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Sudan University of science and Technology

College of Graduate Studies

Sero-prevalence of Hepatitis B Virus among Refugees in Khartoum state

الانتشار المصلي لفيروس التهاب الكبد (ب) لدى اللاجئين في ولاية الخرطوم

A dissertation submitted in partial fulfillment for the requirements of M.Sc. Medical Laboratory Science (Microbiology)

BY

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الآيــة

قال تعالى :

(فَبَدَأَ بِأَوْعِيَتِهِمْ قَبْلَ وِعَاءِ أَخِيهِ ثُمَّ اسْتَخْرَجَهَا مِن وِعَاءِ أَخِيهِ ۚ كَذَٰلِكَ كِدْنَا لِيُوسُفَ ۖ مَا كَانَ لِيَأْخُذَ أَخَاهُ فِي دِينِ الْمَلِكِ إِلَّا أَن يَشَاءَ اللَّهُ ۚ نَرْفَعُ دَرَجَاتٍ مَّن نَّشَاءُ ۗ وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيم)

سورة يوسف: الآية (76)

Dedication

To my lovely mother

To my great father

To my respectful brothers and sisters

To my best friends

To my colleagues

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Firstly, all precise and thank to **Almighty Allah** who blessed me with health and power to carry out this study.

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ABSTRACT

Hepatitis B is an infectious disease caused by Hepatitis B Virus (HBV). It is represent global health problem and infection with this virus may lead to serious consequences such as liver cirrhosis, failure, and liver carcinoma.

The objective of this study was to determine the frequency of HBV among refugees in Khartoum state.

The study was conducted during the period between February to April 2017.

A total of ninety blood samples (n=90) were obtained from refugees at Nefasha camp.

Five ml blood sample was collected from each refugeen. Then serum or plasma was obtained by centrifugation at 3000 rpm for 5 min. The sera was exemined for the presence of HBsAg using Enzyme Linked ImmunoSorbent Assay (ELISA).

The results showed that out of 90 blood samples investigated, 8 (8.9%) were positive for HBsAg. The rest 82(91.1%) were negative.

The study concluded that the prevalence of HBV infection in refugees is relatively high and the level of infection is independent to suggested factors such as: age, sex, family history for hepatitis.

Further studies with large number of samples and more advanced technique are required to validate the results of the present study.

مستخلص الاطروحه

التهاب الكبد الفيروسي ب هو عبارة عن مرض معدي يسببه فيروس التهاب الكبد ب . يعتبر واحد من المعضلات الصحيه عالمياً والاكثر خطوره وانتشاراً اذ أن الاصابه به تؤدي الى تليف الكبد ,الفشل الكبدي وايضاً سرطان الكبد على السواء.

الهدف من الدراسه هو تحديد معدل انتشار فيروس التهاب الكبد ب بين اللاجئين في ولاية الخرطوم في الفترة بين فبراير الى أبريل 2017.

جمعت 90 عينة دم من اللاجئين بمخيمات نيفاشا . حوالي 5 مل من كل المرضى جمعت وتم فصل السيرم بحثاً عن المستضد السطحي لفيروس الكبد ب باستخدام تقنية الإلايزا.

اظهرت النتائج انه من مجموع 90 عينه فحصت فقط 8 (8.9) عينات اظهرت نتائج ايجابيه بينما 82 (91.1) عينه اظهرت نتائج سلبيه.

خلصت الدراسه الي ان عدوى فيروس التهاب الكبد الوبائي بين اللاجئين نسبياً تعتبرعاليه. وانها غير معتمده على العوامل المصاحبه سواء أكان عمر او جنس او التاريخ الاسري للاصابه بهذا الفيروس.

عليه يوصى بدراسات اضافيه بعدد اكبر من العينات بإستخدام تقنيات متقدمه للتحقق من نتائج هذه الدراسه.

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INTRODUCTION AND OBJECTIVES

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1.1. Introduction

Hepatitis B Virus (HBV) represent global health problem worldwide. Currently, more than 350 subjects are chronically infected with HBV (El-Serag ,2012).

Chronic infection with this virus may predispose to serious consequences such as liver cirrhosis, liver failure and hepatocellular carcinoma. Annually, around 500,000 deaths occur due to such infections and their consequences (Davis *et al* .,2008).

Sudan is classified among countries with high hepatitis B surface Ags (HBsAg) endemicity of more than 8% (NCBI .,2008).

The prevalence of HBV varies from less than 1% in developed world to more than 8% in some Asian countries (Shepard *et al* .,2006) .

According to a 2007 United Nations High Commissioner for Refugees (UNHCR) report, 1.9 million, the largest population of Afghan refugees in any single country, are residing in Pakistan (Rajabali *et al* .,2009).

These refugees reside both in camps and urban slums, struggling against impoverishment, overcrowding, poor sanitation, lack of clean water, and of healthcare infrastructure(Connolly *etal* ,2004, Beckwith *et al* .,2009).

Human Immunodeficiency, Hepatitis B and C viruses (respectively, HIV, HCV, and HBV) share similar modes of transmission, that include blood to blood contact, sexual contact with infected person or vertical transmission (mother to child). Pakistan has an overall HIV prevalence of

0.1%, Hepatitis B and C prevalence of 2.4 and 3%, respectively (Khanani *et al* .,2010).

Refugees form a small albeit important fraction of immigrants to the United States but HBV infection is endemic in most areas of the world from where refugees originate.

The prevalence of HBV markers is 20-60% and greater than 60% in intermediate and high endemicity countries, respectively (Gogos *et al* .,2003).

Most countries with high endemicity do not have comprehensive HBV immunization programs for populations at risk (WHO .,2004) .

In addition, conflict, transit, and camping conditions inherent in refugee situations hinder the delivery of adequate healthcare. It is also likely that living conditions in refugee camps directly enhance transmission beyond the usual rates in original countries. The current immunization recommendations for refugee healthcare in the acute phase do not include vaccination against HBV infection (Chris Ugwu *et al* .,2007)**.**

1.2. Rationale

Infections with HBV pose serious healthcare problem, especially in developing countries .(Hussein .,2015)

There is no doubt that migration can change the map of infectious diseases and there is a bilateral effect on the host and moved population. Probably, infectious disease is one of the most challenging risks facing populations (Sharara and Kanj .,2014).

Periodically identifying the prevalence of hepatitis B infection in a large population of refugees is important for assessing the burden of disease and for planning or adjusting prevention and treatment programs. Due to

changes in the patterns of refugee admissions closely linked with changing world events, period prevalence estimates from large aggregate populations of refugees reflect a better picture of the burden of disease in this population (Chris Ugwu *et al* .,2007).

1.3. Objectives

1.3.1. General objectives

To study occurance of Hepatitis B Virus (HBV) among refugees to Khartoum state.

