1.1 Introduction

Hepatitis B is a viral infection that attacks the liver and can cause both acute and chronic disease. Hepatitis B virus (HBV) disease is serious complication worldwide and it's the most common cause of chronic hepatitis or liver cirrhoses and hepatocellular cancer(HPC) [1].

It's estimated that universally more than approximately two billion of people have been infected by hepatitis B virus and 350 million of them have chronic condition [2].Three quarter of the world population lives in highly endemic areas of infection[3]. Sudan is classified among countries of highly endemic hepatitis B infection (HBV)[3].The overall prevalence of HBV in Sudan in 2012 was 4%. The prevalence varies between the 15 states included in the Surveillance, ranging between 0.1% in Nahr Alnile and the Northern state to 15.7% in South Kordofan. In 2013, 17 states were included in the survey and the overall prevalence of HBV was found to be 5%. The prevalence of HBV ranged between 0% the Northern state to 12.3% in White Nile state. In 2014 HBV prevalence was found to be 4.2%. The survey included eighteen states and HBV prevalence ranged between 0.5% in the Northern state and 0.9% in Nahr Alnile to 8.8% in Gadarif.[4]

Hepatitis is one of the transfusion transmissible infection (TTIs) that is still concerned in blood donation in Sudan and other parts of world so to give patients safe blood it must be checked this disease and it's risk factors to prevent transmission of disease by blood to blood contact, during birth , sexual contact and by contaminated instruments(sharing needles)[5].

In this study will be going to determine the sero-prevalence of hepatitis B surface antigen and its risk factors associated with it for transmission HBV infection before to the introduction of blood screening and Vaccination River Nile sate.

Hepatitis B virus infection can lead to acute and chronic hepatitis. There is a phase during which HBsAg cannot be detected in the blood, although hepatitis B infection is present. During this window period detection of antibody to the hepatitis B core antigen (anti-HBc) serves as useful serological marked for hepatitis B infection[6].

1.2 objective

1.2.1 General objectives

To detect the Sero-prevalence of hepatitis B virus antigen among blood donors attending Blood Transfusion Center in Atbara River Nile state in 2017

1.2.2 Specific objectives

1. To detect the presence of hepatitis B virus antigen in blood donor

2. To correlate between the presence of hepatitis B virus antigen and risk factors (blood transfusion, blood donation, surgical operation, smoking, alcoholic intake, injection drugs dose, dental carrier, tattooing, vaccination and illegal sexual intercourse).

3. To get enough information about hepatitis BsAg in Atbara River Nile state blood donors.

4. To compare between ICT method and ELISA method in diagnosis of hepatitis B virus

1.3. Research problems

Since hepatitis b is one of the major viral problems and its endemic in Sudan specially in northern and River Nile state and there no enough information about it in last two years that is why we have chosen this research to get precise and accurate information about its prevalence.

1.4. Justification

Since hepatitis B viral infection is one major underlying factors leading to chronic liver disease and hepatocellular carcinoma and can be transmitted by blood to blood contact and there is no enough information about it in River Nile state this study will be concerned the evaluation of sero-prevalence of hepatitis B virus antigen among blood donors attending blood transfusion center in Atbara River Nile state to help in establish secondary medication in individual patients.

2. Literature review

2.1. Hepatitis B virus

Hepatitis b is a global health defect especially in developing countries and it is estimated universally more than approximately two billion of people have been infected by hepatitis B virus as it mentioned in introduction.

Around 350 million of these people have chronic infection[2]. approximately 5% of the world populations are carriers of HBV defined as being positive for hepatitis B surface antigen. there are four antigenic subtypes of hepatitis B (adw,ayw,adr,ayr) with geographic variation in the distribution of these subtypes, but have no more clinical significance with infection by these different subtypes.[7]

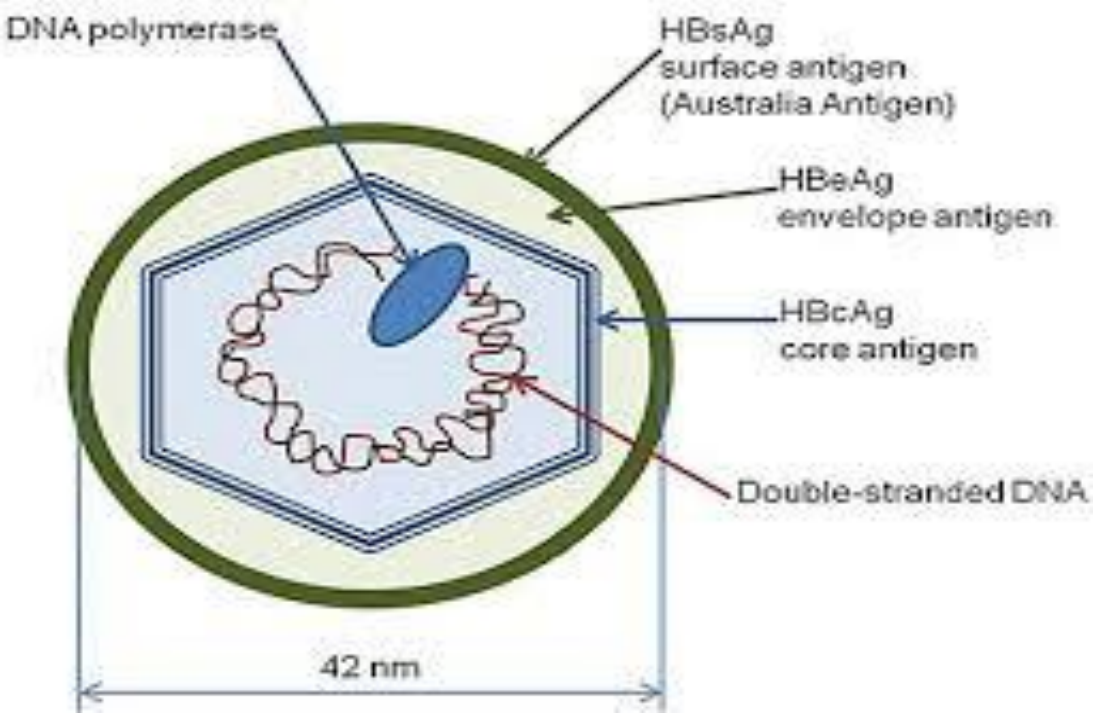
Complications for hepatitis B include progression of hepatocellular carcinoma (HCC) and rarely cirrhosis.[8]

2.2. stability of HBV

Hepatitis B virus is a relatively heat stable virus. It remains viable at room temperature for period, heating to 60ċ for 10 hours inactivate virus by factor 100-1000 fold. It is susceptible to chemical agent, exposure to hypochlorite or 2% glutaraldehyde for 10 minutes will inactivate virus

s 100000 fold though hepatitis B surface antigen may not be destroyed by such treatment. HBsAg is not destroyed by ultraviolet irradiation of plasma or other blood product.[9]

2.3. Chemical structure of HBV

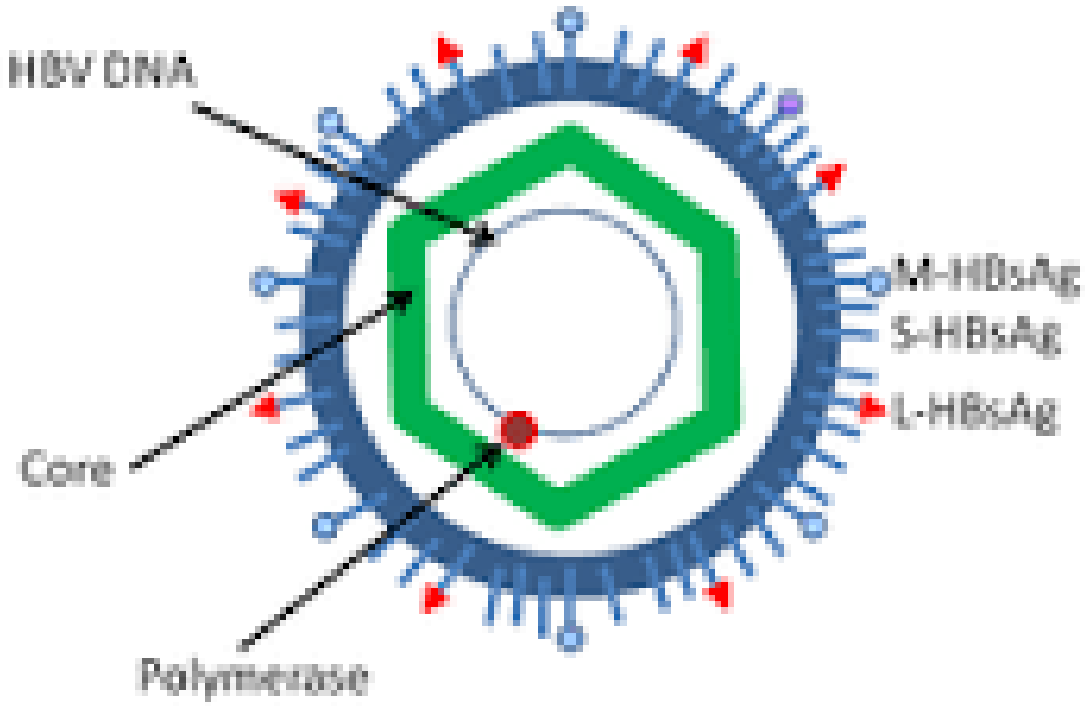


Hepatitis B virus is a number of the hepadnavirus family, the virus particle called Dane particle (virion) consist of an envelope lipid and an icosahedral nucleocapsid core composed of protein [10]. The envelope contains a protein called the surface antigen (HBsAg) which is valuable for laboratory investigation and immunization[11]. HBsAg was known as Australia antigen because it was first described in serum of an Australian aborigine.

