 30.6 % respectively. Of these, 7.1 % (30/438) had a co-infection of *A. lumbricoides* and *T. trichiura*, 2.3 % (10/438) had a co-infection of HBV and *A. lumbricoides*, and 2.7 % (12/438) had a co-infection of HBV and *T. Trichiura* (14). HBV might be a risk factor for *A. lumbricoides* infection, or infection with *A. lumbricoides* could increase the risk of HBV infection. The co-infections may cause an imbalance between Th1 and Th2 cells, and another study found that Th1 cells were suppressed while Th2 cells were enhanced (40).

2.9 HBV Co morbidity with Helicobacter pylori

Huang J *et al.,* had demonstrated that the HBV -related cirrhosis patient group had the highest *H. pylori* infection rate (79.3%). It was significantly higher than that in the chronic HBV. HBV -negative hepatic carcinoma which indicated that *H. pylori* infection rate increased with progression of disease in patients with chronic HBV. This result suggests that *H. pylori* contributes to pathogenesis in coordination with HBV (8).

Qu H *et al* showed that *H. pylori* prevalence in CHB patients (63.9%) was higher than in the healthy donors (43.3%) ($P<0.01$, $\chi^2=66.064$, OR 2.313, 95% CI 1.889-2.832). *H. pylori* prevalence in CHB patients (59.6 %), HBV related cirrhosis patients (77.3%), and HCC patients (80.3%) were all higher than in healthy donors ($P<0.05$), and *H. pylori* prevalence in HBV related cirrhosis patients and HCC patients was clearly higher than that in CHB patients (41).

In study by Al musawi *et.al* found out that when they compared the percentage of positive *H. Pylori* in hepatitis patients and control there was significant differences between hepatitis patients and control. The OR is 14.7 points at the clear risk of particular exposure to *H. Pylori*infection in getting viral hepatitis compared to control group (42).

A study conducted by Anthony *R* in patients with chronic hepatitis B-related HCC, demonstrated that ,the rate of *H. pylori* infection was six times higher than that of healthy controls, though this result was insignificant (pooled OR, 6.02, 95 percent CI, 4.33–8.37; p=0.821).Overall, there was a higher incidence of *H. pylori* in patients with chronic hepatitis B compared with those without chronic hepatitis B (pooled OR, 3.17, 95 percent CI, 2.38–4.22; p<0.01) (43).

A total of 15 case control studies were conducted by Wang J *et al* which showed that all the studies were found to be significant (I2=77.9%). The pooled OR was 3.17 (95% confidence interval (CI) 2.38–4.22;p<0.01) indicating that the *H.pylori*-positive rate in patients with CHB was approximately 3.17 times more than in the healthy population (44).

Fan XG *etal* under took a study on 96 patients with hepatitis B, with the result that 55 (57.3%) were positive for serum IgG anti-*H. pylori* which is significantly greater than in the control group (21,34).

3 Objectives

3.1 General objective

- ✓ To determine the magnitude of HBsAg along with intestinal parasites and *H. Pylori* co-infection among school children in Batu Ziway, Ethiopia.

3.2 Specific objectives

- ✓ To determine the magnitude of HBV in school children in Ziway
- ✓ To determine the magnitude of HBV and intestinal parasite co-infection
- ✓ To determine the magnitude of HBV and *H. pylori*co-infection

Hypothesis: The magnitude of HBsAg is similar with the general population reported in Ethiopia.

4 Materials and Methods

4.1 Study area

Batu (Ziway) town is located in Oromia Regional State, in East Shoa zone, Adami Tulu Jiddo Woreda, at a distance of 160 Km from Addis Ababa. It is located at 7º 56’ North Latitude and 38º 43’ East Longitude with an elevation of 1643 meters above sea level.

Lake Ziway covers an area of 434 square kilometer, with a maximum depth of 4 meter, with approximate annual resource potential of 3 million kilogram live weight of fish of tilapia and barbue. There are also light small scale agro chemical, textile and flower growing hydro chemical industries that contribute to water,soil and air pollution which have invariably negatively impacted on health conditions of inhabitants of Batu. Zeway which 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 and both government and private schools out of which, 14 are primary schools. Batu, primary school, our study site is one of the government primary school.