1.3.2. Specific objective

1. To detect Hepatitis B Virus (HBV) infection among refugees by ELISA techniques.
2. To evaluate the frequency of HBV among refugees.
3. To determine the relationship between the presence of HBV and certain factors such as gender, age, career, social status and history of blood transfusion.

CHAPTER TWO

LITERATURE REVIEW

CHAPTER TWO
LITERATURE REVIEW

2.1. Background

End-stage liver disease represents a major source of morbidity and mortality worldwide. The World Health Organization (WHO) estimates that in 2002 cirrhosis and primary liver cancer caused 783,000 and 619,000 deaths, respectively (WHO. ,2003).

Taken together, these conditions represented approximately one of every forty deaths (2.5%) worldwide.

Among primary liver cancers occurring worldwide, hepatocellular carcinoma (HCC) represents the major histologic type and likely accounts for 70% to 85% of Cases (El Serag .,2001) .

Cirrhosis precedes most cases of HCC, and may exert a promotional effect via hepatocyte regeneration (Bialecki and Bisceglie .,2005 ;Moradpour and Blum .,2005).

Compared with other causes of cirrhosis, chronic infection with hepatitis B virus (HBV) is associated with a higher risk of developing HCC (Bialecki and Bisceglie .,2005 ; Donato F. *et al* .,2004).

Alcohol abuse represents a leading cause of cirrhosis and is also a major contributor to HCC in many parts of the world, with some evidence for synergistic effect in the presence of HBV infection (Gelatti *et al* .,2002 ;Hatten *et al* .,2002) .

Other factors appear to be of regional or local importance (Monto and Wright .,2001; Diaz *et al* .,2005).

For example, dietary aflatoxin exposure in parts of Africa and Asia has been associated with primary liver cancer, especially in hosts with chronic HBV infection (Monto and Wright ,2001).

Population mobility is associated with the introduction of new diseases in the host society (Dasgupta *et al* ,2006) .

With mass population immigration such as when occurs in wars, infectious diseases continue to represent major causes of death and morbidity due to respiratory tract infection, diarrheal diseases, tuberculosis, HIV and an acquired immunodeficiencysyndrome (Gushulak and MacPherson ,2004).

Other diseases that may pose a threat are vaccine-preventable diseases. Each country has its own program of vaccination according to the resources and national need. Movement of individuals from countries with less comprehensive program of vaccination to areas with good preventive program may represent a threat of developing infectious diseases in the distention countries. Additionally, moving from areas with a poor vaccination program such as in post-war Syria to a country with thriving preventive programs such as in Iraq may increase the burden in new habitats (Dasgupta *et al* .,2006).

2.2. Structure and composition

HBV is a member of the hepadnavirus family. It is a 42-nm enveloped virion , with an icosahedral nucleocapsid core containing a partially double-stranded circular DNA genome.

The envelope contains a protein called the surface antigen (HBsAg), which is important for laboratory diagnosis and immunization.

Within the core there is a DNA-dependent DNA polymerase (Warren Levinson .,2010).

Electron microscopy of HBsAg-positive serum reveals three morphologic forms. The most numerous are spherical particles measuring 22 nm in diameter. These small particles are made up exclusively of HBsAg—as are tubular or filamentous forms, which have the same diameter but may be over 200 nm long—and result from overproduction of HBsAg. Larger, 42-nm spherical virions (originally referred to as Dane particles) are less frequently observed. The outer surface, or envelope, contains HBsAg and surrounds a 27-nm inner nucleocapsid core that contains HBcAg. The variable length of a single-stranded region of the circular DNA genome results in genetically heterogeneous particles with a wide range of buoyant densities (Geo F. *et al* .,2010).

The viral genome consists of partially double-stranded circular DNA, 3200 bp in length. Different HBV isolates share 90–98% nucleotide sequence homology. The full-length DNA minus strand (L or long strand) is complementary to all HBV mRNAs; the positive strand (S or short strand) is variable and between 50% and 80% of unit length (Geo F. *et al* .,2010).

There are four open reading frames that encode seven polypeptides. These include structural proteins of the virion surface and core, a small transcriptional transactivator (X), and a large polymerase (P) protein that includes DNA polymerase, reverse transcriptase, and RNase H activities. The S gene has three in-frame initiation codons

and encodes the major HBsAg, as well as polypeptides containing in addition pre-S2 or pre-S1 and pre-S2 sequences. The C gene has two in-frame initiation codons and encodes HBcAg plus the HBe protein, which is processed to produce soluble HBeAg (Geo F. *et al* .,2010).

The particles containing HBsAg are antigenically complex. Each contains a group-specific antigen, *a,* in addition to two pairs of mutually exclusive subdeterminants, *d/y* and *w/r*. Thus, four phenotypes of HBsAg have been observed: *adw, ayw, adr,* and *ayr*. In the United States, *adw* is the predominant subtype. These virus-specific markers are useful in epidemiologic investigations, as secondary cases have the same subtype as the index case (Geo F. *et al* .,2010)

The stability of HBsAg does not always coincide with that of the infectious agent. However, both are stable at –20°C for over 20 years and stable to repeated freezing and thawing. The virus also is stable at 37°C for 60 minutes and remains viable after being dried and stored at 25°C for at least 1 week. HBV (but not HBsAg) is sensitive to higher temperatures (100°C for 1 minute) or to longer incubation periods (60°C for 10 hours). HBsAg is stable at pH 2.4 for up to 6 hours, but HBV infectivity is lost. Sodium hypochlorite, 0.5% (eg, 1:10 chlorine bleach), destroys antigenicity within 3 minutes at low protein concentrations, but undiluted serum specimens require higher concentrations (5%). HBsAg is not destroyed by ultraviolet irradiation of plasma or other blood products, and viral infectivity may also resist such treatment (Geo F. *et al* .,2010).

2.3. Replication

The replication of HBV is unique for several reasons, First, HBV has a distinctly defined tropism for the liver, Its small genome also necessitates economy, In addition, HBV replicates through an RNA intermediate produces and release antigenic decay particles (Murray *et al* .,2002).

The infectious virion attaches to cells and becomes uncoated . In the nucleus, the partially double-stranded viral genome is converted to covalently closed circular double-stranded DNA (cccDNA). The cccDNA serves as template for all viral transcripts, including a 3.5-kb pregenome RNA. The pregenome RNA becomes encapsidated with newly synthesized HBc (Geo F. *et al* .,2010).

Within the cores, the viral polymerase synthesizes by reverse transcription a negative-strand DNA copy. The polymerase starts to synthesize the positive DNA strand, but the process is not completed. Cores bud from the pre-Golgi membranes, acquiring HBsAg-containing envelopes, and may exit the cell. Alternatively, cores may be reimported into the nucleus and initiate another round of replication in the same cell.(Geo F. *et al* .,2010).

