بسم الله الرحمن الرحيم

Shendi University

College Of Post Graduate Studies

And Scientific Reserach

Prevalence Of Hepatitis B and C Viral Infections Among Health Workers In Elmak Nimir University Hospital

A thesis submitted in fulfillment of the requirement for the degree of MSC in Medical Laboratory Sciences (Microbiology)

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الآيـة

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قال تعالى:

﴿ اللّهُ لاَ إِلَهَ إِلاَّ هُوَ الْحَيُّ الْقَيُّوْمُ لاَ تَأْخُذُهُ سِنَةٌ وَلاَ نَوْمٌ لَهُ مَا فِي السَّمَاوَاتِ وَمَا فِي الأَرْضِ مَنْ ذَا الَّذِيْ يَشْفَعُ عِنْدَهُ إِلاَّ بِإِذْنِهِ يَعْلَمُ مَا بَيْنَ أَيْدِيْهِمْ وَمَا خَلْفَهُمْ وَلاَ يُحِيْطُوْنَ بِشَيْءٍ مِنْ عِلْمِهِ إِلاَّ بِمَا شَاءَ وَسِعَ كُرْسِيُّهُ السَّمَاوَاتِ وَالأَرْضَ وَلاَ يَئُوْدُهُ حِفْظُهُمَا وَهُوَ الْعَلِيُّ الْعَظِيْمُ ﴾

صدق الله العظيم

سورة البقرة _ الآيـة (255)

DEDICATION

To those who were the cause of my being come into existence

My Mother…

&

My Father…

To…Those …

Who are my lights in the darkness of the life…

My close friends who help me a lot through this study.

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Finally, I am of course thankful to my family for their support.

Abstract

Background: Health care workers (HCWs) are at risk of acquiring and transporting blood –borne viral infections, particularly hepatitis B (HBV) and hepatitis C (HCV)

Objective: The main objective of this study was to determine the seroprevalence rate of hepatitis B and C virus's infections among the health care workers in Elmak Nimir Hospital, Shendi River Nile State, Sudan.

Methods: This study was conducted during the period from Des 2013 to Des2015. A total of 200 blood samples collected from study group and 200 sample of blood donors as control group screened for HBsAg and antibodies (anti-HC) respectively using the Enzyme-Linked Immunosorbent Assay (ELISA), and liver enzymes assay such as (AST and ALT) were measured.

Result: The prevalence of hepatitis B viral infection used HBsAg as marker was (5/200) 2.5 % compared with (1/200) 0.5% in the control group (P=0.1). While prevalence of HC viral infection was (3/200) 1.5 % compared with (1/200) 0.5 % prevalence in the control group (P=0.3). These differences were statistically insignificant indicating working in Elmak Nimir Hospital did not increase the risk of infection for both viruses. Co- infection with HB and HC viruses did not exist in the examined samples .The infected individuals showed normal liver enzymes (AST and ALT) indicated inactive hepatitis B and C viral infection.

Conclusion:

The prevalence of HBsAg and anti-HCV were higher among HCWs than control group but these differences were not statistically significant. Prevalence of HBsAg and anti-HCV was higher in the study group compared with results done in central Europe and other in USA. Prevalence of HB virus in this study was less than results done in Cameron, Saudi Arabia and other in Egypt , Prevalence of hepatitis B virus in this study equal the other result done in Sudan, while prevalence of HC

virus in this study was higher than results done in Gaza strips and Saudi Arabia. While Prevalence of HC virus in this study was lower than Egypt. There was statistical correlation between HBV infection and marital statuses, long period of occupational, and un graduation, also the correlation between HCV infection and old age. The highest percentage of infection of HB and HC viruses found among cleaning staff

مستخلص الدراسة

خلفية: العاملين في مجال الرعاية الصحي معرضون لخطر اكتساب العدوي وخصوصا التهاب الكبد الفيروسي ب و ج , الهدف من هذه الدراسة هو ايجاد معدل انتشار فيروس التهابالـ ك بد الوبائ ب و ج بين العاملين في مجال الرعاية الصحية بمستشفي المك نمر الجامعي في السودان ولاية نهر النيل شندي , **المنهجية** قد اجريت هذه الدراسة خلال الفترة من ديسمبر 2013 الي ديسمبر 2015 شملت 200 شخص ضمن العاملين بالمستشفي وقورنت ب200 شخص من المتبرعين بالدم كمجموعة ضابطة تم الكشف عن الفيروسات باستخدام اختبار ايلازا وقيست معدلات مستوي انزيمات الكبد بالدم وهي اسبارتيت امينو ترانسفريزواللنين امينو ترانسفريز

النتيجة: اظهرت الدراسة ان الاصابة بالفيروس ب دالا عليه بوجود المستضد السطحي ب للفيروس خمسة مصابين من ضمن مائتين عامل(5/200) بالمستشفي بمعدل اصابة 2.5% في حين تم الكشف عن مدي انتشار الاصابة بالفيروس مستخدمين المستضد السطحي ب في المتبرعين بالدم واظهرت النتايج مصاب واحد من ضمن مائتين متبرع (1/200) بمعدل اصابة 0.5%, هذا الفرق ليس له اهمية احصائية (P=0.1),بينما الاصابة بالفيروس ج ثلاثة مصابين من ضمن مائتين عامل (3/200) بالمستشفي بمعدل أصابة 1.5% , مقارنة بالمتبرعين بالدم مصاب واحد للفيروس ج (1/200) بمعدل اصابة 0.5%, هذا الفرق ليس له اهمية احصائبة (P=0.3) وبالتالي العمل في مستشفي المك نمر لا يزيد من خطر الاصابة بفيروس الكبد الوبائي ب و ج في هذه الدراسة. لا يوجد احد مصاب بالتهاب الكبد الوبائي ب و ج معا. بقياس انزيمات الكبد(اسبارتيت امينو ترانسفريز و اللنين امينو ترانسفريز) للمصابين دلت النتيجة ان هذه الانزيمات في المعدل الطبيعي مما يدل علي ان التهاب الكبدي غير نشطة .