4.2 Study design

Both prospective and retrospective cross- sectional study was conducted on samples collected from Ziway primary school.

4.3 Study period

The study was conducted from October 2018 to January 2019.

4.4 Study variables

4.4.1 Dependent variables

- Magnitude of HBV and comorbidities of intestinal parasites and *H. pylori* infection

4.4.2 Independent variables

- Age
- sex
- Parent education status
- Parent occupation
- Type of residence

4.5 Population

4.5.1 Source of population

The source of population of Zeway primary school children in Batu town is the source population for this study.

4.5.2 Study population

The study population were all school children who were willing to participate based on the inclusion criteria were the study population .

4.6 Sampling Method and Sample size

4.6.1 Sample size

$N = Z2\ P(1-P)/\ D^2$

D^2 = Marigin of errors which is taken as 0.03

Where Z= 95% confidence interval (1.96)

P = Estimated prevalence rate 9.7% (0.097) According to a study in Nigeria (20)

N = minimum sample size

$= (1.96)2.\ 0.097(1-\ 0.097)/\ 0.03^2$

373.

However, we have included 348 serum samples that were available for Laboratory analysis.

4.6.2 Sampling Method

A convenient sampling technique was used from selected sample.

4.7 Inclusion and Exclusion criteria

4.7.1 Inclusion criteria

All data of school children aged 2-14 years old included.

4.7.2 Exclusion criteria

Serum samples with very small volume were excluded and like wise, students who do not have complete data on intestinal parasites and H. pylori status were excluded from analysis.

4.8 Data collection

4.8.1 Socio-demographic and other variables

Socio-demographic data and information concerning intestinal parasites and *H. pylori* infection status were collected by using check lists which is prepared for this study from the original data base that has been used for investigation of atrophy, intestinal helminthes and H. Pylori infection in the same study site.

4.8.2 HBsAg testing

4.8.2.1 Principle of the test

In the murex HBsAg version 3 , the sample was pre incubated in micro wells coated with a mixture of mouse monoclonal antibodies specific for different epitops on the a determinant of HBsAg . Affinity purified goat antibody to HBsAg conjugated to horseradish peroxidase is then added to the sample in the well. During the two incubation step any HBsAg present in the sample is bound to the well in an antibody antigen antibody enzyme complex. In the absence of HBsAg no conjugate was bound. After washing to remove sample and unbound conjugate, a solution containing 3,3',5,5'-tetremethyl benzidine (TMB) and hydrogen peroxide was added to the wells. Wells that contain HBsAg and hence bound conjugate will develop a purple colour which

is converted to orange when the enzyme reaction is terminated with sulphuric acid. The amount of colour can be determined spectrophotometrically and is directly proportional to the amount of conjugate bound and hence the concentration of HBsAg in the sample. Test were interpreted as negative if sample absorbance is less than the cut-off value and are considered non reactive.While,samples giving an absorbance value equal to or greater than the cut-off value were considered as reactive.

4.9 Quality control

As mentioned earlier the data of the blood and stool collected from the data were calculated and analyzed with appropriate quality control measures whichwere taken properly. We have checked the completeness of socio-demographic data and the volume of the stored samples. Both known positive and negative samples were run during sample testing process.

The mean A 450/690 of the negative control must be less than 0.15 or the mean A450 of the negative control is less than 0.2. Cut-off value was calculated by adding 0.05 to the mean of the negative control replicates.

For HBsAg positive control, the result of an assay run is valid if the A450/690 or A450 of the positive control must be more than 0.8 above the mean. A 450/690 or A450 of the negative control. If the above requirements are not met by the negative and positive control the assay run is unsatisfactory and was repeated.

As a post analytical phase all results were valid only when both the positive and the negative controls have passed.Before the result can be released, it was double checked by a second person authorized to release result as mentioned earlier for data of the blood and stool.

4.9.1 Data management and analysis

Data were entered and cleansed before analyses.Then it was analyzed using the Statistical Package for Social Sciences (SPSS 24, USA). We used X^2 test to compare difference between HBV infection and other variables of interest and result was considered statistically significant at $p < 0.05$.