2.4. Transmission

The three main modes of transmission are via blood, during sexual intercourse, and perinatally from mother to newborn. The observation that needle-stick injuries can transmit the virus indicates that only very small amounts of blood are necessary.

HBV infection is especially prevalent in addicts who use intravenous drugs (Warren Levinson .,2010).

However, because blood transfusion is a modern procedure, there must be another, natural route of transmission. It is likely that sexual transmission and transmission from mother to child during birth or breast feeding are the natural routes (Warren Levinson .,2010).

2.5. Epidemiology

HBV infection is prevalent worldwide , representing a global public problem and causing chronic hepatitis, liver cirrhosis and HCC (Hou *etal* ,2005; Hovart and Tegtmeier .,2011).

Annually, around 500,000 deaths occur due to such infections and their consequences . The prevalence of HBV varies from less than 1% in developed world to more than 8% in some Asian countries (Nawfal Hussien *et al* .,2016).

It is estimated about 2 billion people have been infected by HBV, representing approximately 1/3 of the world´s population .while more than 350 million are chronic carriers of HBV(Hou *et al* .,2005).

Sero-prevalence of HBV ranging from as low as 6.8% in central Sudan to as high as 26% in southern Sudan (NCBI .,2008)

The prevalence of HBV markers is 20–60% and greater than 60% in intermediate and high endemicity countries, respectively (Chris Ugwu *et al* .,2007).

In the United States, an estimated 1.25 million people are infected with hepatitis B, and 300,000 new cases occur annually. About 300

of these patients die of acute fulminant hepatitis, and 5% to 10% of infected patients become chronic HBV carriers (Kenneth Ryan and George Ray .,2010).

2.6. Pathogenesis & immunity

Hepatitis is a general term meaning inflammation of the liver. Microscopically, there is spotty parenchymal cell degeneration, with necrosis of hepatocytes, a diffuse lobular inflammatory reaction, and disruption of liver cell cords. These parenchymal changes are accompanied by reticuloendothelial (Kupffer) cell hyperplasia, periportal infiltration by mononuclear cells, and cell degeneration (Geo F.*et al* .,2010).

It is suggested that HBV is not directly cytopathic for the infected hepatocytes (Chisari *et al* .,2009)

Chronic carriers of HBsAg may or may not have demonstrable evidence of liver disease. Persistent (unresolved) viral hepatitis, a mild benign disease that may follow acute hepatitis B in 8–10% of adult patients, is characterized by sporadically abnormal aminotransferase values and hepatomegaly. Histologically, the lobular architecture is preserved, with portal inflammation, swollen and pale hepatocytes (cobblestone arrangement), and slight to absent fibrosis. This lesion is frequently observed in asymptomatic carriers,

usually does not progress toward cirrhosis, and has a favorable prognosis (Geo F. *et al* .,2010)

Chronic carriage is more likely to occur when infection occurs in a newborn than in an adult, probably because a newborn's immune system is less competent than that of an adult's. Approximately 90% of infected neonates become chronic carriers. Chronic carriage resulting from neonatal infection is associated with a high risk of hepatocellular carcinoma(Warren Levinson .,2010).

Chronic active hepatitis features a spectrum of histologic changes from inflammation and necrosis to collapse of the normal reticulum framework with bridging between the portal triads or terminal hepatic veins. HBV is detected in 10–50% of these patients (Geo F. *et al* .,2010).

HBV has significant roles in the development of hepatocellular carcinoma that may appear many (15–60) years after establishment of chronic infection (Geo F. *et al* .,2010).

Lifelong immunity occurs after the natural infection and is mediated by humoral antibody against HBsAg. Antibody against HBsAg (HBsAb) is protective because it binds to surface antigen on the virion and prevents it from interacting with receptors on the hepatocyte. (HBsAb is said to neutralize the infectivity of HBV.) Note that antibody against the core antigen (HBcAb) is *not* protective because the core antigen is inside the virion and the antibody cannot interact with it (Warren Levinson .,2010).

2.7. Clinical significant

HBV is found in highest concentrations in blood and in lower concentrations in other body fluids (e.g., semen, vaginal secretions, and wound exudates). The incubation period from the time of exposure to onset of symptoms is 6 weeks to 6 months. HBV infection can be self-limited or chronic (CDC .,2012).

The clinical picture of hepatitis B is highly variable. The incubation period may be as brief as 30 days or as long as 180 days (mean approximately 60 to 90 days) (Kenneth Ryan and George Ray .,2010).

Acute hepatitis B is usually manifested by the gradual onset of fatigue, loss of appetite, nausea and pain, and fullness in the right upper abdominal quadrant. Early in the course of disease, pain and swelling of the joints and occasional frank arthritis may occur. Some patients develop a rash. With increasing involvement of the liver, there is increasing cholestasis, and hence clay-colored stools, darkening of the urine, and jaundice. Symptoms may persist for several months before finally resolving (Kenneth Ryan and George Ray .,2010).

Chronic infection with hepatitis B virus either may be asymptomatic or may be associated with a chronic inflammation of the liver(chronic hepatitis), leading to increases the incidence of hepatocellular carcinoma (EL-Serag and Rudolph .,2007).

2.8. Laboratory diagnosis

Diagnosis is based on clinical and laboratory findings, It is impossible to differentiate HBV infection on clinical ground alone; so, differentiate diagnosis should be established on the results of laboratory testing. Both serological and molecular methods are available and used to distinguish between acute and chronic infections (CDC .,2012).

2.8.1. Specimens

Serum or plasma can be used for detection of serologic and molecular markers of HBV infection. Plasma is separated from blood collected in containers with EDTA or citrate dextrose as anticoagulant. Heparinized plasma is unacceptable for nucleic acid analysis as heparin interferences with Taq polymerase in PCR (Hovart and Tegtmeier .,2011)

2.8.2.1. Direct detection

In general HBV antigens are detected using highly sensitive techniques that used either solid-phase immune assays or microparticles to capture the protein.By definition, HBsAg persist for more than 6 months in the presence of HBe Ag or anti- HBc antibodies (Hovart and Tegtmeier .,2011).

2.8.2.2. Nucleic acid detection

Detection and/or quantitation of HBV DNA are useful in the initial characterization of HBV infection and monitoring of chronic infection , especially in patients on antiviral therapy.Many of assays that detect HBV DNA are oligo primers that recognize a conserved

sequence within HBV precore/core gene.Conventional PCR and real time PCR are commonly used to detect and quantify HBV DNA, respectively (Hovart and Tegtmeier .,2011).

2.8.2.3. Serological tests

Several commercial assays are available to detect HBV-specific antibodies, which determine the stage of the disease and immunity due to vaccination.