There are two forms of surface antigen particle a spherical particle that measure 20-25nM in diameter and elongated tubular particle that measure more than 500nM in length[12].

1. HB core antigen (HBcAg)

2. Hepatitis Be antigen (HBeAg) which consist of three component referred to as HBeAg/1 HBeAg/2 and HBeAg/3.



Proteins of HBV encodes by viral genome includes

1. S. gene encodes the surface antigen
2. C. gene encodes the core antigen and the e antigen
3. P. gene encodes the polymerase
4. X. gene encodes the x protein. HBx is an activator of viral RNA transcription and may be involved in the on cogenesis because it can inactivate the p53 tumor suppressor protein [(11)]

Viral replication takes place predominantly and to a lesser extent in stem cells in the pancreas, bone marrow, and spleen. The intact virion is a double shelled particle with an envelope of hepatitis B surface antigen (HBsAg), an inner nucleocapsid of core antigen (HBcAg), and an active polymerase enzyme that is linked to a single molecule of doulble stranded HBV DNA[(13)].

2.4. HBV subtypes

The human hepatitis B virus has the smallest genome among the eukaryotic viruses and it is composed of circulate double stranded DNA with a nick in the non coding chain and along gap with the fixed 5 terminal end in the coding one [(14)].

Three HBV associated antigen are known including the surface antigen (HBsAg). The HBsAg has a group specific antigenic

determinants (A) and two pairs of mutually exclusive sub types – specific determinant "d" or "y" and "r" or "w" therefore it can be classified into 4 major sub types [adr,adw, ayw, ayr] the cloning in the bacterial cells has been performed and the primary structure of HBV DNA determined for the following HBsAg sub types: 1.ayw 3182bp 2.adw3221bp 3.adw3200bp and four variants of 4.adr(HBsAg)[7]

2.5 genetic variation of hepatitis B virus

Three main types of HBV sequence variability are known.

The first type of variability reflects the HBV genotype or local strain genotype. Specific variability is on a short time scale stably transmitted within the host population and present from the beginning of infection in an individual.

The second type of variability arises spontaneously during replication of HBV due to biochemical processes of the host cell or due to infidelity of the viral replication machinery. These mechanisms provide a basis for both evolutionary virus drift and selection of specific variants in a patient.

The third type of viral sequence variability is the result of a selection process due to a selective advantage of variants during the course of HBV infection in patients [15].

2.6. Clinical types of hepatitis B Virus infection

2.6.1. acute phase

The incubation period is 1-6 months in the acute phase of hepatitis B infection. Anicteric hepatitis is the predominant form of expression for this disease.

The most of people are asymptomatic but patients with anicteric hepatitis have a greater tendency to develop chronic hepatitis. Patients with symptoms are resembled with patients who develop icteric hepatitis. Icteric hepatitis is associated with a prodromal period during which a serum sickness like syndrome can occur.

The symptomatology is more constitutional and includes:

- Anorexia
- Nausea
- Vomiting
- Low grade fever
- Myalgia
- Fatigability
- Disordered gustatory acuity and smell sensation
- Right upper quadrant and epigastria pain.

Patients with fulminant and subfulminant hepatitis may present with the following:

- Hepatic encephalopathy
- Somnolence
- Disturbances in sleep pattern
- Mental confusion
- Coma
- Gastrointestinal bleeding
- Coagulopathy

2.6.2 chronic phase

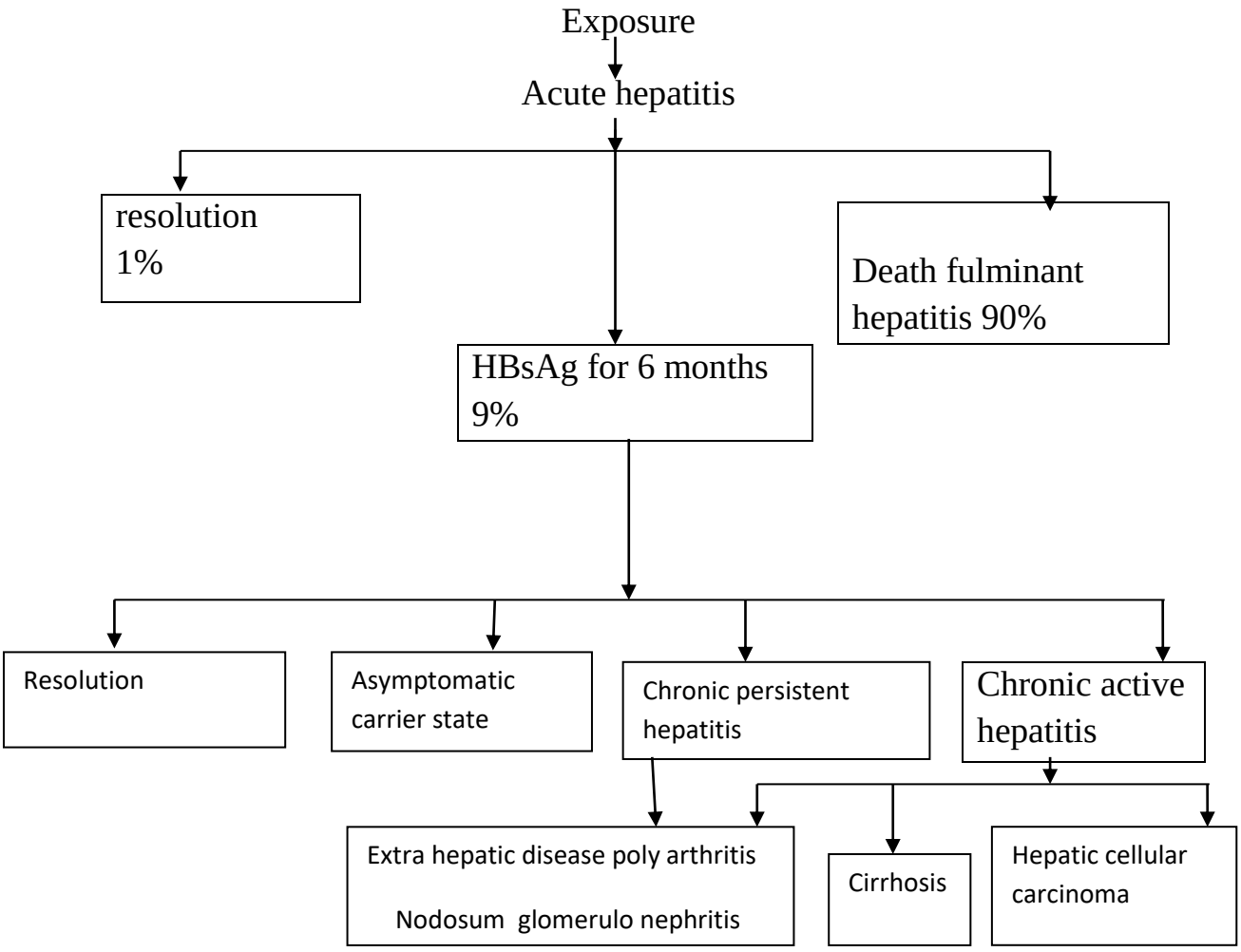
Chronic patients in hepatitis can be immune tolerant or have an inactive chronic infection without any evidence of active disease; they are also asymptomatic.

Patients with chronic active hepatitis especially during the replicative state, may complain of symptoms including:

- Symptoms similar to those of acute hepatitis
- Fatigue
- Anorexia
- Nausea
- Mild upper quadrant pain or discomfort

If progressive liver disease is present, the following symptomatology my appear:

- Hepatic decompensation
- Hepatic encephalopathy
- Somnolence
- Disturbances in sleep pattern
- Mental confusion
- Coma
- Gi bleeding
- Coagulopathy[16].



2.7 clinical and serological course of HBV infection

Hepatitis B virus has eight genotypes whish have distinct geographical distribution .studies comparing differences in the clinical outcomes of infections caused by strain whish genotype related variation in the HBV genome have largely compared genotypes B ,C ,A and D but not all four genotypes .the present study include 196 HBV .infected patient attending and infections disease outpatient clinic in Sweden .the HBV DNA and were analyzed for each patient .HBV DNA was defected in 144 patients and the HBV genotype and the core promoter and pre core sequences were determined for the isolation from 101 of these patient among the patient who might be considered most likely to be non viromic.

Namely anti HBe .possible HBV carries with normal alanine aminotransferase (ALT) level 65% had detectable HBV DNA and were thus viremic among viremic patient HBe Ag .positive patient were more likely to have elevated (ALT) level than anti HBe positive patint ,HBV genotypes A to F were represented in the study and their distributions coincided accurately with number of genotype D infected patient were anti HBe positive and had elevated (ALT) levels 42% of genotype D infected patient but 5% patient infected with mutation in the core promoter and the pre core regions were significantly correlation with elevated (ALT) levels in the patients .the different were not age related .therefore in this appears to be associated with more active disease.[17]

2.8 Epidemiology of hepatitis B virus infection

2.8.1 Global epidemiology of HBV:

The burden of hepatitis B virus disease and efforts to control infection with determine the future size of the population requiring treatment of HBV infection to quantity the current prevalence of infection and to reexamine the epidemiology of HBV infection .a structured review was conducted that focused on available primary literature for over 30 countries worldwide.