5. Results

5.1 Sociodemographic Characteristics of school children and their parents

A total of 348 children aged 4-14 years participated in this cross sectional study at Batu primary schools in the Ziway town. Amongst them 169 (48.5%) were males and 179 (51.5%) were females. The most sampled population was children with age group of 5-9 (49.6%) , followed by 10-14 (47.2%). The majorities were residents in Ziway town (338, 97.12%). About 31.9% of the mothers have no formal education. Table 1 summarizes socio-demographic and maternal characteristics of the study participants.

Table 1: Distribution of socio-demographic and maternal characteristics of the study population in Ziway school children, Ethiopia, 2019

Variables	**Number**	**Percent (%)**
Sex		
Male	169	48.4
Female	179	51.6
Total	348	
Age (years)		
<5	11	3.2
5-9	170	48.9
10-14	167	47.9
Total	348	
Maternal Education		
Formal	237	68.1
Non-formal	111	31.9
Total	348	
Maternal Occupation		
House wife	152	44.0
Farming & related	7	2.0
Trading & related	58	16.8
Government employee	6	1.7

Others	123	35.6
Total	346[b]	
Residence		
Urban	338	99.06
Rural	10	0.04
Total	348	

Formal education- Mothers, who can read, write or learned to higher levels.

Non formal education - Mothers who can't read or write.

b. N.B We had two missed information from maternal occupation of school children and it makes the total 346.

5.2. Magnitude of HBsAg

Out of 348 samples analyzed, thirteen (13) representing 3.74 % of the study population were positive for HBsAg (Figure1). Children aged 10-14 had highest magnitude of 9(5.5%).However, there exists no statistical evidence at 95 % significant level to show the age of the participants had effect on their HBV status. With regards to sex distribution of the children screened, out of 13 positive children , 6 (3.4%) were females while 7(4.2%) males were positive, though the difference was not statistically significant (P >0.05) (Table 2).

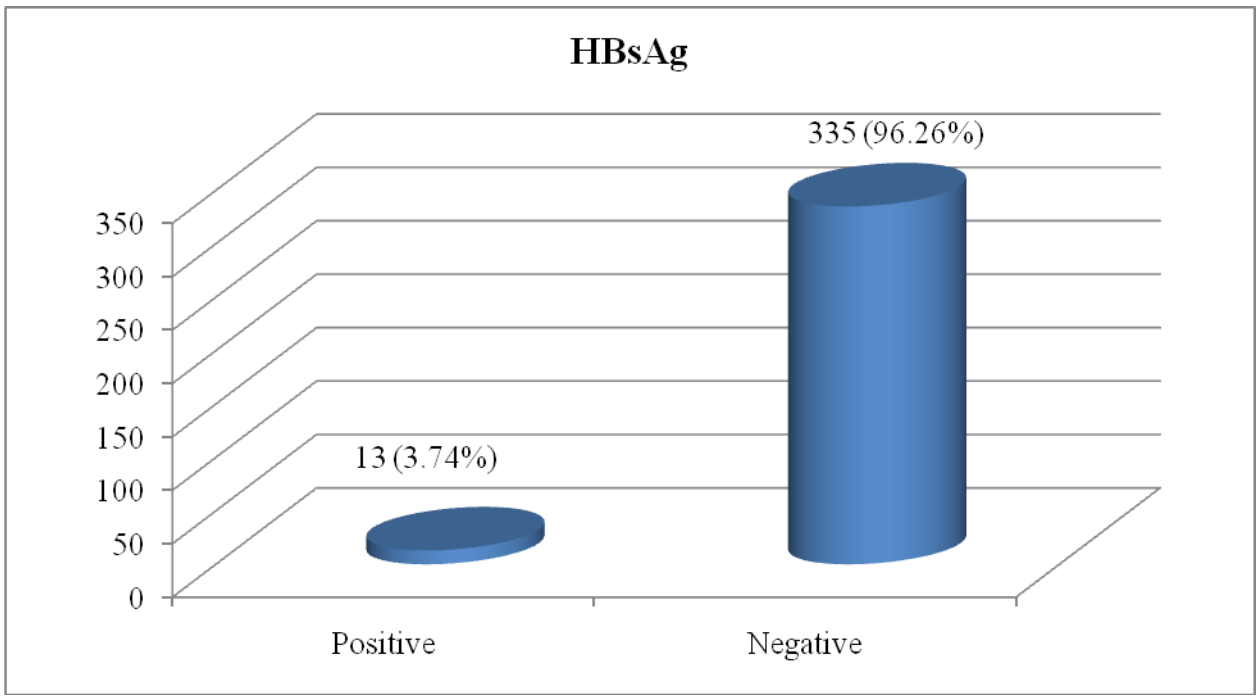

Figure 1.Magnitude of HBsAg sero-positivity among school children at Ziway town, Ethiopia, 2019