There is a period of several weeks when HBsAg has disappeared but HBsAb is not yet detectable. This is the window phase.At this time, the HBcAb is always positive and can be used to make the diagnosis(Warren levinson .,2010).

The development of anti-HBs is associated with elimination of infection and protection against reinfection. Anti-HBc is detected early in the course of disease and persists in serum for years. It is an excellent epidemiologic marker of infection, but is not protective (Kenneth Rayan and George Ray .,2010)

The laboratory diagnosis of acute hepatitis B is best made by demonstrating the IgM antibody to HbcAg in serum, since this antibody disappears within 6 to 12 months of the acute infection. Almost all patients who develop jaundice are anti-HBc IgM–positive at the time of clinical presentation. Past infection with hepatitis B is best determined by detecting IgG anti-HBc, anti-HBs, or both, whereas vaccine induces only anti-Hbs(Kenneth Rayan and George Ray .,2010).

2.9. Prevention of HBV infection

A vaccine for hepatitis B has been available since 1982 (Geo F. *et al* ,210). Prevention involves the use of either the vaccine or hyperimmune globulin or both. The vaccine is highly effective in preventing hepatitis B and has few side effects. It is indicated for people who are frequently exposed to blood or blood products, such as certain health care personnel (e.g., medical students, surgeons, and dentists), patients receiving multiple transfusions or dialysis, patients with frequent sexually transmitted disease, and abusers of illicit intravenous drugs. Travelers who plan a long stay in areas of endemic infection, such as many countries in Asia and Africa, should receive the vaccine. The United State Public Health Service recommends that all newborns and adolescents receive the vaccine (Warren Levinson .,2010).

At present, booster doses after the initial three-dose regimen are not recommended. However, if antibody titers have declined in immunized patients who are at high risk, such as dialysis patients, then a booster dose should be considered(Warren Levinson .,2010).

Hepatitis B immune globulin (HBIG) contains a high titer of HBsAb because it is prepared from sera of patients who have recovered from hepatitis B. It is used to provide immediate, passive protection to individuals known to be exposed to HBsAg-positive blood, e.g., after an accidental needle-stick injury(Warren Levinson .,2010).

Combination of HBIG and vaccine significantly reduces vertical transmission (Kenneth Rayan and George Ray .,2010).

2.10. Treatment

Acute HBV does not usually require treatment and most adults clear the infection spontaneously. Early antiviral treatment may be required in fewer than 1% of people, whose infection takes a very aggressive course or who are immunocopromised (Hollinger and Lau .,2006).

As from 2008 , there are seven medications licensed for treatment of HBV infection in the United States; Lamivudine (Epivir), Adefovir (Hepsera), Tenofovir (Viread),Telbivudine (Tyzeka) and Enteccavir(Baraclude), and the two immune system modulators interferon Alpha-2a and PEGylated interferon Alpha-2a (Albert and Caporaso .,2011).

2.11. Previous study:

Many studies have been published in the last two decades addressing various aspects of HBV infection in Sudan, such as its prevalence among blood donors, health care workers, pregnant women, virus genotypes and its relation to hepatocellular carcinoma. These studies indicate that the disease is endemic in the Sudan and of major public health importance (Elduma and Saeed .,2011).

In spite of it’s a first time for focusing prevalence of HBV infection among Refugees in Sudan .

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design

3.1.1. Types of study

This is a descriptive cross-sectional study conducted to detect sero-prevalence of Hepatitis B virus infection among Refugees to Khartoum state.

3.1.2. Study area

Refugees living in Nefasha camp (Western Omdurman near Alraghi hospital).

3.1.3. Study duration

The study was conducted during the period from February to May 2017.

3.1.4. Study population

Refugees at Khartoum state.

3.2. Sample size and sampling technique

A total of ninety (n-90) Refugees were enrrolled in this study. Five ml of blood samples were collected from each patients.

3.3. Ethical consideration

Ethical approval to conduct this study was obtained from the College of medical laboratories of the Sudan University of Science and

Technology (SUST). After explaining the study and its goal, a verbal consent was taken from the study recruits before proceeding with the study and collecting blood samples.

3.4. Sample collection

Blood samples were collected from each person (Refugee)after their consented. The venipuncture technique were used for collection; the available vein was located, then skin was cleaned by 70% (v/v) ethanol, sterile syringe(5ml) was used to collect 5 ml of blood, then the blood was dispensed in a sterile EDTA blood container.

3.5. Laboratory work

3.5.1. Prepration of specimen

Blood samples were centrifuge at 3000 rpm for 5-10 minutes to obtain plasma. Then obtained plasma were preserved at -20^{o}c until the serological analysis.

3.5.2. Sample analysis

The samples were analyzed for the presence of HBsAg by commercially available Enzyme –linked immunosorbant assay "HBsAg ELISA" kit(Fortress Diagnostic Limited, unit 2C Antrim technology park, Antrim BXE0742A United Kingdom).

The assay was performed following the instructions of the manufacture. Positive and negative controls were included in each assay. According to the information included in the kit´s insert, the immunoassay used has specificity 99.94%.

3.5.3. Principle of the assay

The test is an enzyme-immunoassay based on a sandwich principle. Polystyrene micro titer strip wells have been coated with monoclonal anti-HBs (antibody to HBsAg). Patient's serum sample is added to the micro wells. During incubation, the specific immune-complex formed in the case of presence of HBsAg in the sample, is captured on the solid phase. After washing to remove sample serum patients, second antibody conjugated to the enzyme HRP and directed against different epitope of HBsAg is added to the wells. During the second incubation steps, these HRP conjugated antibodies will be bound -to any anti-HBs-HBsAg complexes previously formed during the first incubation, and the unbound HRP conjugate is then removed by washing. After washing to remove unbound conjugate, chromogen solutions containing TMB and urea peroxidase are added to the wells. In the presence of antibody-antigen-antibody HRP sandwish immune-complex, the colorless chromogens are hydrolyzed by the bound HRP conjugate to a blue colored product. The blue color turns yellow color after stopping the reaction using the stop solution. The color was read as optical density in order to determine the result of the test. Wells containing samples negative for HBsAg remain colorless.

3.5.4. Procedure

All reagents and specimens were settled to reach room temperature, 20µl of specimen diluents was added to each well except the blank, then 100µl of positive control, negative control and specimen were

added to their respective wells. The plate was covered with plate cover and incubated 60 minutes at 37ºc. At the end of incubation period , 50µl of HRP-conjugate was added to each well except the blank; the plate was covered and incubated for 30 minutes at 37ºc . By the end of incubation period each well was washed 5 times with diluted wash buffer. Finally 50 µl of chromogen A and B solutions were added to each well including blank, then the plate was incubated at 37ºc for 15 minutes and stop solution was added.