The prevalence of chronic HBV infection continues to be highly variable ,ranging over 10% in some Asian and western specific countries to under 0.5% in the united states and northern European countries .the current

global estimated of the number of HBV infected individual is 350 million [18]

The prevalence of the chronic HBV infection various greatly in different part of the world, and it could be categorized as high , intermediately and low epidemiology. The age at the time of infection is associated with the epidemiology of hepatitis B infection.

2.8.2 Highly Endemicity

The prevalence of HBV infection varies markedly throughout regions of the world [19]

Hepatitis B is highly endemic in developing regions with large population such as south east Asia, china, sub-Saharan Africa and Amazon basin where at least 8% of the population are hepatitis b virus chronic carrier. Most infection occur during infancy or childhood (asymptomatic), there is a little evidence of acute disease related to hepatitis B virus, but the rate of chronic liver disease and liver cancer in adult are high [20].

2.8.3 Intermediate endemicity

Hepatitis B is moderately endemic in part of the eastern and southern Europe, middle east, Japan and part of south America. Between 10-60% of the population have evidence of infection, and 2-7% are chronic carriers , acute disease related to hepatitis B virus is common in these area because many infection occur in adult, however the high rate of chronic infection are maintained mostly by infection occurring in infants and children [21]

2.8.4 Low endemecity

The endemicity of hepatitis B virus is low in most developed areas, such as north America north and western Europe and Australia. In these regions, hepatitis B virus 5-7% of population and only 0.5-2% of the population are chronic carriers [22]

2.9 hepatitis B virus transmission

Major source of HBV is blood and its products but also HBV found in semen, saliva, tears ,breast milk ,vaginal and menstrual secretion ,amniotic fluid and CSF in acutely ill patient and also in carriers.

1 .transfusion of blood and blood product containing HBV

2.use of hepatitis B virus contaminated prickets, needle, or syringes.

3. close personal contact with a person with hepatitis B.

4. congenital or vertical from carrier mother is common

5. saliva and semen are responsible for venereal transmission[9,12]

2.10 diagnosis of HBV:

The diagnosis of HBV infection require the evaluation of patients blood for HBs Ag, hepatitis B surface antibody (HBsAb).although the presence of HBsAg indicate that the person is infectious ,the presence of HBsAb indicate recovery and immunity from HBV infection or successful immunized on against HBV .

HBcAb appears of the onset of acute HBV infection , but may also indicate chronic HBV infection.

2.10.1 Diagnosis of HBV

The diagnosis of HBV infection is typically based on the evaluation of serological and virological markers of HBV in serum as well as the evaluation of biochemical and histological markers of the liver [٣١].

2.10.2 Biochemical Assays

The biochemical assessment of liver function include: total and direct bilirubin (TSB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, albumin, globulin and coagulation profile [٤٠].

2.10.3 Serological Diagnosis of HBV

The most useful detection methods are ELISA for detecting HBV antigens or antibodies [٣٠]. The detection of serological markers are based on Ag-Ab reaction, therefore divided into two classes, detection of Ags or detection of Abs.

2.10.3.1 Detection of HBV Antigens

2.10.3.1.1 Detection of HBsAg

Hepatitis B surface antigen is the first serological marker to appear after infection. Its persistence for more than 6 months indicates CHB infection [٣١]. HBsAg appears at average of 6-8 weeks after exposure, 1-3 weeks before ALT becomes abnormal and 3-5 weeks before onset of symptoms or jaundice [٢٤]. This Ag can be detected by many techniques; the commonly used are radioimmunoassay (RIA) and enzyme immunoassay. Since many immunoassays use monoclonal antibodies directed against the "a" determinant, amino acid substitution in this region may account for false-negative results in immunoassay [٣٣]. Thus, diagnosticians and healthy care industry need to increase their awareness of HBsAg mutation and how these mutants may alter current diagnostic and treatment algorithms [٢٨].

2.10.3.1.2 Detection of HBeAg

The presence of HBeAg indicates active viral replication. However, its absence cannot be assumed to equate to absent of viral replication because HBeAg is not detectable in patients with HBeAg-negative HBV infection [٣١].

Highly sensitive assays such as passive hemagglutintion and RIA have demonstrated that HBeAg appears simultaneously or within a few days of the appearance of HBsAg in all or almost primary infection [٣٠]. In CHB infection, HBeAg may persist for years prior to seroconversion to anti-HBe [٣٥].

2.10.3.1.3 Detection of HBcAg

Hepatitis B core antigen is an intracellular antigen that is expressed in infected hepatocytes. It is not detectable in serum [٣٢].

2.10.4 Detection of HBV Antibodies

2.10.4.1 Anti-HBs Antibodies

Anti-HBs replace HBsAg as AHB infection is resolving. It generally persists for life time in over 80 % of patients and indicates immunity [٢٩]. Occasionally, anti-HBs and HBsAg are both detectable in patients with CHB infection, a finding of no known significance [٣١]. Anti-HBs may not be detectable until after a window period of several weeks to months [٢٦]. This marker is acquired through natural HBV
infection, vaccination, or passive antibody immunization [٣٢].

Level of circulating anti-HBs is used to determine the effectiveness of vaccination and in USA an antibody level of 10 ml u / ml or higher indicates immunity [٣٧].

2.10.4.2 Anti-HBc Antibodies

It is the first antibody to appear. Demonstration of anti-HBc in serum indicates HBV infection, current or past. Anti-HBc IgM is present in high titer during acute infection and usually disappears within 6 months, and although it can persist in some cases of chronic infection, this test may therefore reliably diagnose AHB infection [٢٩]. High levels of IgM-specific anti-HBc are frequently detected at the onset of illness because this antibody is directed against the 27 nm internal core component of HBV and it is appearance in the serum indicate viral replication [٣٠]. Anti-HBc IgG predominates after 6 months and generally persists indefinitely in patients who have recovered from HBV infection. Anti-HBc IgG present in virtually at all patients who ever been exposed to HBV [٢٩و٣٢]. Anti-HBc total is used with anti-HBs and HBsAg for screening populations at risk [٣٧].

2.10.4.3 Anti-HBe Antibodies

Hepatitis Be Ag is replaced by anti-HBe, signaling the start of resolution of disease. Anti-HBe levels often are no longer detectable after 6 months [30].

Its presence in CHB infection indicates the onset of the non-replicative phase [٣٥,٣٢]. Generally HBeAg seroconversion to anti-HBe has been considered the end point for HBV therapy for HBeAg-positive (wild type) patients, because it has shown to be associated with a lower risk for disease progression, although not protective against a later development of HCC [٣١].

2.10.5 Molecular Diagnosis

The amount of HBV-DNA in serum is a measure of the level of viral replication. Previously, serum HBV-DNA testing was performed using non-amplified hybridization. These assays (Dot blot hybridization, Liquid hybridization, North blot and branched DNA assays) have a limit of quantification of 105-106copies / ml and should no longer be used for routine management of patients with CHB infection [٣١]. HBV-DNA assays provide very useful adjunct information concerning viral replication, especially in situations when patients' serological profiles fall outside of classical pattern. The molecular testing of HBV

consists of two categories (i) HBV-DNA quantification assays that measure the amount of HBV-DNA in peripheral blood, which reflects the level of HBV replication (viral load) in the liver (ii) assays that identify sequences or motifs of clinical or pathophysiological importance in the HBV genome[٣٤].

2.10.5.1 Polymerase Chain Reaction Technique (PCR)

Hepatitis B virus DNA detection based on a nested PCR approach can detects as few as 102 -103genome copies [٣٨]. It is at least 10 times more sensitive than dot blot assays for HBV-DNA [٤١].

In contrast to PCR assay that measure HBV-DNA titers only after the PCRcycle (end point measurement), Real-time PCR technology, based on continuous quantitative monitoring during the exponential phase of the PCR reaction, it is able to measure viral loads over a large dynamic rang [٣٧]. New developments (Taq Man technology, molecular beacons) that decrease the number of handling steps reduce contamination, and increase throughput and the accuracy of quantification will further enhance the utility of these assays [٣٦].