Table 2.Magnitude of HBsAg sero-positivity by age and sex among school children at Ziway town, Ethiopia,2019. The detailed result is illustrated in table one

Variables	Total	HBsAg Positive		HBsAg Negative		P value
		Number	**Percent (%)**	**Number**	**Percent (%)**	
Sex						0.690
Male	169	7	4.14	162	95.85	
Female	179	6	3.35	173	96.64	
Total	348	13	3.74	335	96.26	
Age (years)						0.263
<5	11	0	0.0	11	100.0	
5-9	170	4	2.4	166	97.6	
10-14	167	9	5.5	158	94.5	
Total	348	13	3.74	335	96.26	

5.3. Magnitude of HBsAg co- morbidity with *Helicobacter pylori* and Intestinal parasites

In order to estimate the comorbidity of HBsAg and H. pylori infection, 333 school children were qualified and 14 (4.2 %) were positive for *H. pylori* stool antigen. While 343 school children had parasitological data and which makes the magnitude of intestinal parasites, 6.7 % (23/343). All H. pylori infected and intestinal parasites infected children were negative for HBsAg (Figure 2 and 3).

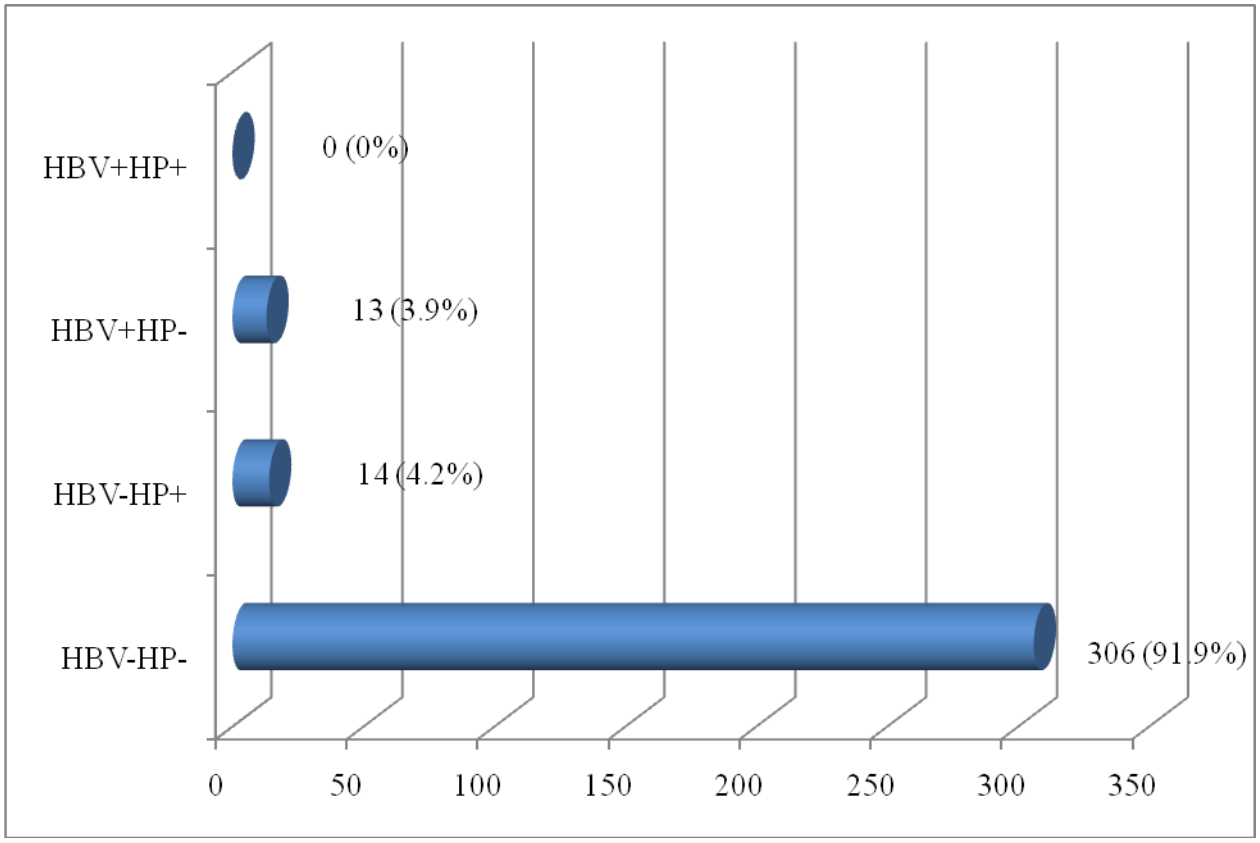

Figure 2. Magnitude of HBsAg co- morbidity with *Helicobacter pylori* among school children at Ziway town, Ethiopia, 2019 (n=333)

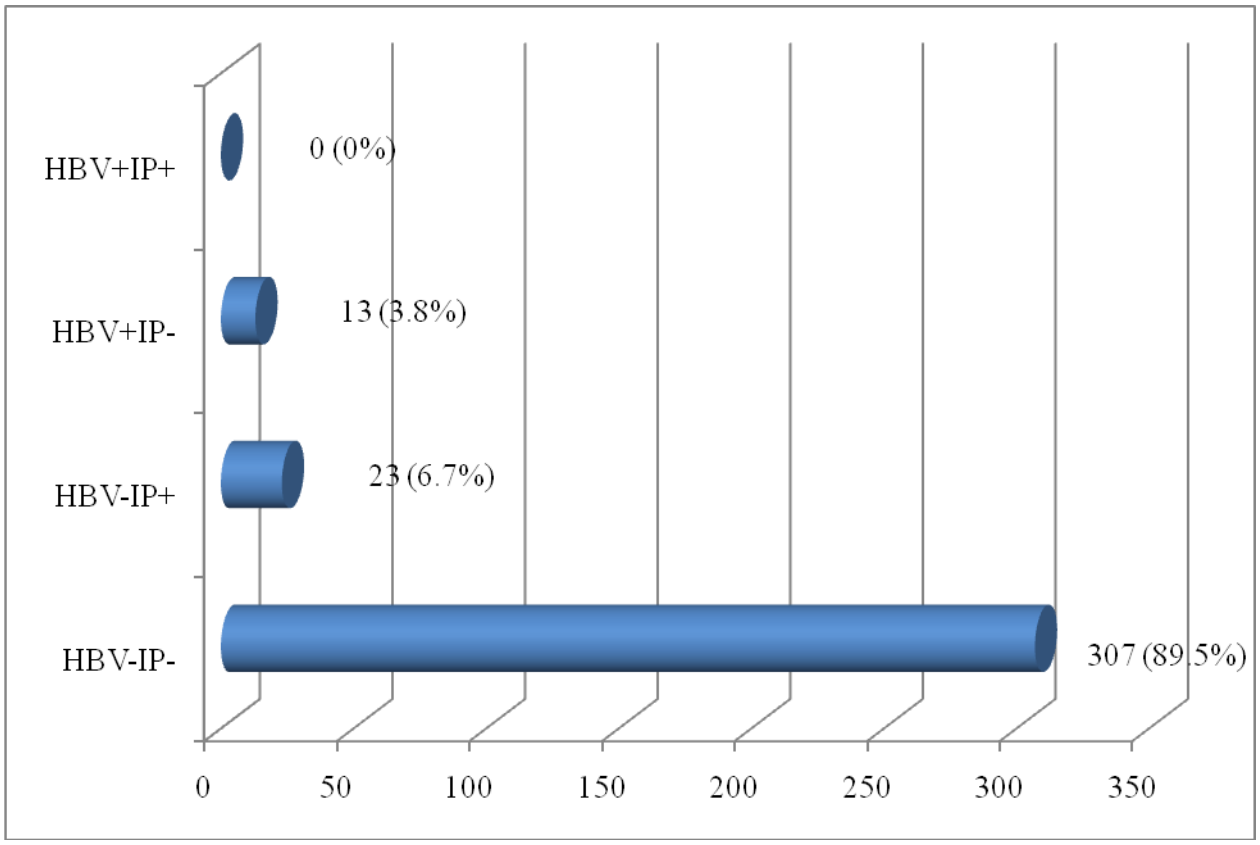

Figure 3. Magnitude of HBsAg co- morbidity with Intestinal parasites among school children at Ziway town, Ethiopia, 2019 (n=343).