خلاصة

وجد ان انتشار فيروس الكبد الوبائي ب و ج في العاملين بالمستشفي اعلي من المجموعة الضابطة وهذا الفرق ليس له اهمية احصائية . ان انتشار فيروس الكبد الوبائي ب و ج في هذه الدراسة اعلي مقارنة مع نتايج دراسات اجريت في وسط اوربا واخري اجريت في الولايات المتحدة الامريكية. ان المستضد السطحي ب في هذه الدراسة اقل من دراسات اجريت في كل من الكميرون والمملكه العربية السعودية ومصر,وتطابقة نتيجة مدي انتشار فيروس الكبد ب في هذه الدراسة مع دراسات اخري اجريت في السودان بينما الاصابة بفيروس الكبد ج في الدراسة اعلي من دراسات اجريت في قطاع غزة والمملكه العربية السعودية واقل من دراسات اجريت في مصر.وجدت علاقة زات اهمية احصائيه في حالة فيروس الكبد ب بين (الحالة الاجتماعية - طول الفترة المهنية - وغير المتعلمين) بينما وجدت اهمية العلاقة الاحصايئة في حالة فيروس الكبد ج عند كبار السن . الاصابة بالتهاب الكبد الفيروسي ب و ج كانت نسبة الاصابة اعلي في عمال النظافة .

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List of abbreviation

AST	Aspirtate aminotransferase
ALT	Alanine aminotransferase
CCC	Covalently closed circular
CTL	Cytotoxic T-cell
CDC	Centers for Disease Control
DNA	Deoxyribonucleicacid
ER	Endoplasmic reticulum
ELISA	Enzyme Linked Immunosorbent Assay
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HCC	Hepatocellular carcinoma
HCWs	Health care workers
HBsAg	Hepatitis B service antigen
HLA	Human leucocytes antigen
HBcAg	Hepatitis B core antigen
HBeAg	Hepatitis B e antigen
HAV	Hepatitis A virus
HDV	Hepatitis D virus
HEV	Hepatitis E virus
HGV	Hepatitis G virus
HBIG	Hepatitis B immunoglobulin
IDU	Intravenous drug use
MGN	Membranous glomerulonephritis
MTCT	Mother to child transmission
MS	Multiple sclerosis
PC	Plasma cells
PCR	polymerase chain reaction
RNA	Ribonucleicacid
RIBA	Recombinant Immuno Blot Assay
HRP	Horseradish peroxidase
WHO	world health organization

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Chapter one

1. Introduction:

Viral hepatitis is a global public health problem affecting million of people every year, causing disability and death. Overall, Around 500 000 000 people are chronically and infected with hepatitis B virus (HBV) or hepatitis C virus (HCV). Approximately 1 000 000 people die each year's (2.7 % of all deaths) from causes related to viral hepatitis, most commonly including liver cancer. An estimated 57 % of cases of liver cirrhosis and 78 % of cases of primary liver cancer result from HBV or HCV infection.[1] HBV infection has a worldwide distribution. It is estimated that more than 2 billion people have been infected. Of these approximately 240 million are chronically infected and at risk of serious illness and death from cirrhosis and hepatocellular carcinoma (HCC), disease that are estimated to cause 500 000 – 700 000 deaths each year worldwide.[2] About 150 million people are chronically infected with HCV. More than 350 000 people are estimated to die from HCV related liver disease each year's.[1] chronic hepatitis B and C virus infections are leading causes of chronic liver disease and hepatocellular carcinoma in the United State. In 2007 chronic liver disease and cirrhosis were listed among the 15 leading causes of death in the United State.[1-3-4] Hepatocellular carcinoma accounted for an estimated 18910 deaths in the United States in 2010.[1-4] At least one half of which were associated with HCV infection.[4] Recent trend analyses have documented a decline in the rate of death associated with HBV, but an increase in deaths associated with HCV through 2004.Models have predicted a 2-fold increase in HCV-related deaths, with direct medical costs exceeding $6.7 billion between years2010- 2019.[1-4] health care workers (HCWs) are directly at risk of transmission of blood borne pathogens through their

handling of contaminated body fluids.[5] sharps injuries among health care workers in Indonesia were estimated to be approximately 44 % of HBV and 47 % of HCV.[6] In Cameroon Healthcare workers study done of HBsAg was 6.3% and HCV was 1.7% [7] also in Egypt has a high prevalence of HCV infection (17 - 26%) besides Hubei, Mongolia and Pakistan [8].Sudan is classified among the countries with high hepatitis B virus seroprevalence. Exposure to the virus varied from 47 - 78 % with hepatitis B surface antigen prevalence ranging from 6.8 % in central Sudan to 26 % in Southern Sudan.[9] previous studies in Sudan among health care workers a study done in Omdurman the seroprvalence of HBsAg was 2.4% while anti-HCV 0% [10]. There is no study dealing with prevalence of HBV and HCV among health workers of El Mak Nimir Hospital, so I decided to hold such responsibility seeking help for good.

1.2 Rational:

1) Hepatitis B and C are predisposing factors to chronic liver disease and hepatocellular carcinoma.