2.10.6 Viral Load

Researchers have developed assays that detect and accurately measure HBVDNA. These assays detect the viral genome and measure the level of circulating virus in an infected individual. The level of HBV-DNA is often referred to as “viral load”; predict response to anti- viral therapy based on pretreatment viral load [٣١]. The National Institutes of Health Workshop on Management of Hepatitis B recommended that the treatment be considered for patients with detectable HBV-DNA by non amplified assays (i.e. > 105 copies / ml or 20,000 IU / ml). However, some HBeAg-negative patients have fluctuating HBV-DNA levels

that decrease to less than (105copies / ml) [٤٢]. Furthermore, the threshold HBV-DNA level associated with progressive liver disease is unknown. In the panel’s experience, patients can have advanced liver disease even if they have serum HBV-DNA levels persistently less than 20,000 IU / ml, thus, the clinical significance of low HBV-DNA levels is uncertain and should be individualized [٣١]. Real-time PCR assays have become more widely available and preferred in the initial evaluation of patients, and even, more importantly in the monitoring of both treated and untreated patients. The current lack of standardization between assays, which make it difficult to compare data

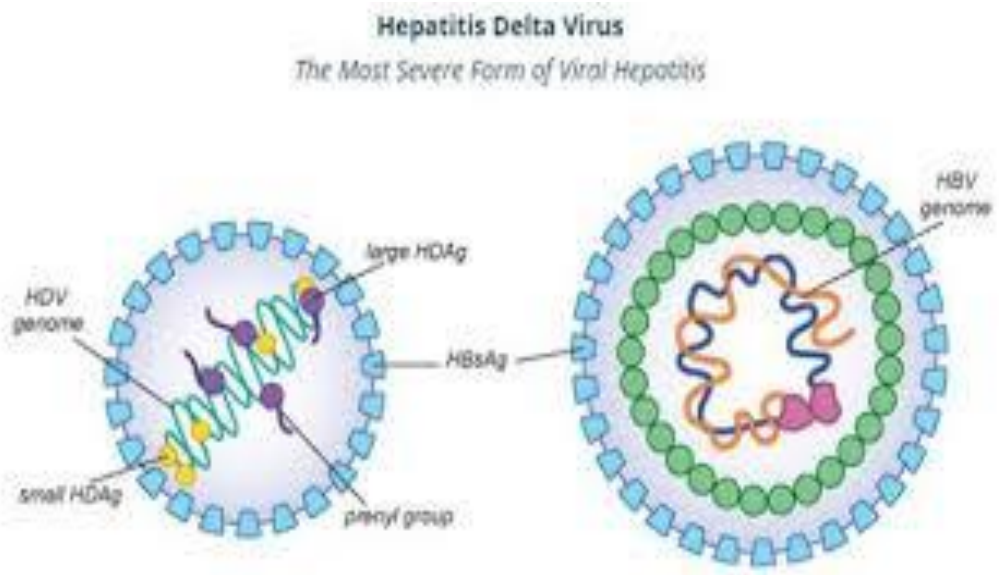
from different laboratories, will be resolved with the introduction of an HBV-DNA standard [٣٩]. In the future all results should be reported in IU / ml (IU / ml equals approximately 5.6 genome / ml) [٣١].

2.10.7.Active Immunization for HBV

Hepatitis B is a vaccine-preventable disease, but although global control of hepatitis B is achievable. Hepatitis B vaccine is the first and currently the only vaccine against a major human cancer. Vaccination is the most effective tool in preventing the transmission of HBV and HDV [٤٢].

2.11 hepatitis delta virus (HDV) :

The delta antigen particle is a recently discovered RNA molecule that measure 35-37nm in diameter .it resembles a large HBsAg particle .



The hepatitis delta virus or HDV is a RNA defective virus composed of core presenting the delta specific antigen ,encapsulated by HBsAg that require the helper function of HBV to support its replication .infection by HDV occurs in the presence of acute our chronic HBV infection .when acute delta and acute HBV simultaneously occur, the illness becomes severe and clinical and biochemical features may be indistinguishable from those of HBV infection alone ,in constant, a patient with chronic HBV infection can support HDV replication indefinitely usually with a less severe illness appearing as a clinical exacerbation .

The determination of HDV specific serological markers (HDVAg, HDVAb, HDVIgM, and HDVIgG) represents in these cases an important tool to the clinician for the classification of the etiological agent , for the follow up of infected patients and their treatment .the detection of HDV total antibodies allows the classification of the illness and the monitoring of the seroconversion event.[13]

3. Material and methodology

3.1. Study design

This is cross sectional descriptive study, this study was conducted in period from April to June in 2017 to detect the Sero-prevalence of hepatitis B surface antigen among blood donors.

3.2. Study area

The study was conducted at Atbara hospital in central blood bank which is located in Atbara town in north Sudan. Atbara is a town in northern Sudan, 300km northeast of Khartoum.

3.3. Study population

Blood donors attending in central bank of Atbara. Donors with normal hemoglobin and pass physical examination was included and Donors with abnormal hemoglobin and not pass physical examination was excluded.

3.4 Sample size

A total of 91 donor samples were collected from various capillary bloods for ICT viral scanning according to the formula of Stephen Thompson that is $n = \frac{N \times p(1-p)}{[[N-1 \times (d^2 \div z^2)] + p(1-p)]}$ where n is sample size, N is population size, p and d is constant 0.05 that is degree of accuracy and z is degree of standard that is 1.96 and conforming the positive results were done by ELISA. Capillary blood will be collected using sterile lancet after cleaning capillary puncture area with 70% ethanol for viral screening by immune chromatography test (ICT) and venous blood by using sterile disposable plastic syringe , the venous blood was used to detect serum antigen

Sample was Centrifugated at 1300rpm for 15 minutes to obtain serum.

3.5. Data collection

Primary data was collected by using questionnaire, taking 2cc (2ml) of blood sample from each donor.

3.6 study variables

blood transfusion, blood donation, surgical operation, smoking, alcoholic intake, injection drugs dose, dental carrier, tattooing, vaccination and illegal sexual intercourse are all study variables.

3.7. Data analysis

Data was analyzed by using statistical package for social science (SPSS).

3.8. Ethical consideration

Ethical approval for our study was given by the University of El-sheik Abdullah El-badri ethics committee and permission from ministry of health to use blood donors from blood transfusion center at Atbara River Nile state was granted as well.

Information about the study was also delivered to the blood donors and consent was obtained then face to face interview conduction.

Informed written consent was obtained from blood donors who was agree to participate in the study.

3.9. Methodology

3.9.1 Sampling

Individuals those were diagnosed as hepatitis B were selected and data was collected by using self-administrated pre-coded questionnaire which was specially designed to obtain information that help in study.

3.9.2 .ICT Method

3.9.2.1. ICT principle

The HBsAg rapid test cassette is a qualitative solid phase two side sandwich immune assay for the detection of HBsAg in serum or plasma. the membrane is pre coated with anti HBsAg antibody on the test line region of the cassette. During testing the serum or plasma specimen reacts with the particle coated with anti HBsAg antibodies. The mixture migrated upward on the membrane chromatographically by capillary action to react with anti HBsAg antibody on the membrane and the generate are colored line. The presence of this colored line in the test region indicated a positive result, while it is absence indicated a negative result. To serve as a procedural control, a colored line will always appear in the control line region indicated that proper volume of specimen has been added and membrane wicking has occurred. [23]

3.9.2.2. Materials provided

- Test cassette
- Droppers
- Package insert

3.9.2.3 Materials required but not provided

- Specimen collection containers
- Centrifuge
- Timer
- Gloves
- Syringe
- Cotton
- Tunicate

3.9.2.4 Procedure

Step1: remove the cassette from sealed foil pouch and use it as soon as possible. Best results will be obtained if the assay is performed immediately after opening the foil pouch.

For serum or plasma specimen:

Spet2: Hold the dropper vertically and transfer three drops of serum or plasma (approximately 120microleter) to the specimen well of test device and start the timer. See illustration below.

Step3: wait for the colored line is appeared. The result should be read at 15-30minutes. Do not interpret the result after 30 minutes

3.10 ELISA

3.10.1 Principle of ELISA

The test is an enzyme immunoassay based on a sandwich principle. Micro titer wells have been coated with monoclonal and anti hepatitis B surface (antibody to HBsAg), which constitutes the solid base antibody. The test sample is incubated in such well: HBsAg, if present in the sample, will bind to the solid base antibody subsequently a guinea pig anti HBs, which has been labeled with enzyme horse reddish peroxidase(HRP), is added. With a positive reaction this labeled antibody bound to any solid base antibody HBsAg complex previously formed. Incubation with enzyme substrate produces a blue in the test well which turns yellow when the reaction is stopped sulfuric acid. If the sample contain no HBsAg, the labeled cannot be bound specifically and only allow background color develops.

Assay principle schemes: double antibody sandwich ELISA

Ab(p)+Ag(s)+(Ab)ENZ ⟶ [Ab(p)-Ag(s)-(Ab)ENZ] ⟶ blue ⟶ yellow(+).

Ab(p)+(Ab)ENZ ⟶ [Ab(p)] ⟶ no color (-).

Incubation	immobilized complex	coloring results
60min	15min	

3.10.2. ELISA material

Kit contents	**Volume**
Micro well plate 96 tests	1 plate (12x8 well strips per plate)
Negative control	1x1ml
Positive control	1x1ml
HRP conjugate reagent	1x7ml
Stock wash buffer	1x30ml (dilute 1 to 20 with distilled water before use . once diluted stable for two weeks at 2-8ċ).
Chromogen solution A	1x8ml (ready to use and once open, stable for one month at 2-8ċ).
Chromogen solution B	1x8ml (ready to use and once open, stable for one month at 2-8ċ).
Stop solution	1x8ml
Plastic sealable bag	1 unit
Plate cover	1 sheet
Package inserts	1 copy

Additional material and instruments required but not provided

1. Freshly distilled or deonized water
2. Disposable gloves and timer.
3. Appropriate waste container for potentially contaminated material.
4. Disposable v shaped troughs.
5. Dispensing system and / or pipette (single or multi channel). Disposable pipette tips.
6. Absorbance or clean towel.
7. Dry incubator or water bath, 37±0.5ċ.
8. Micro shaker for dissolving and mixing conjugate with sample.
9. Micro well plate reader, single wave length 450nm or dual wave length 450nm and 630nm.
10. Micro well aspiration/ wash system.