3.5.5. Quality control and calculation of the results

Reagent, standard and control were checked for storage, stability and prepration before starting work. Each microplate was considered separetly when the results was calculated and interrelated; the results were calculated by relating each specimen absorbance (A) to the cut off (c.o) of the plate.

Calculation cut off value (C.O) =NC*2.1 (NC is mean of three negative controls).

The OD value of the blank well must be less than 0.08 at 450nm.

The OD value of the positive control must be more than 0.08 at 450nm.

The OD value of the negative control must be less than 0.1 at 450nm.

3.5.6. Interpretation of results

Positive more than cut off value.

Negative less than cut off value

CHAPTER FOUR

RESULTS

CHAPTER FOUR

RESULTS

A total of 90 blood specimens were collected from refugees peopl e resident in a refuegees camp.

The specimens obtained from forty two (42) males and forty eight (48) females were analyzed for Hepatitis B Virus (Table 1).

About eight (8) were HBV positive, giving percentage of HBV a mong refugees in Khartoum state to 8.9% (Table 2 and fig 1).

About four (4) positive obtained from male giving the percentage of HBV among refugees in Khartoum state of male to be 4.4% (T able 3 and Fig 2).

About four (4) were HBV positive, giving percentage of HBV am ong refugees in Khartoum state of female to be 4.4% (Table 3 and Fig 2).

About seven (7) participants have a history of blood transfusion, t hree (3) of them are male and four (4) are females, and no one hav e infected with HBV (Table 4).

About six (6) positive participants are married, two (2) are males and four (4) females are infected. Two (2) males are single partici pants infected with HBV and there is no single female infected (T able 5).

About nine (9) have a history of hepatitis, three (3) males and six (6) females, and there is no infection among participants who hav

e history of hepatitis (Table 6).

About twenty six (26) have a surgery in the past, nine (9) of them are male and seventeen (17) are females, and there is no infection among participants who have history of surgery (Table 7).

About twenty eight (28) have a history of jaundice, twelve (12) of them are male and sixteen (16) are females, one (1) female and on e (1) male have an infection with HBV (Table 8).

About thirty eight (38) suffering from fatigue, sixteen (16) of the m are males and twenty two (22) are females, two (2) males from them have an infection with HBV (Table 9).

No one have a self injection with intravenous (IV) drug.

No one have a renal failure.

About twenty five (25) suffering from fever, five (5) are males an d twenty (20) are females, one (1) female are infected (Table 10).

About thirty six (36) have abdominal pain, thirteen (13) are males and twenty three (23) are females, one (1) male and one (1) femal e are infected (Table 11).

About four (4) have sharing razors and shaving machine, one (1) male and three (3) females, and no one have an infection with HB V (Table 12).

Table 1. Distribution and frequency of specimens according to the gender.

Sex	Frequency	Percent%
Male	42	46.7
female	48	53.3
Total	90	100

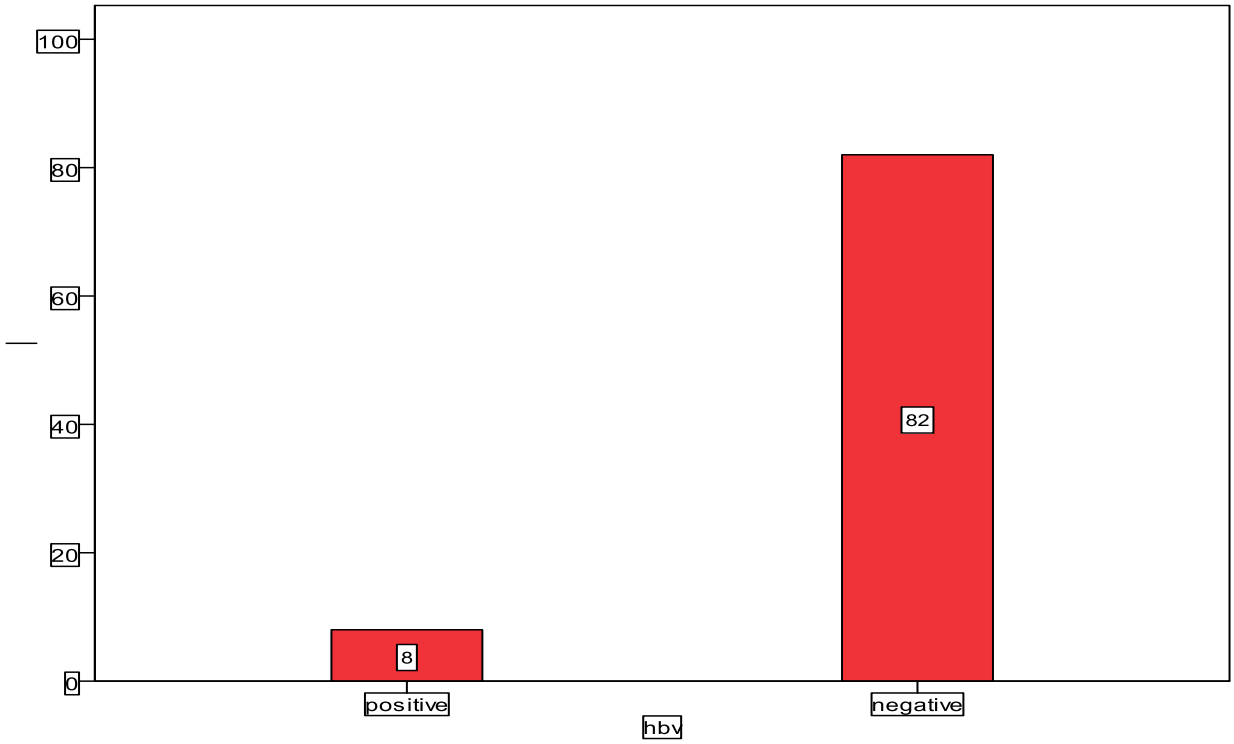

Fig (1): Frequency of the result.

Table (2): Frequency and percentage of HBV result

HCV	Frequency	Percentage%
Positive	8	8.9
Negative	82	91.1
Total	90	100

Table (3): Frequency of HBV according to gender.