2) There are no studies conducted in Almak Nimir University Hospital- Shendi among health workers for prevalence of Hepatitis B and C viral infections.

3) This study well be of great benefit for prevention and control of these viral infections in Almak Nimir University Hospital medical workers by introducing programs of regular screening and vaccination.

1.3 Objectives:

1.3.1: General objective:

-To study the prevalence of Hepatitis B and C viral infections among Elmak Nimir Hospital University health workers- Shendi.

1.3.2: Specific objectives:

- To find out the prevalence of HB viral infection in Hospital health workers.
- To find out the prevalence of HC viral infection in Hospital health workers.
- To find out the impact of viral hepatitis infections to the liver by testing liver enzyme such as AST and ALT.
- To identified the risk factor which increase transmission of Hepatitis B and C viral infections in hospital workers.

Chapter two

2. Literature review:

2.1 Hepatitis:

Hepatitis is a general term meaning inflammation of the liver and can be caused by a variety of different viruses such as hepatitis A, B, C, D and E since the development of jaundice is a characteristic feature of liver disease, a correct diagnosis can only be testing patients sera for the present of specific antiviral antigens or antibodies.[11-12-13]

2.1.1 Hepatitis A:

Formerly known as infectious hepatitis is an acute infectious of the liver caused by the hepatitis A virus (HAV),[14] Hepatitis A virus (HAV), classified as hepatovirus, is a small, un enveloped symmetrical RNA virus which shares many of the characteristics of the picornavirus family.[20] Is usually transmitted by the faecal-oral route, either through person-to-person contact or ingestion of contaminated food or water. Certain sex practices can also spread HAV. Infections are in many cases mild, with most people making a full recovery and remaining immune from further HAV infections. However, HAV infections can also be severe and life threatening. Most people in areas of the world with poor sanitation have been infected with this virus. Safe and effective vaccines are available to prevent HAV infection.[1] Hepatitis A virus enters the body by ingestion and intestinal infection. The virus then spreads, probably by the bloodstream, to the liver, a target organ. Large numbers of virus particles are detectable in feces during the incubation period, beginning as early as 10–14 days after exposure and continuing. The incubation period of hepatitis A is 3–5 weeks, with a mean of 28 days.Viral hepatitis type A (previously named infectious or epidemic hepatitis) occurs endemically in all parts of the world, with frequent

reports of minor and major outbreaks. The exact incidence is difficult to estimate because of the high proportion of subclinical infections and infections without jaundice, differences in surveillance, and differing patterns of disease. The degree of under reporting is believed to be very high. Hepatitis. [20] Hepatitis A can be prevented by vaccination, good hygiene and sanitation.[14]

2.1.2. Hepatitis B:

The Global prevalence of HBV infection is higher than that of HCV. Globally, HBV causes 60-80% of the world's primary liver cancers.[16] Hepatitis B virus infection is estimated to be the cause of 30% of cirrhosis and 53% of liver cancer in the world. Approximately 15-40% of patients with chronic HBV will develop cirrhosis, end-stage liver failure or hepatocellular carcinoma (HCC) in their lifetime. [9]

2.1.3. Hepatitis C:

The first demonstration that most cases of transfusion-associated hepatitis were caused by neither hepatitis A virus (HAV) nor hepatitis B virus (HBV), the only two known human hepatitis viruses at the time, came in 1975. This new form of disease was called non-A non-B hepatitis and the presumed etiologic agent, non-A non-B hepatitis virus.[15-17]Like many viruses, the hepatitis C virus is gradually inactivated outside the body of a host. The presence of heat can have a drastic impact on the virus's lifespan outside the body. The virus can remain infectious outside a host for about sixteen days at 25°C and two days at 37°C, while it can remain active for more than six weeks at temperatures less than or equal to 4°C. When heated to temperatures of 60°C and 65°C, however, the hepatitis C virus can be inactivated in eight and four minutes, respectively.[18]

2.1.4. Hepatitis D:

Hepatitis Delta Virus (HDV) is a defective virus that is only infectious in the presence of active HBV infection. HDV infection occurs as either coinfection with HBV or superinfection of an HBV carrier. Coinfection usually resolves. Superinfection, however, causes frequently chronic HDV infection and chronic active hepatitis. Both types of infections may cause fulminate hepatitis. [16] The routes of transmission of hepatitis D are similar to those for hepatitis B. Infection is largely restricted to persons at high risk of hepatitis B infection, particularly injecting drug users and persons receiving clotting factor concentrates. Worldwide more than 15 million people are co-infected. HDV is rare in most developed countries, and is mostly associated with intravenous drug use. However, HDV is much more common in the immediate Mediterranean region, sub-Saharan Africa, the Middle East, and the northern part of South America.[19] Preventing acute and chronic HBV infection of susceptible persons by vaccination will also prevent HDV infection.[16]

2.1.5. Hepatitis E:

Hepatitis E virus (HEV) . the causes of enteric- ally transmitted non A non B hepatitis non enveloped, single strand RNA viruses with 7.5 kilo base genome, which shares many biophysical and biochemical features with caliciviruses, it cause large epidemics of acute hepatitis in the subcontinent of India, central and southeast Asia, the middle east, part of Africa and elsewhere. The average incubation period is slightly longer than for hepatitis A with mean of six weeks, the highest rate found in young adults and high mortality rates up to 20% have been reported in women during pregnancy.[20] Like HAV, is transmitted through

consumption of contaminated water or food. HEV is a common cause of hepatitis outbreaks in the developing world and is increasingly recognized as an important cause of disease in developed countries. HEV infection is associated with increased morbidity and mortality in pregnant women and newborns. A safe and effective vaccine against HEV was licenced in January 2012 but is not yet widely available. It is estimated that one third of the world's population has been infected with hepatitis E virus. However, the true prevalence of hepatitis E is unknown**.** [1]