3.10.3 ELISA method

Step 1. Reagent preparation:

allow the reagent and samples to reach room temperature (18-30ċ)for at least 15-30minutes. Check the wash buffer concentrate for the presence of salt crystal. If the crystals have formed in the solution, re-solubilize by warming at 37ċ until crystals dissolve. Dilute the stock wash buffer 1-20 with distilled or de ionized water. Use only clean vessels to dilute the buffer.

Step 2. Numbering wells:

set the strips needed in strip holder and sufficient number of wells including three negative controls (eg. B1,C1,D1), two positive controls (eg. E1,F1) and one blank (eg. >E1,F1) and one blank (eg. A1, neither samples nor HRP conjugate should be added in to the blank wells). If the result will be determined by using double wavelength plate reader, the requirement for use of blank well could be omitted. Use only number of strips required for the test.

Step3. Adding sample and HRP conjugate:

add 50µl of positive control, negative and specimen into their respective wells. Note: use a separate disposal pipette tip for each specimen, negative and positive controls as to avoid cross contamination. Add 50µl HRP conjugate to each well except the blank and mix by tapping the plate gently.

Step4. Incubation:

cover the plate with plate cover and incubate for 60 minute at 37ċ. It is recommended to use thermostat controlled water tank to assure the temperature stability and humidity during the incubation. If dry incubator is used, do not open the door frequently.

Step5. Washing:

add the end of incubation, remove and discharge the palate cover. Wash each well five times with diluted wash buffer. Each time, allow the micro wells to soak for 30-60second. After the final washing cycle turn the plate

down on to blotting paper of clean towel, and tap it to remove any remainders.

Step6. Coloring:

dispense 50µl of chromagen A and 50µl of chromagen B solution into each well including the blank, and mix by tapping the plate gently. Incubate the plate at 37ċ for 15 minutes avoiding light. The enzymatic reaction between the chromagen solutions and the HRP conjugate produce blue color in positive control and HBsAg positive sample wells.

Step7. Stopping reaction:

Using a multichannel pipette or manually, add 50µl stop solution into each well and mix gently. Intensive yellow color develops in positive control and HBsAg positive sample wells.

Step8. Measuring the absorbance:

Calibrate the plate reader with the blank well and read the absorbance add 450nm. If dual filter instrument is used, set the reference wavelength at 630nm. Calculate the cut off value and evaluate the results (note: read the absorbance within 5 minutes after stopping the reaction). [25]

Step 9 cut off calculation: cut off = $\frac{3\text{control negatives}}{3}$+0.05

Positive >0.1

Negative<0.1

According to this equation the two donors became 1.6 and 1.2 which means positive test results.

Results

This Analytical prospective descriptive study which was conducted in Atbara during the period of April to June 2017.The study Aimed to detect the Sero-prevalence of hepatitis B virus antigen among blood donors attending blood transfusion center at Atbara River Nile state .

Our results showed that 2.2% of all blood donors in Atbara town during the period from March to June were positive for hepatitis B virus.

After statistical analysis by SPSS Also statistical analysis shows that there was no significant association between hepatitis B results and study risk factors including blood donation, blood transfusion, surgical operation, tattooing, alcoholic intake, vaccination, drug abuse and smoking.

All of specimen were taken from male among total specimen of 91samples, 2.2% of them were positive while other samples were negative.

All of blood donors according to:

- Age group 18-28(67%), 29-38(29%) and 39-48(4%) showed in table 4.1.2,
- Gender they were all from male as it showed in table 4.1.3,
- Residence they are all from Atbara residences as it showed in table 4.1.4
- blood donation 49% of them were donated blood while 51% were not as it showed in table 4.1.5,figure 4.1.2
- blood transfusion 12% of them were transfused blood while 88% other were not as it showed in table 4.1.6. figure 4.1.3
- smoking 39% of them were smoking while 61% were not as it showed in table 4.1.7 , figure 4.1.4
- surgical operation 14% of them were surgically operated while 86% were not as it showed in table 4.1.8, figure 4.1.5
- Tattooing 4% of them were tattooing while 95% were not as it while the missed 1% showed in table 4.1.9, figure 4.1.6
- alcoholic intake 14% of them were alcoholing while 86% were not as it showed in table 4.1.10, figure 4.1.7
- vaccination 8% of them were vaccinated while 92% were not as it showed in table 4.1.11, figure 4.1.8
- drug abusers 12% of them were used drug abusers while 88% were not as it showed in table 4.1.12, figure 4.1.9

according to co-relation between presence of hepatitis B antigen and all risk factors there is no relationship between the presence of hepatitis B virus antigen and risk factors including blood donation, blood transfusion, smoking, surgical operation, tattooing, alcoholic intake, vaccination and drug abuse and showed in table (4.2.1, 4.2.2, 4.2.3, 4, 2.4, 4.2.5, 4.2.6, 4.2.7, 4.2.8) respectively were p values of them were (0.166, 0.096, 0.258, 0.559, 0.942, 0.559, 0.860 and 0.596) respectively.