Gender	Frequency	Percentage%
Males	4	4.4
Females	4	4.4
Total	8	8.9

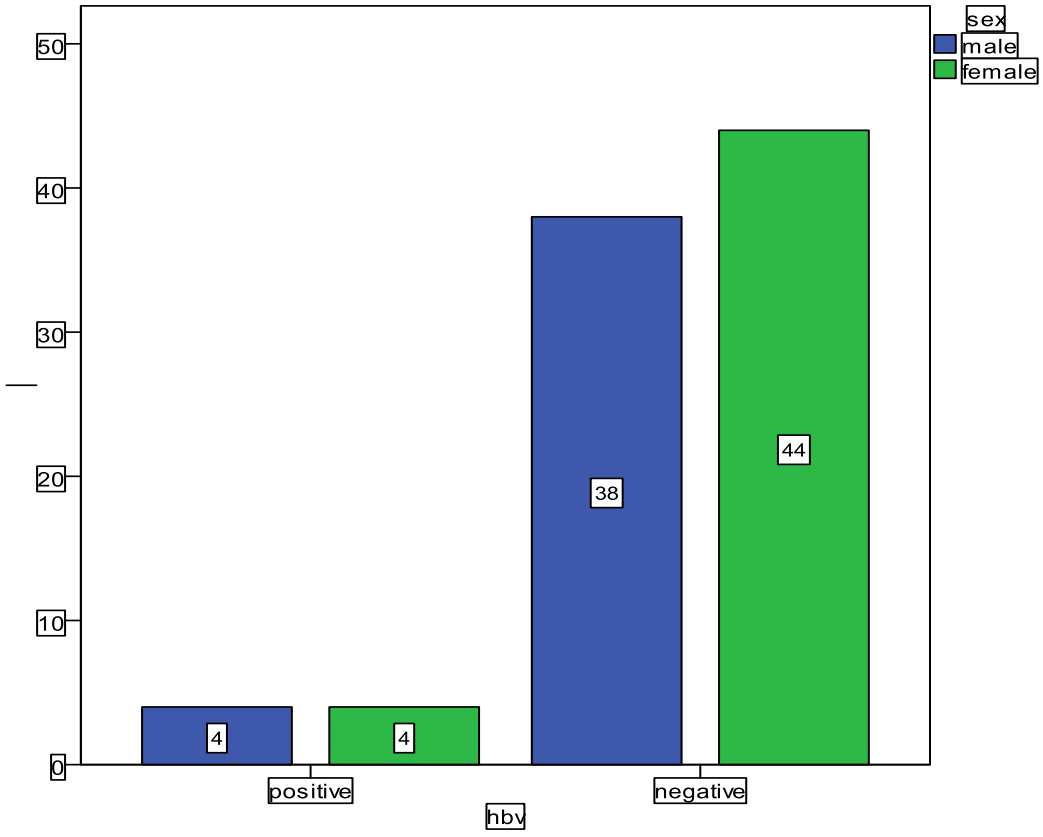

Fig (2): Frequency of HBV according to gender.

Table (4): Frequency of HBV according to history of blood transfusion.

Gender	History of transfusion +ve HBV	History of transfusion -ve HBV	No History of transfusion +ve HBV	No History of transfusion -ve HBV	Total
Male	0	3	4	35	42
Female	0	4	4	40	48
Total	0	7	8	75	90

Table (5): Frequency of HBV according to marital status.

Gender	Married +ve HBV	Married -ve HBV	Single +ve HBV	Single -ve HBV	Total
Male	2	37	2	1	42
Female	4	42	0	2	48
Total	6	79	2	3	90

Table (6): Frequency of HBV according to family history of hepatitis.

Gender	History of hepatitis +ve HBV	History of hepatitis -ve HBV	No History of hepatitis +ve HBV	No History of hepatitis -ve HBV	Total

Male	0	3	4	35	42
Female	0	6	4	38	48
Total	0	9	8	73	90

Table (7): Frequency of HBV according to history of surgery.

Gender	History of surgery +ve HBV	History of surgery -ve HBV	No history of surgery +ve HBV	No history of surgery -ve HBV	Total
Male	0	9	4	29	42
Female	0	17	4	27	48
Total	0	26	8	56	90

Table (8): Frequency of HBV according to history of jaundice.

	history of	history of	No histor	No histor	

Gender	jaundice +ve HBV	jaundice -ve HBV	y of jaundice +ve HBV	y of jaundice -ve HBV	Total
Male	1	11	3	27	42
Female	1	15	3	29	48
Total	2	26	6	56	90

Table (9): Frequency of HBV among people who have fatigue.

Gender	History of fatigue +ve HBV	History of fatigue -ve HBV	No History of fatigue +ve HBV	No History of fatigue -ve HBV	Total
Male	2	14	2	24	42
Female	0	22	4	22	48
Total	2	36	6	46	90

Table (10): Frequency of HBV among people who have fever.

Gender	Fever +ve HBV	Fever -ve HBV	No fever +ve HBV	No fever -ve HBV	Total
Male	0	5	4	33	42
Female	1	19	3	25	48
Total	1	24	7	58	90

Table (11): Frequency of HBV among people who have abdominal pain.

Gender	Abdominal pain +ve HBV	Abdominal pain -ve HBV	No abdominal pain +ve HBV	No abdominal pain -ve HBV	Total
Male	1	12	3	26	42
Female	1	22	3	22	48
Total	2	34	6	48	90

Table 12: frequency of HBV among people who have sharing razors and shaving machines

Gender	Sharing of razors and shaving machine +ve HBV	Sharing of razors and shaving machine -ve HBV	No sharing of razors and shaving machine +ve HBV	No sharing of razors and shaving machine -ve HBV	Total
Male	0	1	4	37	42
Female	0	3	4	41	48
Total	0	4	8	78	90