2.1.6 Hepatitis G:

GB virus C (GBV-C), formerly known as hepatitis G virus (HGV), is a virus in the Flaviviridae family and a member of the Peg virus genus,[21] is known to infect humans, but is not known to cause human disease. There have been reports that HIV patients co infected with GBV-C can survive longer than those without GBV-C, but the patients may be different in other ways. There is current active research into the virus' effects on the immune system in patients co infected with GBV-C and HIV.[22]

2.1.2 Hepatitis B:

Hepatitis B is a serious and common infectious disease of the liver, affecting millions of people throughout the world.[11-12-13-16] Hepatitis B has also been called type B hepatitis, homologous serum jaundice.[12-13-16] More than 2000 million people alive today have been infected with HBV at some time in their lives. Of these, about 350 million remain infected chronically and become carriers of the virus.[12-16] Three quarters of the world's population live in areas where there are high levels of infection. Every year there are over 4 million acute clinical cases of HBV, about

25% of carriers,1 million people a year, die from chronic active hepatitis, cirrhosis or primary liver cancer.[16] HBV may be the cause of up to 80% of all cases of hepatocellular carcinoma worldwide, second only to tobacco among known human carcinogens.[11-16-] During the incubation phase of the disease (6 to 24 weeks), patients may feel un well with possible nausea, vomiting, diarrhea, anorexia and headaches. Patients may then become jaundiced although low grade fever and loss of appetite may improve. Some times HBV infection produces neither jaundice nor obvious symptoms.[11-13]. Most adult patients recover completely from their HBV infection, but about 5 to 10 %, will not clear the virus and will progress to become asymptomatic carriers or develop chronic hepatitis possibly resulting in cirrhosis and /or liver cancer.[13] Rarely, others may develop fulminant hepatitis and die. Hepatitis B is a vaccine – preventable disease.[16]

2.1.2.1 Signs and symptoms:

2.1.2.1.1 Acute hepatitis B:

The acute form of the disease often resolves spontaneously after a 4-8 week illness. Most patients recover without significant consequences and without recurrence. However, a favorable prognosis is not certain, especially in the elderly who can develop fulminating, fatal cases of acute hepatic necrosis. Young children rarely develop acute clinical disease, but many of those infected before the age of seven will become chronic carriers.[11-12-13] The incubation period varies usually between 45 and 120 days, with an average of 60 to 90 days. The variation is related to the amount of virus in the inoculums, the mode of transmission and host factors.[11-12-16] In patients with clinical illness, the onset is usually insidious with tiredness, anorexia, vague abdominal discomfort, nausea

and vomiting, sometime arthralgias and rash, often progressing to jaundice. Fever may be absent or mild.[12-13] The enteric phase of acute viral hepatitis begins usually within 10 days of yellowish discoloration of the mucous membranes, conjunctivae, sclera and skin. Jaundice becomes apparent clinically when the total bilirubin level exceeds 20 to 40 mg/dl. It is accompanied by hepatomegaly and splenomegaly. About 4-12 weeks thereafter, the jaundice disappears and the illness resolves with the development of natural protective antibodies (anti-HBs), in about 95% of adult. [11] Acute hepatitis B is characterized by the presence of anti-HBc IgM serum antibodies converting to IgG with convalesced recovery, and the transient (<6months) presence of HBsAg, HBeAg, and viral DNA, with clearance of these markers followed by seroconversion to anti-HBsAg and anti-HBeAg. More than 90% of adult-onset infection cases fall in to this category. The remaining 5-10% of adult-onset infection and over 90% of cases of neonatal infection become chronic, and may continue for the life span of the patients.[12-16] In most cases, no special treatment or diet is required, and patients need not be confined to bed. A small percentage of persons die from acute HBV.[16]

2.1.2.1.2 Chronic hepatitis B:

Following acute HBV infection, the risk of developing chronic infection varies inversely with age. Chronic HBV infection occurs among about 90% of infants infected at birth, 25-50% of children infected at 1-5years of age and about 1-5% of persons infected as older children and adults. Chronic HBV infection is also common in persons with immunodeficiency.[11-12-13] Up to 20% of the chronic persistent hepatitis cases progress to cirrhosis. This a serious liver disease associated with chronic and often widespread destruction of liver substance occurring over a period of several years.[16] Surprisingly, some of the patients

infected persistently may have no clinical or biochemical evidence of liver disease. While others may show signs of easy fatigability, anxiety, anorexia and malaise.[11-12] The severe pathological consequences of persistent HBV infection include the development of chronic hepatic insufficiency, cirrhosis, and hepatocellular carcinoma (HCC). In addition, HBV carriers can transmit the disease for many years.[12-13-16] In cirrhosis, liver cells die and are progressively replaced with fibrotic tissue leading to nodule formation. The internal structure of the liver is deranged leading to the obstruction of blood flow and decrease in liver function. This damage is caused by recurrent immune responses stimulated by the presence of the virus. Because liver inflammation can be totally symptomless, progression of inflammation to cirrhosis can occur without the knowledge of the patient.[16] Chronic hepatitis B is a prolonged (>6 months) infection with persistent serum levels of HBsAg and IgG , anti-HBcAg and the absence of an anti-HBsAg antibody response. HBV DNA and HBeAg are often detectable at high concentrations, but may disappear if viral replication ceases or if mutations occur that prevent the synthesis of viral precore protein precursor of HBeAg.[11-13-16] Three phase of replication occur during the course of HBV infection, especially in patients with chronic hepatitis B:

(1) high replicative phase. In this phase HBsAg, HBeAg and HBV DNA are present and detectable in the sera. Aminotransferase levels may increase, and moderate inflammatory activity is histologically apparent. The risk of evolving to cirrhosis is high.