6. Discussion

This cross-sectional study aimed at determining the magnitude of HBsAg and co morbidity with intestinal parasites and *H-Pylori* stool antigen among school-aged children in Ziway town, central Ethiopia.

6.1. HBV magnitude

According to repeated observation, experimental results obtained in varied regions of the world there is well established criteria of HBV prevalence rates: low endemic rates (less than 2 %) intermediate rate of 2-8 %; and high of greater than 8 % (24) .Similarly, high endemicity of hepatitis B is defined as HBsAg prevalence of more than 7 % in a given population size.

In this study the magnitude of HBsAg ,3.74 % was depicted among school children which is graded as intermediate endemicity. This result has corresponded with other studies that were conducted in different continents including Nigeria (45) and Kenya (46). The finding was lower compared to the finding of 6.65 *%* from Chinese children as reported by Yang *et al* in 2017 (47).

However, other studies in Addis Ababa revealed high prevalence of HBV compared to the current study (48) though the study population are not the same. The difference in sample size and population could also contribute varation in magnitude of HBsAg . Moreover, this study was conducted after one and half decades of the previous study and through time the people awareness is improving due to the effort of the health extension workers and vaccination efforts that has been made so far in Ethiopia(48).

The findings in this study is also lower than most reported prevalence studies in Nigeria considering that it is a hyper-endemic setting for HBV as described in a study by Isa MA *et al,*2015 (49) and by Sadoh *et al*, 2014 (50). The latter study was among Nigerian children admitted to a children's emergency room and this may contribute to the difference noted (51).

The finding in this study was also higher than the 2.1 % reported among HIV uninfected children in Tanzania by Muro *et al,* 2013 (52) and Southern Africa each and 0.5 % in Ibadan, Southwest Nigeria, respectively (53).

The study population in the current study was for the children aged 2-14 years while most of the other studies cited across the entire childhood age groups from 0 to 18years. The three main chronological phases of the natural history of HBV infection are immune tolerance, immune clearance and low replication phases, particularly in the older children and adolescents (54).This may partly be the reason for the seemingly low prevalence of HBV infected children observed in the current study despite the fact that Ethiopia is a non-hyper-endemic country.

More males tested positive to HBsAg compared with females in the current study though not statistically significant. Similar findings have been reported by other workers including Ndako J et al., 2010) (54). There was age difference between the ages of the participants who tested positive to HBsAg in the current study. This finding has revealed that the age groups 10-14 years were more infected than other age groups. The reason for this might be partly due to the reason that at this age when children are likely to start involving themselves in sexual act. Moreover, the current study showed that males were highly infected than females, though not statistically significant, which are in contrary with previous studies by Pungpapong *et al.,* 2007(55); Sarwar *et al.,* 2010 (56) and Zhang *et al.*, 2011) (57).

The reason for this might be, the active involvement of males in activities like unprotected and multi-partner sex practice. However, females are restricted to household activities that reduced the chance being influenced to sexual acts . The role of the universal HBV vaccination in the prevention against and /or reduction in the incidence and prevalence of HBV infection has been well documented in the literature (58).

One culprit that is mentioned regarding HBV infection and its endemic proportions in sub-saharan Africa is the tropical as well as temperate zones, but with particular reference to tradition- bound societies is circumcision. This is exacerbated by scarification marker, traditional birth giving and inadequate health facilities (21). This could potentially exposed them for possible transmission of blood born infectious agents including HBV.

6.2. HBVco-morbidity with Helicobacter pylori and intestinal parasite infections

In recent years, researchers have paid particular attention to the relationship between *H. pylori* infection and liver diseases, especially viral hepatitis.A study which included 147 patients with liver cirrhosis found that *H. pylori* infection was significantly more frequent among patients with post inflammatory liver cirrhosis infected with hepatitis C virus or hepatitis B virus (59).