Table 4-1-1 sero- prevalence of hepatitis B virus antigen among blood donors

Item	Percentage
Positive	2.2%
Negative	97.8%

Figure 4-1-1sero prevalence of hepatitis B virus antigen among blood donors

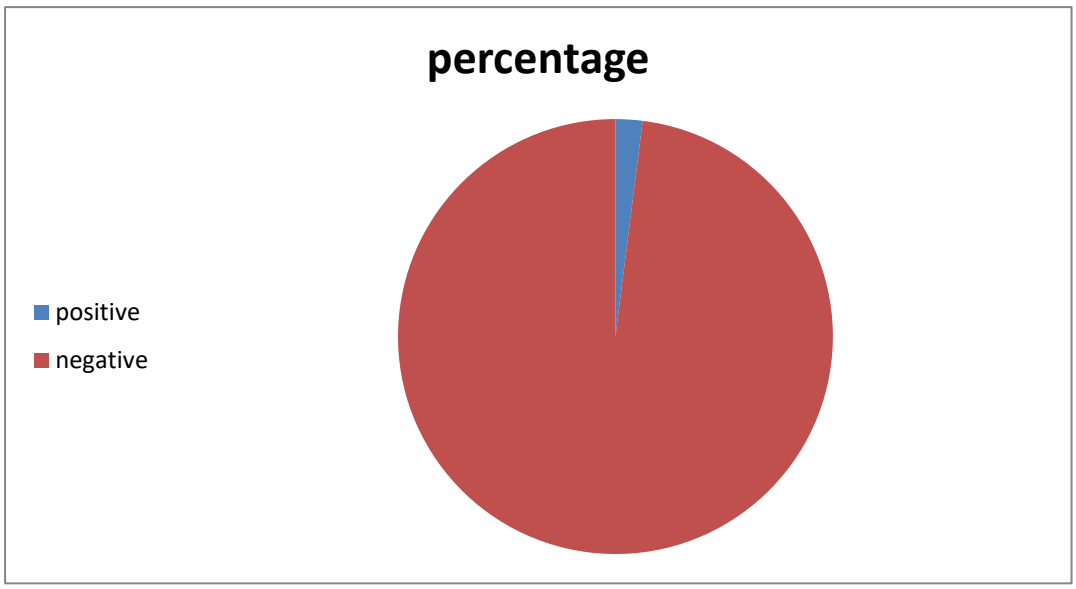


Table 4-1-2 show frequency percent of blood donors among age

Age	Percentage
18-28	67%
29-38	29%
39-48	4%

Table 4-1-3 show frequency percent of blood donors among gender

Gender	Percentage
Male	100%
Female	0

Table 4-1-4 show frequency percent of blood donors among residence

Residence	Percentage
Atbara	100%
Other	0

Table 4-1-5 show frequency percent of blood donation

Item	Percentage
Yes	49%
No	51%

Figure 4-1-2 show frequency percent of blood donation

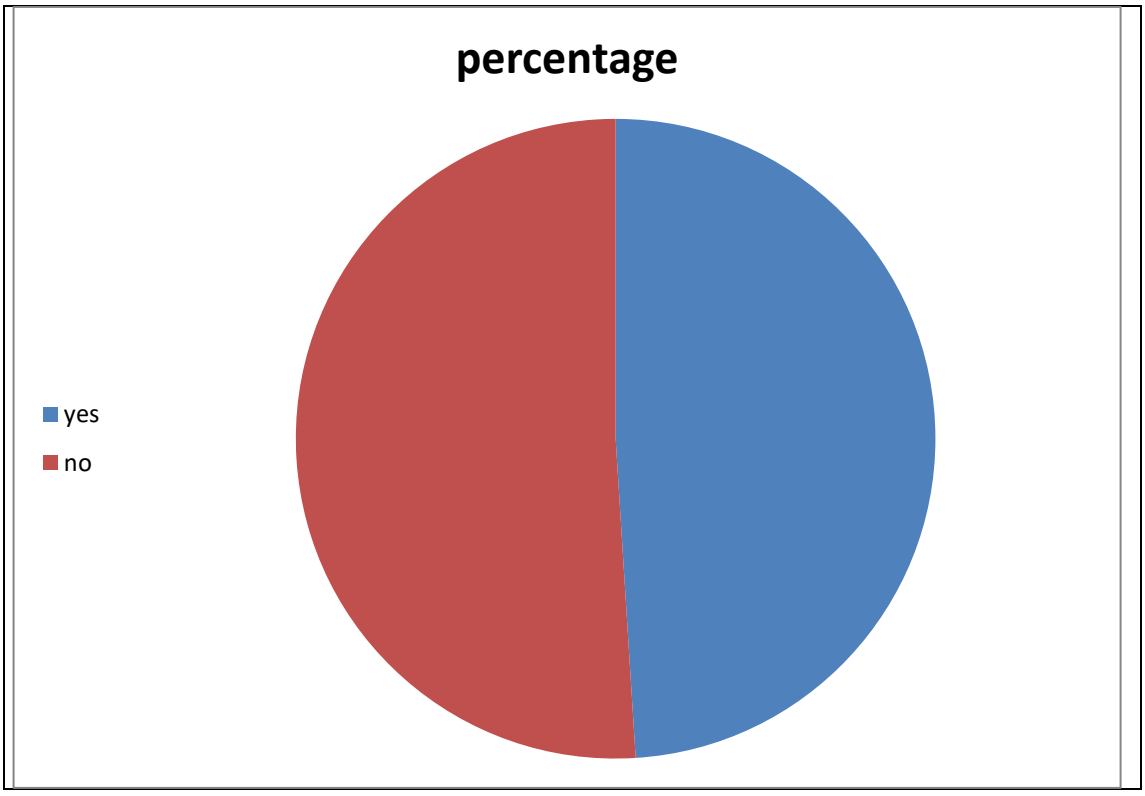


Table 4-1-6 show frequency percent of blood transfusion

Item	Percentage
Yes	12%
No	88%

Figure 4-1-3 show frequency percent of blood transfusion

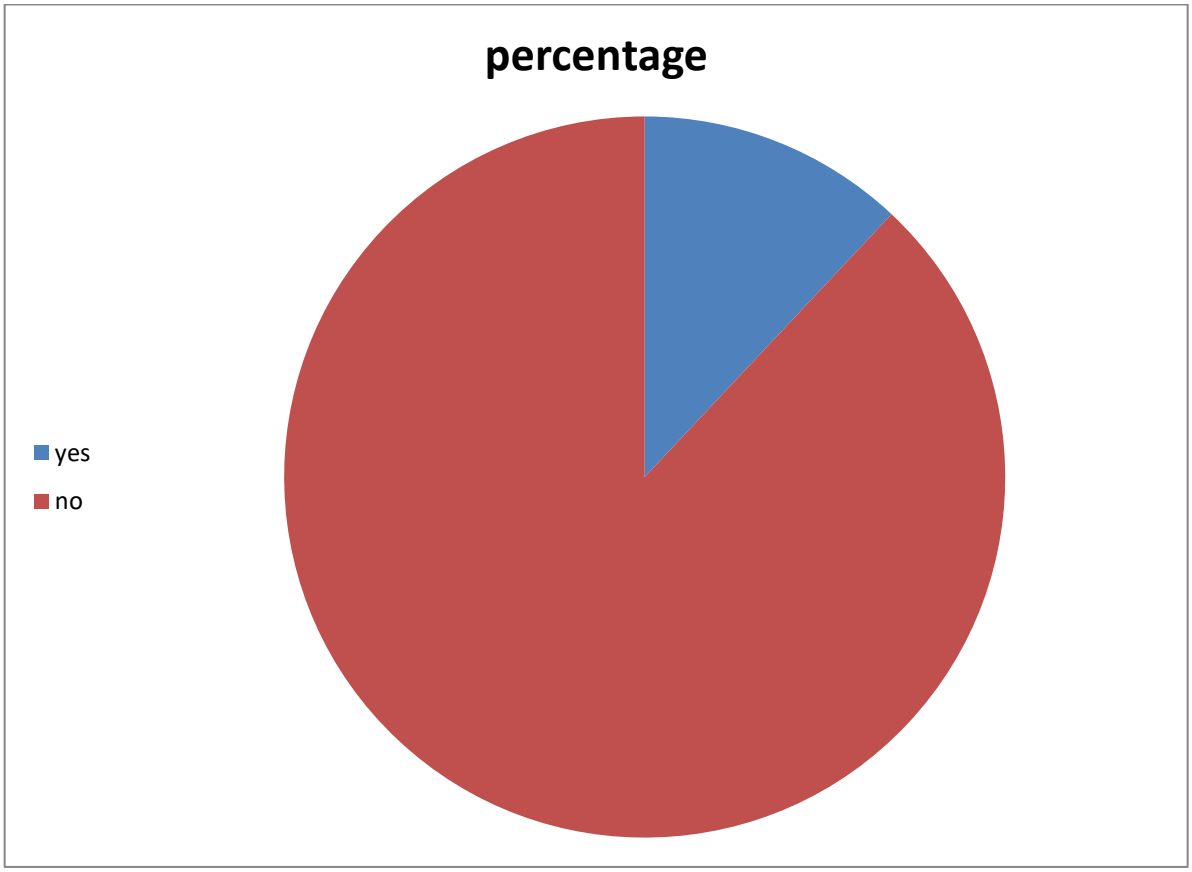


Table 4-1-7 show percentage frequency of smoking in blood donors

Item	Percentage
Yes	39%
No	61%

Figure 4-1-4 show percentage frequency of smoking in blood donors

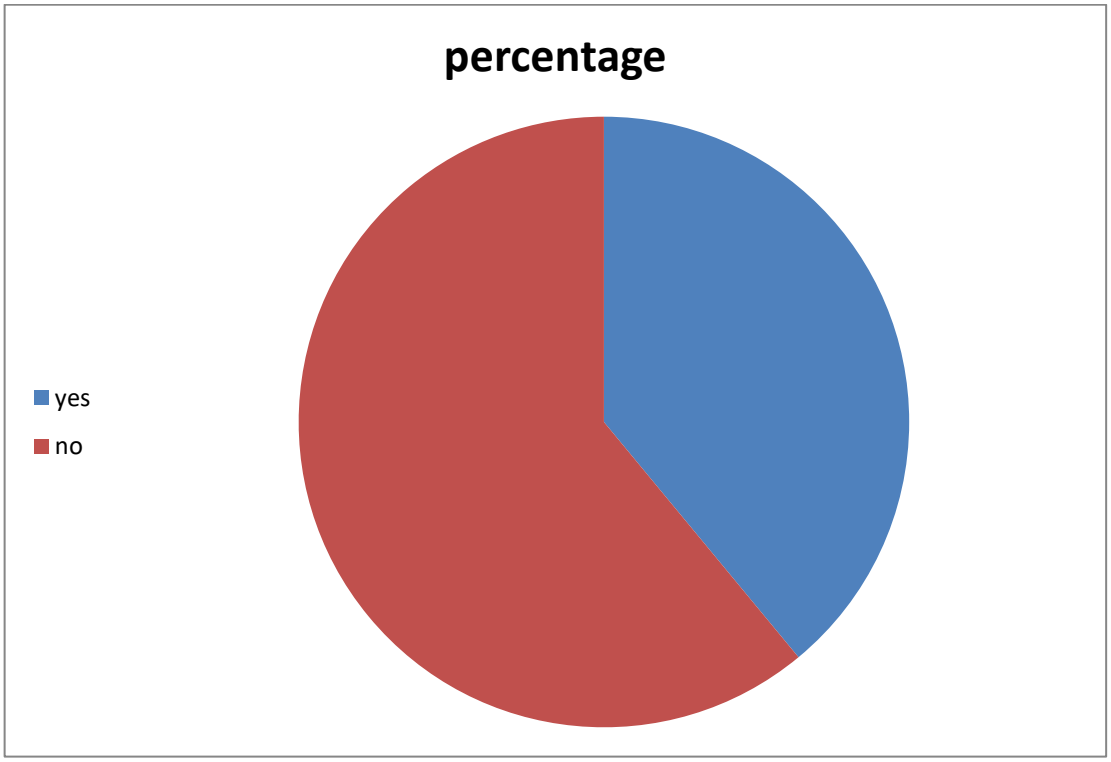


Table 4-1-8 show frequency percentage of surgical operation in blood donors

Item	Percentage
Yes	14%
No	86%

Figure 4-1-5 show frequency percentage of surgical operation in blood donors

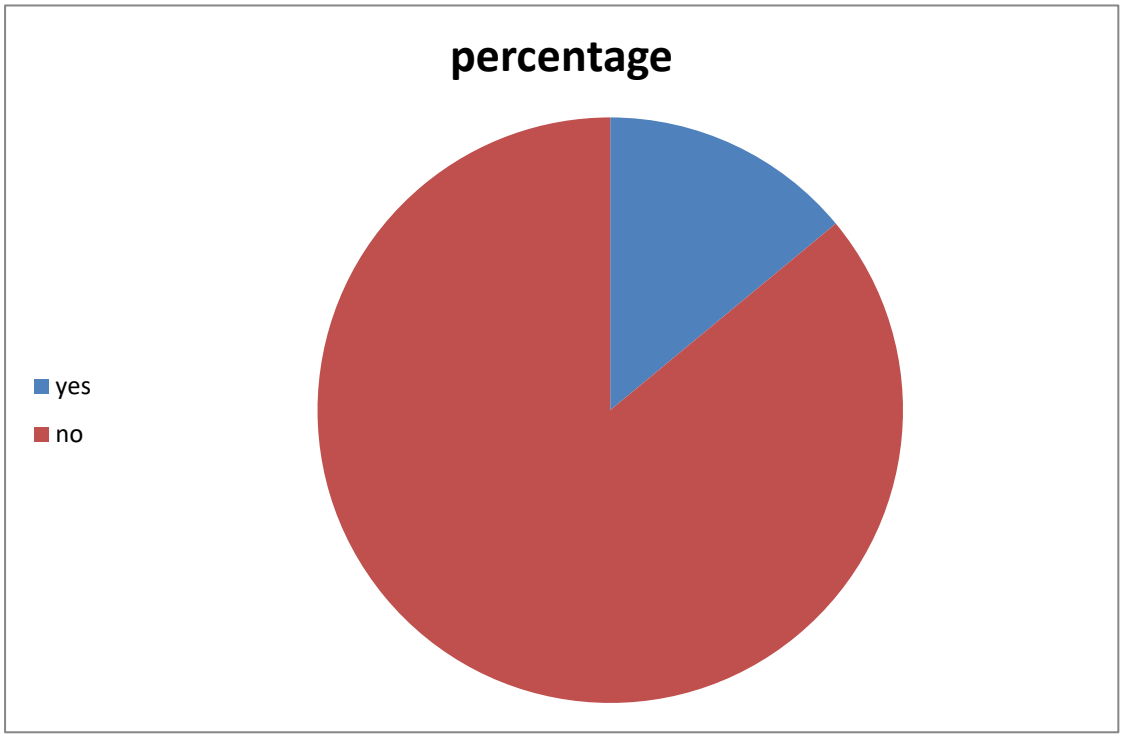


Table 4-1-9 show frequency percentage of tattooing in blood donors

Item	Percentage
Yes	4%
No	95%
Missed	1%

Figure 4-1-6 show frequency percentage of tattooing in blood donors

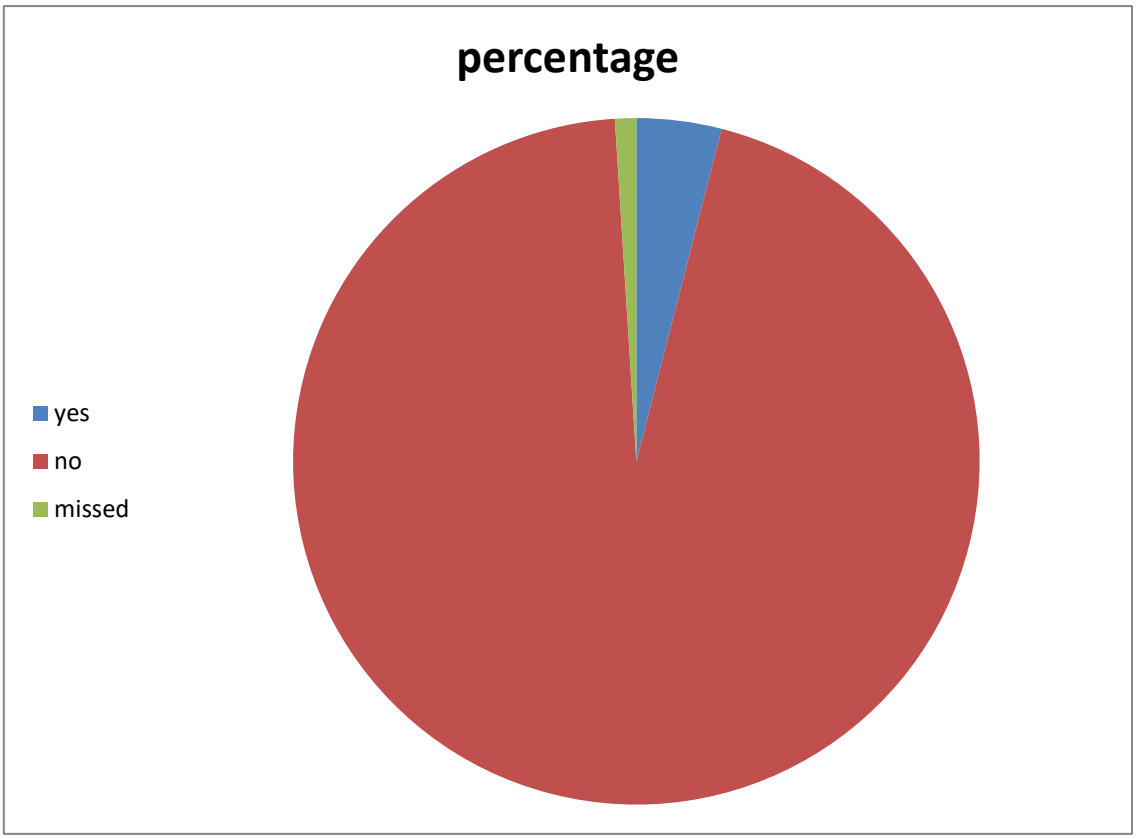


Table 4-1-10 show frequency percentage of alcoholic intake in blood donors

Item	Percentage
Yes	14%
No	86%

Figure 4-1-7 show frequency percentage of alcoholic intake in blood donors

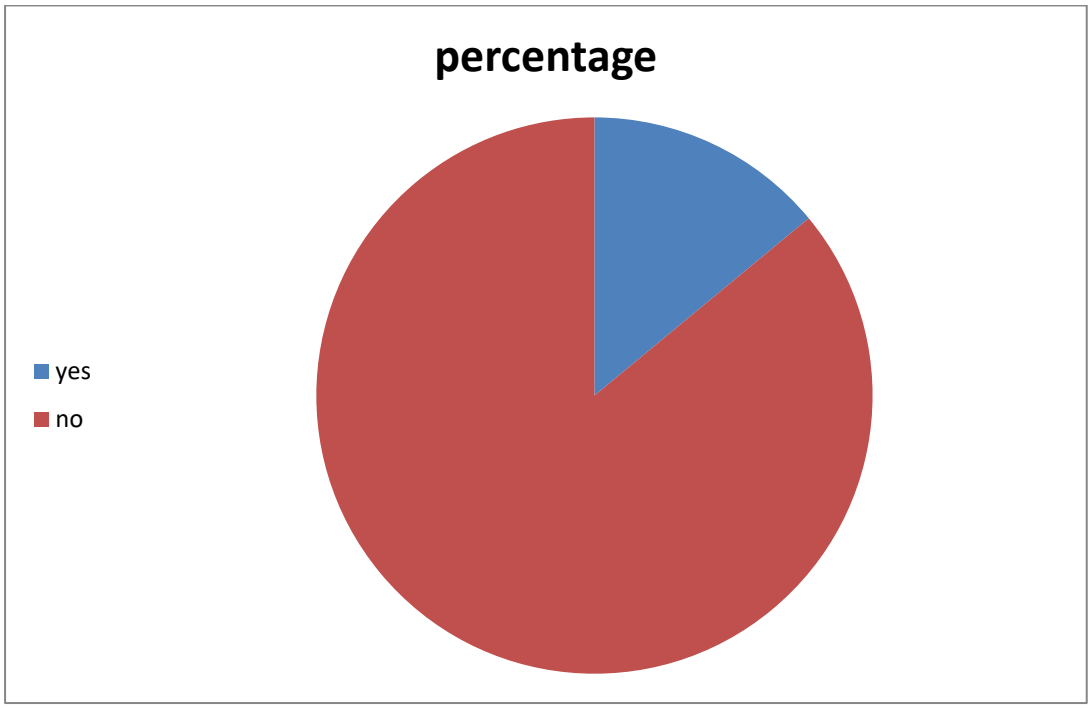


Table 4-1-11 show frequency percentage of vaccination

Item	Percentage
Yes	8%
No	92%

Figure 4-1-8 show frequency percentage of vaccination

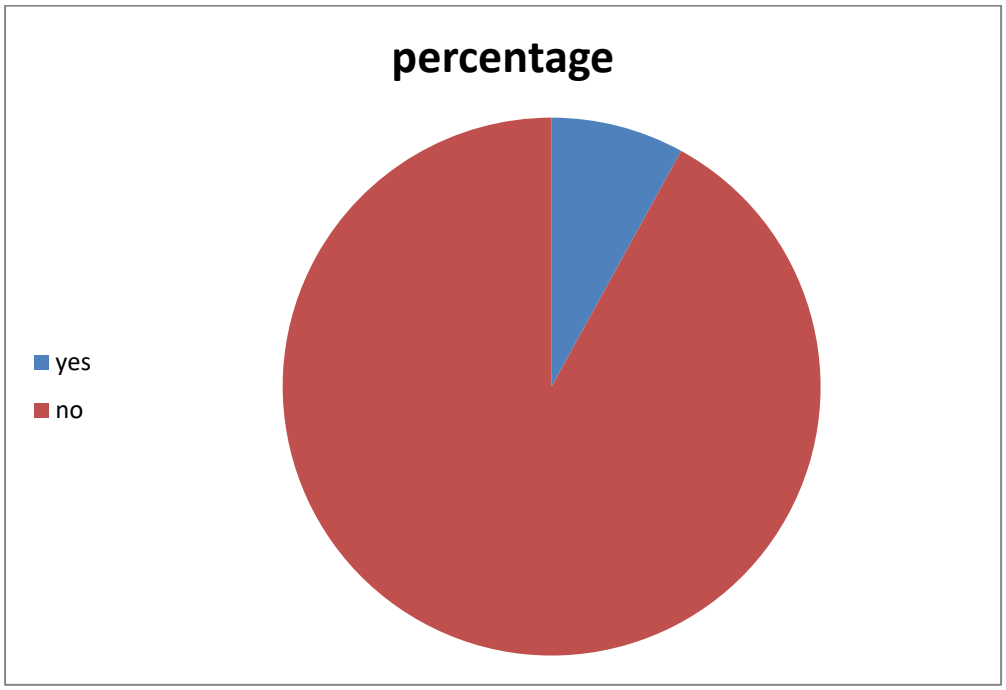
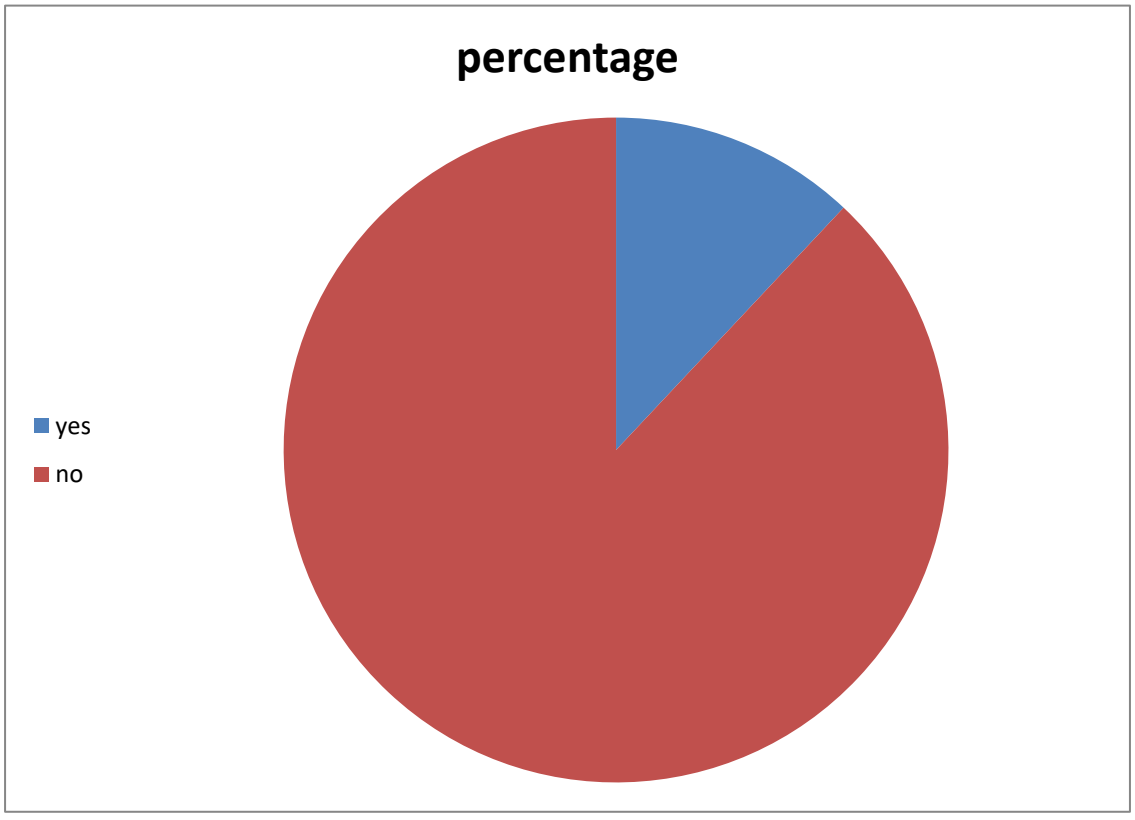


Table 4-1-12 show frequency percentage of drug abuse

Item	Percentage
Yes	12%
No	88%

Figure 4-1-9 show frequency percentage of drug abuse



Comparisons by cross tabulation according to hepatitis B results

Table 4-2-1 co-relation between presence of hepatitis B virus antigen and blood donation

Hepatitis B result	participant's blood donations		Total
	Yes	No	
Negative	44	45	89
Positive	0	2	2
Total	44	47	91