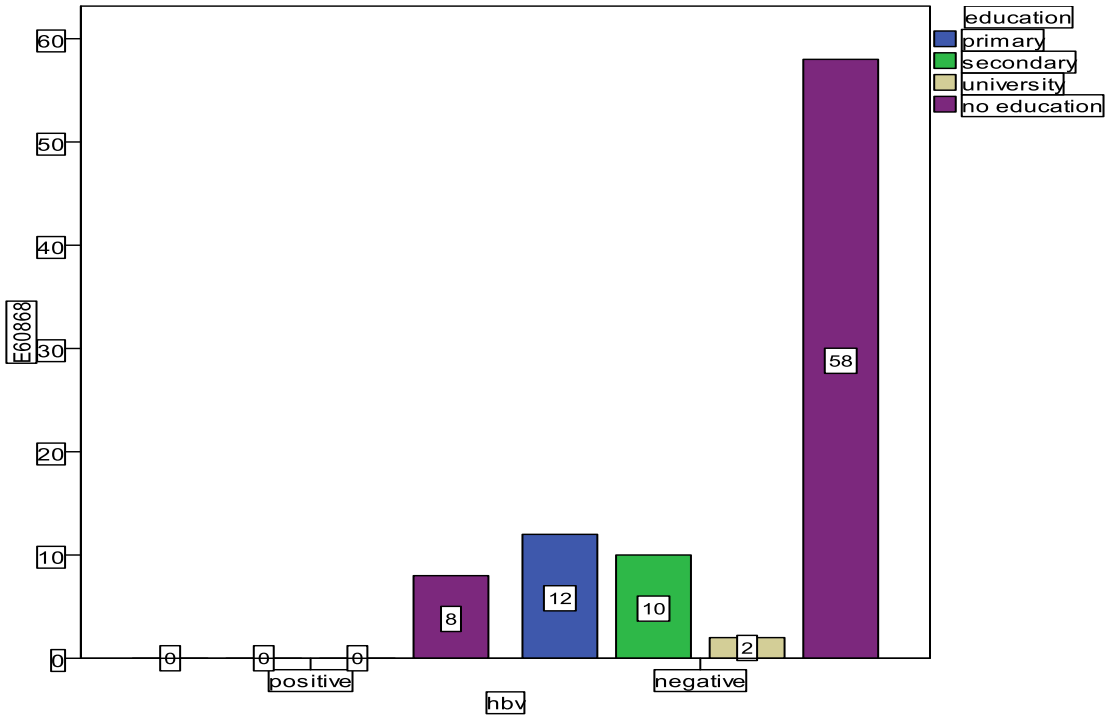

Fig (3): Frequency of HBV according to education.

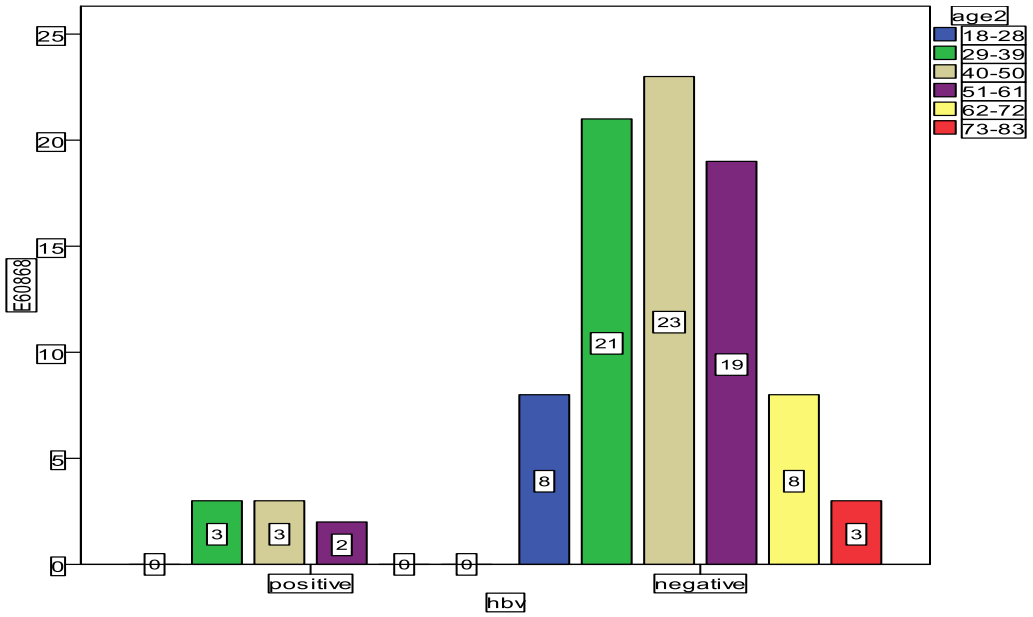

Fig (4): Frequency of HBV according to age group.

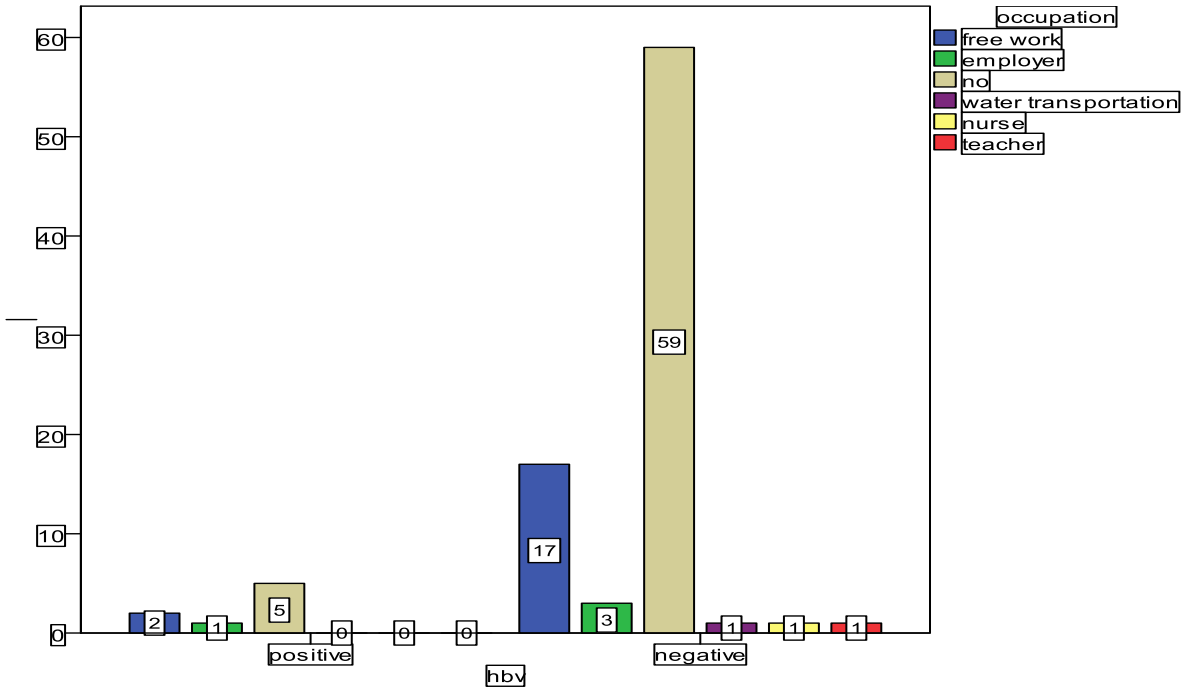

Fig (5): Frequency of HBV according to work.

CHAPTER FIVE

DISCUSSION

CHAPTER FIVE
DISCUSSION

Discussion

There is no doubt that migration can change the map of infectious diseases and there is a bilateral effect on the host and moved population.

Probably, infectious disease is one of the most challenging risks facing populations (Sharara and Kanj .,2014).

Infections with HBV pose serious healthcare problem, especially in developing countries.

Recently, some of the developing countries started ambitious projects to combat these infections.

This study aimed to determine the prevalence of HBV in refugees at Khartoum state. The study revealed that the prevalence of HBsAg among Sudanese refugees was (8.9%).

This result is higher to that obtained by Nawfal R Hussein, *et al* (2016) and Arshad Quddus, *et al* (2005) who reported that prevalence of HBsAg among Syrian and Afgan refugees was 3.86% and 8.3% respectively.