In the current study, however, there was no association between *H.pylori* and HBV and also intestinal parasites and HBV in the study area. However, in China including South-Western-China reported that there is relationship between *H. pylori* and HBV-related liver disease and intestinal parasite and HBV, respectively. In a case-control study performed in China from 2010 to 2015 to investigate the seroprevalence of *Helicobacter pylori* infection in patientswith chronic hepatitis B, significantly higher *H. pylori* infection was detected in patients than controls. *H. pylori* infection was more prevalent in cirrhosis patients with complications than in patients without complications (36). While evidences are showing association between HBV and *H. pylori*, the observation that none of the current study participants had HBsAg and *H. pylori* coinfcetion that calls for further research activities on this area on large sample size. Only 13 of the children were HBsAg positive in this study. Most of the studies involved patients unlike the current study.

Both HBV and H. pylori have different markers to indicate current and or past infection, the limited markers we used both for HBV and H. pylori infection could have an effect to underestimate the magnitude of both diseases and the absence of comorbidites.

7. Strength and Limitation

The number of sero positive children was small making detection of comorbidity with intestinal parasites and H. *pylori* difficult. The study was carried out on stored serum samples collected for other studies on the association of allergy and *H pylori* as well as parasitic infections. We have attempted to search for the existence of HBsAg and *H. pylori* and Intestinal parasites comorbidity in the study site.

8.1. Conclusion

In this study, the magnitude of HBsAg markers found to be in the intermediate enedemicity as most other previous studies in Ethiopia.

Children aged 10-14 years had highest magnitude of HBSAg (5.5 %). However, there exists no statistical evidence at 95 % significant level to show the age of the participants had effect on their HBV status. With regards to sex, out of 13 positive subjects screened, 6 (3.4%) were females. No comorbid state seen between HBsAg and *H.pylori* or intestinal parasite infection in this study.

8.2. Recommendations

Since we have used a single marker for HBV infection, large scale studies are required to know the real burden of hepatitis B virus infection among school children along with various markers of H. pylori.

Awareness creation should be provided periodically for the school communities and families of students to safe guard the society and take preventive measures towards HBV infection.

Though we did not showed any comorbid condition among HBsAg, *H.pylori* and intestinal infection among school children , future studies could also address this issues by including larger sample size and better study design. .

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Annex I. HBsAg ELISA TEST

Materials and Equipment required

- ✓ Refrigerator
- ✓ Distilled water
- ✓ Micro plate (ELISA) reader
- ✓ ELISA kits
- ✓ ELISA washer
- ✓ TTI forms
- ✓ Computer
- ✓ Pipette tips
- ✓ Micropipette (single & multi
- ✓ channel)
- ✓ Waste container
- ✓ Beaker
- ✓ Timer
- ✓ Printer
- ✓ Micropipettes rack
- ✓ Incubator
- ✓ Paper towel
- ✓ Trough
- ✓ Test tubes
- ✓ Marker
- ✓ Centrifuge
- ✓ Test tube rack
- ✓ pipette rack
- ✓ Paper
- ✓ Scissors

- ✓ Pen (Blue & Red)
- ✓ tips rack

Safety:

Use Personal protective equipment and consider all materials used in the assay as potentially infectious. TMB substrate should not come in to contact with metal or bench surface.

Principle and Procedural Steps:

ELISA tests are carried out as per the instructions given in the package insert of the kit.

Principle:

This HBsAg ELISA kit uses antibody sandwich ELISA method in which polystyrene microwell strips are pre-coated with monoclonal antibodies specific to HBsAg. Serum or plasma sample is added to the micro well together with a second antibody conjugated with horse radish peroxides (HRP) and directed against a different epitope of HBsAg. During incubation, the specific immune complex formed in case of presence HBsAg in the sample is captured on the solid phase. After washing to remove sample serum proteins and unbound HRP conjugate, chromogen solutions containing tetramethylbenzen (TMB) and urea peroxides are added to the wells. In presence of the antibody- Antigen antibody (HRP)"sandwich" immune complex, the colorless chromogens are hydrolyzed by thebound HRP- conjugate to a blue colored product. The blue color turns yellow after stopping the reactionwith sulfuric acid. The amount of color can be measured and is proportional to the amount of antigen inthe sample. Wells containing samples negative for HBsAg remain colorless.