Chi-Square Tests

P value is 0.166 so there is no significance between hepatitis B results and blood donation

Table 4-2-2 co-relation between presence of hepatitis B virus antigen and blood donation

Hepatitis B results	participants' blood transfusions		Total
	Yes	No	
negative	10	79	89
positive	1	1	2
Total	11	80	91

Chi-Square Tests

P value is 0.096 so there is no significant relationship between hepatitis result and blood transfusion

Table 4-2-3 co-relation between presence of hepatitis B virus antigen and smoking

Hepatitis B results	participant's smoking situation		Total Yes
	Yes	no	
Negative	35	54	89
Positive	0	2	2
Total	35	56	91

Chi-Square Tests

P value is 0.258 so there is no significant relationship between hepatitis B result and smoking

Table 4-2-4 co-relation between presence of hepatitis B virus antigen and surgical operation

Hepatitis B results	participant's surgical operations		Total
	Yes	no	
Negative	13	76	89
Positive	0	2	2
Total	13	78	91

Chi-Square Tests

P value is 0.559 so there is no significant relationship between hepatitis B result and surgical operation

Table 4-2-5 co-relation between presence of hepatitis B virus antigen and tattooing

	participant's tattooing state			Total
	Yes	No		
Negative	4	84	1	89
Positive	0	2	0	2
Total	4	86	1	91

Chi-Square Tests

P value is 0.942 so there is significant relationship between hepatitis B result and participant's tattooing.

Table 4-2-6 co-relation between presence of hepatitis B virus antigen and alcoholic intake

Hepatitis B results	participant's alcoholic situations		Total
	Yes	No	
Negative	13	76	89
Positive	0	2	2
Total	13	78	91

Chi-Square Tests

P value is 0.559 so there is no significant relationship between hepatitis B results and alcoholic intake

Table 4-2-7 co-relation between presence of hepatitis B virus antigen and vaccination

	participant's vaccination situations		Total
	Yes	No	
Negative	7	82	89
Positive	0	2	2
Total	7	84	91

Chi-Square Tests

P value is 0.680 so there is no significant relationship between hepatitis B results and participant vaccination.

4-2-8 co-relation between presence of hepatitis B virus antigen and drug abuse

	participants' drug abuse situations		Total Yes
	Yes	No	
Negative	11	78	89
Positive	0	2	2
Total	11	80	91

Chi-Square Tests

P value is 0.596 so there is no significant relationship between hepatitis B result and participants' drug abuse.

5.1: Discussion