(2) Low replicative phase. This phase is associated with the loss of HBeAg, or a decrease or loss of HBV DNA concentration, and with the appearance of anti-HBe. Histologically, a decrease in inflammatory

activity is evident. Serologic changes like the loss of HBV DNA and HBeAg are referred to as seroconversion.

(3) Non replicative phase. Markers of viral replication are either absent or below detection level, and the inflammation is diminished. However if cirrhosis has already developed, it persists indefinitely. [16].

Extrahepatic manifestations of hepatitis B are seen in 10-20%of patients as transient serum sickness-like syndrome with fever, skin rash and polyarthritis.[11-12-13] Acute necrtizing vasculitis (polyarteritis nodosa). Membranous glomerulonephritis, is present in both adults children.[11-13] Popular acrodermatitis of childhood (ianotti-crosti syndrome).[12-16]

2.1.2.2 Virology:

2.1.2.2.1 Structures:

Hepatitis B is caused by the hepatitis B virus (HBV), an envelope virus containing a partially double stranded, circular DNA genome, and classified within the family hepadnavirus.[11-12-13-16] The virus particle, (virion) consists of an outer lipid envelope and an icosahedralnucleocapsid core composed of protein. These virions are 42 nM in diameter and are sometime referred to as Dane particles [24] . Membranous glomerulonephritis is the most common form.Other immune-mediated hematological disorders, such as essential mixed cryoglobulinemia and aplastic anemia.[23] The nucleocapsid encloses the viral DNA and a DNA polymerase that has reverse transcriptase activity.[25] The outer envelope contains embedded proteins that are involved in viral binding of, and entry into, susceptible cells. The virus is one of the smallest enveloped animal viruses, but pleomorphic forms exist, including filamentous and spherical bodies lacking a core. These particles are not infectious and are composed of the lipid and protein that

forms part of the surface of the virion, which is called the surface antigen (HBsAg) and is produced in excess during the life cycle of the virus.[26]

2.1.2.2.2 Genome:

The genome of HBV is made of circular DNA, but it is unusual because the DNA is not fully double-stranded. One end of the full length strand is linked to the viral DNA polymerase. The genome is 3020–3320 nucleotides long (for the full-length strand) and 1700–2800 nucleotides long (for the short length-strand).[27] The negative-sense (non-coding) is complementary to the viral mRNA. The viral DNA is found in the nucleus soon after infection of the cell. The partially double-stranded DNA is rendered fully double-stranded by completion of the (+) sense strand and removal of a proteinmolecule from the (-) sense strand and a short sequence of RNA from the (+) sense strand. Non-coding bases are removed from the ends of the (-) sense strand and the ends are rejoined. There are four known genes encoded by the genome, called C, X, P, and S. The core protein is coded for by gene C (HBcAg), and its start codon is preceded by an upstream in-frame AUG start codon from which the pre-core protein is produced. HBeAg is produced by proteolytic processing of the pre-core protein. The DNA polymerase is encoded by gene P. Gene S is the gene that codes for the surface antigen (HBsAg). The HBsAg gene is one long open reading frame but contains three in frames start (ATG) codons that divide the gene into three sections, pre-S1, pre-S2, and S. Because of the multiple start codons, polypeptides of three different sizes called large, middle, and small (pre-S1 + pre-S2 + S, pre-S2 + S, or S) are produced.[28] The function of the protein coded for by gene X is not fully understood but it is associated with the development of liver cancer. It stimulates genes that promote cell growth and inactivates growth regulating molecules.[29]

2.1.2.2.3 Replication:

The HBV virion binds to a receptor at the surface of the hepatocyte. Viral nucleocapsids enter the cells and reach the nucleus, where the viral genome is delivered [16]. In the nucleus, second-strand DNA synthesis is completed and the gaps in both strands are repaired to yield a covalently closed circular (ccc) super coiled DNA molecule that serves as a template for transcription of four viral RNAs that are 3.5, 2.4, 2.1, and 0.7 kb long.[12-13] These transcripts are polyadenylated and transported to the cytoplasm, where they are translated into the viral nucleocapsid and precore antigen (C, pre-C), polymerase (P), envelop L (large), M(medium), S (small), and transcriptional transactivating proteins(X).[12-13-16] The 3.5 kb species, spanning the entire genome and termed pregenomic RNA(pg RNA), is packaged together with HBV polymerase and a protein kinase into core particles where it serves as a template for reverse transcription of negative-strand DNA. The RNA to DNA conversion takes place inside the particles. The new, mature, viral nucleocapside can then follow two different intracellular pathways, one of which leads to the formation and secretion of new virions, whereas the other leads to amplification of the viral genome inside the cell nucleus [16]. In the virion assembly pathway, the nucleocapsids reach the endoplasmic reticulum (ER), where they associate with the envelope proteins and bud into the lumen of the ER, from which they are secreted via the Golgi apparatus out of the cell. In the genome amplification pathway, the nucleocapsids deliver their genome to amplify the intra nuclear pool of covalently closed circular DNA (cccDNA). The X protein contributes to the efficiency of HBV replication by interacting with different transcription factors, and is capable of stimulating both cell proliferation and cell death.[11-16]