The prevalence of HBsAg in a population largely depends on immunization coverage against hepatitis B and prevalence of unsafe injection practices.

Lack of immunization services against hepatitis B in the refugees camps and the preference for injections for common illnesses are the main apparent factors leading to this high level of HBsAg prevalence in this refugee population (Arshad Quddus *et al* .,2005).

In addition, lack of safe health care delivery largely contributes to viral transmission particularly in the perinatal period and in pediatric populations.

Estimates suggest that contaminated needles account for 8–16 million HBV infections each year in developing countries (Kane *et al* ,1999).

This study also indicated that in Sudan, the surveyed refugees did not have a policy for hepatitis B vaccination. In fact, vaccination for HBV was obtained by the individuals themselves independent of the Ministry of Health.

5.2. Conclusion

The study concluded that the prevalence rate of HBV among refugees in Khartoum state was relatively high.

There is no relationship between included factors (age, gender, marital status, educational levels, family history of hepatitis, history of jaundice and general symptoms of disease) and prevalence of HBV.

5.3. Recommendation

- First of all, our study has some limitations; the sample size was relatively small for such a study. Probably, screening all the refugees was more desirable. However, limited resources were the main obstacles to perform mass screening.

  Secondly, the risk factor associated with such infections was not studied. It is important to mention that this study should be considered preliminary and more studies are needed to

investigate risk factors associated with the infections and other infectious diseases.

- To eradicate the infection among refugees a vaccination program must be set place by the Ministry of Health for all of refugees to Sudan.
- The Ministry of Health could consider offering subsidized or free

  Hepatitis B vaccination to refugees. In addition education on infection, infection control and other strategies need to be strengthened.

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APPENDICES

APPENDICES

Questionnaire

Prevalence of HBV among refugees in Khartoum state

*Age: …….. Sex: ……

*Tribe: ……... Education:…….....

*Occupation: ……..……. ……..

*Head country: …… Date of migration: ……..

*Recent address: ……..... Serial No: …….

*Marital status:

1) Single ( ) 2) Married ( )

*Needle sticks per year:

1) Yes ( ) 2) No ( )

*Sharing Razor and razor blade and shaving machine:

1) Yes ( ) 2) No ( )

*History of blood transfusion:

1) Yes ( ) 2) No ( )

*Family History with hepatitis:

1) Yes ( ) 2) No ( )

*Self injection with I.V drugs:

1) Yes ( ) 2) No ( )

*Haemodialysis:

1) Yes ( ) 2) No ( )

*Surgery:

1) Yes ( ) 2) No ( )

*History of jaundice:

1) Yes ( ) 2) No ( )

*Symptoms:-

*Fatigue:

1) Yes ( ) 2) No ( )

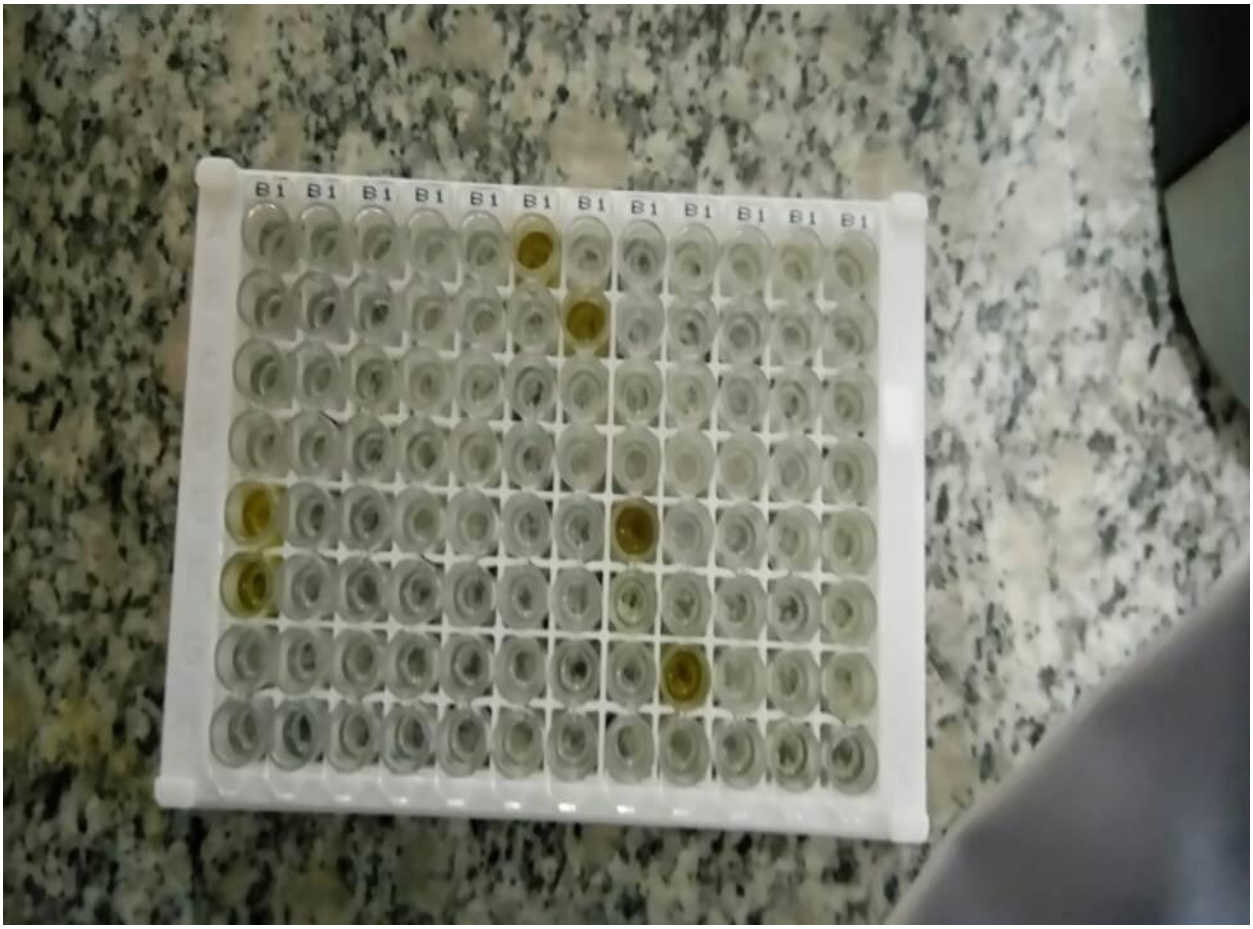
*Fever:

1) Yes ( ) 2) No ( )

*Abdominal pain:

1) Yes ( ) 2) No ( )

*Others: ……



Result of ELISA plate