This Analytical prospective descriptive study which was conducted in Atbara during the period of March to June 2017.The study Aimed to detect the Sero-prevalence of hepatitis B virus antigen among blood donors attending blood transfusion center at Atbara River Nile state .

Our results showed that 2.2% of all blood donors in Atbara town during the period from March to June were positive for hepatitis B virus.

After statistical analysis by SPSS Also statistical analysis shows that there was no significant association between hepatitis B results and risk factors including blood donation, blood transfusion, surgical operation, tattooing, alcoholic intake, vaccination, drug abuse and smoking.

There is deference results determined in previous studies that were conducted in river Nile state like in 2012 the sero-prevalence of hepatitis B in state were 0.1% and in 2014 it was 0.9% so sero-prevalence in this study is 2.2% which showed high prevalence comparing to those previously conducted.

The highest prevalence of hepatitis B virus in Sudan was determined in South Kordofan in 2012 which was 15.7% this deference is May according to geographical state and other factors.

5.2: Conclusion

This study showed that the seroprevalence of HBVAg among blood donors 2(2.2%).

The correlation between the presence of hepatitis B virus antigen and risk factors showed insignificance relationship between them.

5.3 Recommendations

- ❖ Further studies should be done in this topic with large sample size and another study area to get accurate results.
- ❖ Depend on the hepatitis B virus result of diagnosis in blood banking lab.
- ❖ Quality control should be performed during practical in blood banking lab.
- ❖ Avoid donors that under risk of transfusion disease.
- ❖ Use advanced method and confirmatory test for diagnosis

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Appendix (1)

Questionnaire

El-sheikh Abdullah El-Badri University

Sero-prevalence of hepatitis B virus antigen among blood donors attending blood transfusion center in Atbara River Nile state

General questionnaire:

1. Name: ……..

2. Age: ……

3. Gender: male ☐ female ☐

4. Residence: ……

5. Job: ……

Specific questionnaire

1. Blood donation:
   Yes ☐ No ☐
2. Blood transfusion:
   Yes ☐ No ☐
3. Smoking:
   yes ☐ NO ☐
4. Surgical operation:
   yes ☐ No ☐
5. Tattooing:
   yes ☐ No ☐
6. Alcoholic intake:
   Yes ☐ No ☐
7. Vaccination of HBV
   Yes ☐ No ☐
8. Drug abuse ☐ NO ☐

Results for hepatitis

1. Positive ☐ ٢ .Negative ☐