2.1.2.2.4 Serotypes and genotypes:

The virus is divided in to four major serotypes (adr, adw , ayr, ayw) based on antigenic epitopes presented on its envelope proteins, and in to eight genotypes (A-H) according to overall nucleotide sequence variation of the genome. The genotypes have a distinct geographical distribution and are used in tracing the evolution and transmission of the virus. Differences between genotypes affect the disease severity, course and likelihood of complications, and response to treatment and possibly vaccination.[30]

2.1.2.3 Mechanisms:

2.1.2.3.1 Pathogenesis:

HBV infection contracted early in life may lead to chronic hepatitis, then to cirrhosis, and finally to HCC, usually after a period of 30 to 50 years. Once infected with HBV. Males are more likely to remain persistently infected than women, who are more likely to be infected transiently and to develop anti-HBs.[16] Three mechanisms seem to be involved in liver cell injury during HBV infections. The first is a HLA class 1 restricted cytotoxic T-cell (CTL) response directed at HBcAg/HBeAg on HBV-infected hepatocytes. A second possible mechanism is a direct cytopathic effect of HBcAg expression in infected hepatocytes.[12-13-16] A third possible mechanism is high level expression and inefficient secretion of HBsAg.[13] Eradication of HBV infection depends on the coordinate and efficient development of humoral and cell mediated immune responses against HBV protein. Antibodies secreted by plasma cells (PC) derived from antigen specific B cells(which usually recognize

viral antigens in their native conformation) are mostly responsible for the neutralization of free circulating viral particles, Cytotoxic T cells (CTL) that recognize endogenous viral antigens in the form of short peptides associated with human leukocyte antigen(HLA) class 1 molecules on the surface of the infected hepatocytes (HC) are the main effectors for the elimination of intracellular virus. They can do this by at least two different mechanisms: direct attachment to the cell membrane, causing the infected cell to undergo apoptosis: and the releases of soluble cytokines that can down- regulate viral gene expression, leading to the elimination of intracellular virus without destruction of the effect cell. Both hum oral and cytotoxic functions are more or less stringently regulated by the helper effect of the CD4+Tcells (TH) that recognize exogenous viral antigens, released or secreted by liver cells, in the form of short peptides that associate with HLA class11 molecules in the endosomal compartment of professional antigen-presenting cells such as B cells, macrophages, and dendritic cells.[16]

2.1.2.3.2 Transmission:

Currently, there are four recognized modes of transmission:

(1) From mother to child at birth (perinatal).

(2) By contact with an infected person (horizontal).

(3) By sexual contact.

(4) By parenteral (blood to blood) exposure to blood or other infected fluids.[12-16]

There is no convincing evidence that airborne infections can occur and faeces are not a source of infection, since the virus is inactivated by enzymes of the intestinal mucosa or derived from the bacterial flora. HBV is not transmitted by contaminated food or water, insects or other vectors.[11-13] HBsAg has been found in all body secretions and excretions.

However, only blood, vaginal and menstrual fluids, and semen have been shown to be infectious.[11-12-13-16] Transmission occurs by percutaneous and permucosal exposure to infective body fluids. Percutaneous exposures that have resulted in HBV transmission include transfusion of unscreened blood or blood products, sharing unsterilized injection needles for iv drug use, haemodialysis, acupuncture, tattooing and injuries from contaminated sharp instruments sustained by hospital personnel.[11-12-13-31] infection may also be transmitted between household contacts and between sexual partners, either homosexual or heterosexual, and in toddler aged children in groups with high HBsAg carrier rates.[11-12] The most important mode of HBV transmission globally is preinatal, from the mother to her newborn baby. If a pregnant woman is an HBV carrier and is also HBeAg-positive, her newborn baby has 90% likelihood to be infected and become a carrier. Of these children, 25% will die later from chronic liver disease or liver cancer.[11-16] and by sexual intercourse with multiple partners or with persons who have multiple partners can be dangerous. Hepatitis B is the only sexually transmitted infection for which there is a protective vaccine.[12] HBV is stable on environmental surfaces for at least 7 days and indirect inoculation of HBV can occur via inanimate objects like toothbrushes, baby bottles, toys, razors, eating utensils, hospital equipment and other objects, by contact with mucous membranes or open skin breaks.[16] HBV does not cross the skin or the mucous membrane barrier. Some break in this barrier, which can be minimal and insignificant, is required for transmission. [13] Other risk factors for developing HBV infection include working in a healthcare setting,[31]The natural reservoir for HBV is man. Closely related hepadnaviruses have been found in woodchucks and ducks, but they are not infectious for humans. HBV is about 100 times more infectious than HIV[16]

2.1.2.4 Diagnosis:

2.1.2.4.1 Large-scale screening for HBV infection:

Diagnosis of hepatitis is made by biochemical assessment of liver function. Initial laboratory evaluation should include : total and direct bilirubin, ALT(alanine aminotransferase),AST(aspirtate aminotransferase), alkaline phosphatase, prothrombin time, total protein, albumin, globulin, complete blood count, and coagulation studies.[11-13] Diagnosis is confirmed by demonstration in sera of specific antigens and/or antibodies. Three clinical useful antigen-antibody systems have been identified for hepatitis B:

(1) hepatitis B surface antigen (HBsAg) and antibody to HBsAg (anti-HBs).

(2) antibody (anti-HBc IgM and anti-HBc IgG) to hepatitis B core antigen (HBcAg).