Procedural steps:

- ✓ Bring all reagents to room temperature (18-24oc) at least for 30 minute before the assay.
- ✓ Receive serum/plasma from ABO serology unit
- ✓ Inspect samples for appropriate labeling and volume.

- ✓ Sequence the samples according to the pack number order
- ✓ Write test sample numbers on HBsAg screening Protocol
- ✓ prepare washing buffer solution (look kit insert for preparation)
- ✓ Perform the tests according to kit insert
- ✓ Add 50µl negative control to A1, B1 and C1
- ✓ Add 50µl positive controls to D1 and E1
- ✓ Add 50µl donor serum/plasma to the rest wells starting from F1
- ✓ Add 50µl enzyme conjugate to all wells including controls
- ✓ Seal and Incubate at 37^{o}c for 30minutes
- ✓ Wash one cycle and tap on absorbent paper and repeat 5 times for a total of 6 wash
- ✓ Tap the plate on the paper towel to remove any remaining fluid
- ✓ Add 50µl chromogenA first
- ✓ Add 50µl chromogenB
- ✓ Seal and Incubate at 37^{o}c for 10minutes in Dark
- ✓ Turn the reader "on" to stabilize the lamp
- ✓ Add 50µl stopping solution
- ✓ Load the plate on the reader and select HBsAg for DIALAB program from "SESSION/MENU"
- ✓ Read the absorbance at 450nm and (630nm reference wave lengths).
- ✓ Read the test by pressing start key
- ✓ Print test results
- ✓ Evaluate and validate the results
- ✓ Make sure that the printout is read with appropriate program
- ✓ Inspect the reading result with color developed on the plate.
- ✓ Make sure that mean absorbance of negative control must be < 0.1 and the mean
- ✓ absorbance of positive control must be > 0.6
- ✓ Reject and rerun if the test is invalid
- ✓ If the test is valid Calculate cutoff results according to the criteria provided in the Kit
    - insert. Cutoff value = NCx * 2.1
- ✓ Use 0.05 instead of the actual mean In case the mean of negative control replicates is <
    - 0.05,

- ✓ Take Gray zone 10% to the Negative direction of cutoff valueGray zone = (NCx * 2.1)*0.9
- ✓ Interpret Sample with OD greater or equal to gray zone is reactive
- ✓ Interpret Sample with OD less than gray zone is nonreactive
- ✓ Encircle new reactive pack numbers with red pen on protocol
- ✓ Record reactive pack numbers on the space provided on protocol
- ✓ Collect your reactive bags
- ✓ Register your reactive pack numbers on
- ✓ Accept for validity of the test by laboratory case team coordinator
- ✓ Approve the validity of the test by quality officer
- ✓ Put the reactive Blood and Blood product in quarantine until their test result is confirmed
- ✓ File result print outs & respective protocol
- ✓ Prepare untested samples for the next test including reactive samples and their respective segments
- ✓ Re-test reactive Donations both from test tube and respective bag segment on the next test.
- ✓ Underline twice with red pen repeated reactive pack numbers on
- ✓ Record repeat reactive pack numbers on the space provided on
- ✓ Discard all repeat reactive blood and Blood products after acceptance and approval
- ✓ Recheck the test results prior to releasing blood and blood products
- ✓ Release non reactive blood and Blood product for distribution on safe blood product release form
- ✓ Archive your entire sample and store at least for five years
- ✓ If test tube and segment have discordant result asses the problem
- ✓ Check that correct test tube and segment is tested
- ✓ Rerun the test from the same pack number if not tested from the correct sample
- ✓ repeat all batches from test tube if the discrepancy is from test tube
- ✓ Repeat all batches from segment if the discrepancy is from bag segment
- ✓ Test repeat reactive samples with rapid test and report the results for post donation counselingpurpose

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.

M.Sc. candidate: Roza Girma (B.Sc.)

Signature:_____

Date of submission: _____

This proposal has been submitted with our approval as advisors.

Advisor: Aster Tsegaye (MSc, PhD, Associate Professor of Immuno-hematology)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Kassu Desta (MSc, PhD fellow, Associate Professor of Medical Microbiology)

Signature: _____

Date: _____