(3) hepatitis B e antigen (HBeAg) and antibody to HBeAg (anti-HBe).[16] Tests specific for complete virus particles or DNA and DNA polymerase-containing virions, and for HDAg and HDV RNA in liver and serum are available only in research laboratory.[13] HBsAg can be detected in the serum from several weeks before onset of symptoms two months after onset. HBsAg is present in serum during acute infections and persists in chronic infections. The presence of HBsAg indicates that the person is potentially infectious.[11-12-13] very early in the incubation period. Pre-S1 and pre-S2 antigens are present. They are never detected in the absence of HBsAg. Hepatitis B virions, HBV DNA polymerase, and HBeAg are then also detected. The presence of HBeAg is associated with relatively high infectivity and severity of disease.[11-13] Anti-HBc is the first antibody to

appear. Demonstration of anti-HBc in serum indicates HBV infection. Current or past IgM anti-HBc is present in high titer during acute infection and usually disappears within 6 months, although it can persist in some cases of chronic hepatitis. This test may therefore reliably diagnose acute HBV infection. IgG anti-HBc generally remains detectable for a lifetime. [11-12-13] Anti-HBe appears after anti-HBc and its presence correlates to a decreased infectivity. HBeAg in the resolution of the disease.[11-12] Anti-HBs replaces HBsAg as the acute HBV infection is resolving. Anti-HBs generally persist for a lifetime over 80% of patients and indicate immunity. [11-12-13]

2.1.2.4.2 Small scale screening for HBV:

Immunofluorescence.studies,insitu hybridization, immunohistochemistry, and thin- section microscopy are used to examine pathological specimens for the presence of HBV- associated antigens or particles, providing information about the relationship between HBV DNA replication and HBV gene expression. Within the hepatocytes, HBsAg localizes in the cytoplasm, and HBcAg is seen in the nucleus and or the cytoplasm, detection of complete virions in the liver is uncommon.[11] DNA hybridization technique and RT-PCR assays have shown that almost all HBsAg/HBeAg- positive. Patients have detectable HBV DNA in their serum, whereas only about 65% of the HBsAg/antiHBe- reactive patients are positive. All patients who recover from acute hepatitis B are negative for HBV DNA. On the other hand, some patients infected chronically who have lost their HBsAg remain HBV DNA positive.[11-13]

2.1.2.5 Stability:

The stability of HBV does not always coincide with that of HBsAg, exposure to ether, acid (pH 2.4 for at least 6 h), and heat (98^{O}C for 1 min: 60 ^{O}C for 10 h) does not destroy immunogenicity or antigenicity. However, inactivation may be incomplete under this condition if the concentration of virus is excessively high. Antigenicity and probably infectivity are destroyed after exposure of HBsAg to 0.25 sodium hypochlorite for 3 min.[11] Infectivity is lost after autoclaving at 121^{O}C for 20 min, or dry heat treatment at 160^{O}C for 1h.[11-13] HBV is inactivated by exposure to sodium hypochlorite (500 mg free chlorine per liter) for 10 min, 2% aqueous glutaraldehyde at room temperature for 5 min , heat treatment at 98^{O}C for 2min, Sporicidin (Ash, Dentsply, York, PA) (PH 7.9), formaldehyde at 18.5 g/l (5% formalin in water), 70% isopropylalcohol, 80% ethyl alcohol at 11^{O}C for 2 min, Wescodyne (a iodophor disinfectant ,American Sterilizer Co, Erie, PA) diluted 1:213, or combined β-propriolactone and UV irradiation.[11-16] HBV retains infectivity when stored at 30^{O}C for at least 6 months and when frozen at -15^{O}C for 15 years. HBV present in blood can withstand drying on surface for at least a week.[11-13-16]

2.1.2.6. Vaccination:

HB 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. Vaccines are composed of the surface antigen of HBV (HBsAg), and are produced by two different methods: plasma derived or recombinant DNA. When administered properly, hepatitis B vaccine induces protection in about 95% of recipients.[16] A

course of two to three (2-3) vaccine injections are given, the second injection at least one month after the first dose and the third injection being administered six months after the first dose, or four injections at 0,1,2, and 12 months.[11-13-16] A safe and effective vaccine against HBV infection has been available for 20 years. HB vaccine is effective in preventing HBV infections when it is given either before exposure or shortly after exposure; At least 85-90% of HBV- associated deaths are vaccine-preventable. [16]

2.1.2.6.1Plasma-derivedvaccines:

These vaccines, derived from the plasma of HBsAg –positive donors, consist of highly purified, formalin- inactivated and/ or heat -inactivated, alum-adsorbed, hepatitis B subvirion particles (22nm) of HBsAg that are free of detectable nucleic acid, and, therefore, noninfectious.[11-12] The first plasma -derived HB vaccines manufactured in the USA and in France were licensed in1981-1982 . They contain 20µg/ml HBsAg and the preservative thimerosal at a concentration of 1:20:000.[11-13-16] plasma-derived HB vaccines are no longer produced in North America or Western Europe, but several hundred million doses are produced in the Republic of Korea, China, Vietnam, Myanmar, Indian, Indonesia, Iran and Mongolia.[11-12-13] More than 200 million doses of plasma-derived vaccines have been distributed globally, and the safety record is impressive. Local reactions are generally insignificant clinically and are limited to mild pain or discomfort at The injection site in up to 25% of the vaccine recipients.[11-16]

2.1.2.6.2 RecombinantDNAyeast–derived or mammalian cell-derived vaccines

In the mild- 1980s, an alternative, genetically engineered vaccine became available. The new technologies offer manufacturers a shorter production

cycle (12 instead of 65 weeks), batch- to batch consistency, and continuous supply of material, allowing the replacing of plasma-derived vaccines available on the market.[11-13] In recombinant DNA technology, the S gene (pre-S1,preS2,S) is cloned and isolated, inserted in to an expression plasmid and introduced in to yeast(S. cerevisiae) or mammalin (Chinese hamster ovary, CHO) cells. The desired protein(s) is (are) expressed and assembled in to 22 nm antigenic particles.[11-12-13] The only mammalian cell-derived vaccine available is GenHevac B® GenHevac B® contains both preS2 and S proteins.[16] The two major yeast-derived hepatitis B vaccines that licensed in most countries are Engerix-B®(Smithkline Beecham, 1992) and Recombivax HB®(Merck & Co). Both recombinant products contain non glycosylated HBsAg particles (only S protein) that have been physicochemically purified, adsorbed on aluminium hydroxide, and preserved with thimerosal. Only Recombivax HB® is treated with formaldehyde.[11] Vaccine batches should be stored at 2-8ºC but not frozen. Freezing destroys the potency of the vaccine since it dissociates the antigen from the adjuvant alum interfering with the immunogenicity of the preparation.[11-16] the vaccine is thermo stable and neither reactogenicity nor immunogenicity are altered after heating at 45ºC for 1 week or 37ºC for 1 month.[12]

2.1.2.6.3 Recommended populations:

Here is a list of groups for whom pre exposure vaccination is recommended. If all were immunized, the incidence of hepatitis B would decrease rapidly.[11] Infants (universal immunization), infants and adolescents not vaccinated previously (catch-up vaccination), persons with occupational risk (exposure to blood- contaminated environments) and students of health-care professions before they have blood contact, Haemodialysis patients. (vaccination before dialysis treatment is

recommended), sexually active men or women, populations with a high incidence of disease, international travelers to areas of high HBV ende micity if specific at- risk circumstances exist, transplant candidates before transplantation, susceptible injecting drug abusers, household contacts and sex partners of HBV carriers, recipients of frequent and/or large volumes of blood or blood components, clients and staff of institutions for the developmentally disabled and susceptible contacts in day-care programs who are at increased risk from HBV carrier clients with aggressive behavior or special medical problems that increase the risk of exposure.[16]

2.1.2.6.4 Response to vaccination:

Following the primary course of 3 vaccinations, a blood test may be taken after an interval of 1–4 months to establish if there has been an adequate response, which is defined as an anti-hepatitis B surface antigen (anti-Hbs) antibody level a above 100 mIU/ml. Such a full response occurs in about 85-90% of individuals. An antibody level between 10 and 100 mIU/ml is considered a poor response, and these people should receive a single booster vaccination at this time, but do not need further retesting.[32] People who fail to respond (anti-Hbs antibody level below 10 mIU/ml) should be tested to exclude current or past Hepatitis B infection, and given a repeat course of 3 vaccinations, followed by further retesting 1–4 months after the second course. Those who still do not respond to a second course of vaccination may respond to intradermal administration [33] or to a high dose vaccine [34] or to a double dose of a combined Hepatitis A and B vaccine.[35] Those who still fail to respond will require hepatitis B immunoglobulin (HBIG) if later exposed to the hepatitis B virus.[32] Factors that may reduce the immunogenicity of hepatitis

vaccines include age above 40 years, gender, weight, genetics, haemodialysis, HIV infection, immunosuppression, tobacco smoking, subcutaneous injection, injection into the buttocks, freezing of vaccine, and accelerated schedule.[11-13] and also in alcoholics, especially if with advanced liver disease.[13] May respond less well and require larger or more frequent doses of vaccine.[32]

2.1.2.6.5 Duration of protection:

The duration of vaccine- induced immunity is uncertain but it is definitely long term (>15 years). At present there is no recommendation for the administration of booster dose, although demonstrate a need for boosters.[16]It is now believed that the hepatitis B vaccine provides indefinite protection. However, it was previously believed and suggested that the vaccination would only provide effective cover of between five and seven years,[36] but subsequently it has been appreciated that long-term immunity derives from immunological memory which outlasts the loss of antibody levels and hence subsequent testing and administration of booster doses is not required in successfully vaccinated immunocompetent individuals.[37] Hence with the passage of time and longer experience, protection has been shown for at least 25 years in those who showed an adequate initial response to the primary course of vaccinations,[38] and UK guidelines now suggest that for initial responders who require ongoing protection, such as for healthcare workers, only a single booster is advocated at 5 years.[32]

2.1.2.6.6 Vaccine safety:

Side effects are local, of low intensity and short duration, involving a generally clinically insignificant soreness at the injection site or a mild to

moderate fever for 1-2 days following injection.[11-12-13-] Persons allergic to vaccine components should follow the recommendations for the use of HBIG.[13] Neither pregnancy nor lactation should be considered a contraindication to vaccination of women.[12-16] Hypersensitivity reactions can be expected in some individuals who are allergic to yeast antigens. The yeast derived vaccine is not recommended for such individuals.[12-13] Both plasma -derived and yeast- derived hepatitis B vaccines and safe for the prevention of HBV infection.[11-13] There is no scientific evidence that hepatitis B vaccine causes or exacerbates multiple sclerosis (MS) or other central nervous system demyelinating diseases.[11-12-16]

2.1.2.7 Treatment:

Currently there is no treatment available for acute hepatitis B. Symptomatic treatment of nausea, anorexia, vomiting, and other symptoms may be indicated.[16] Treatment of chronic hepatitis B is aimed at eliminating infectivity to prevent transmission and spread of HBV, at halting the progression of liver disease and improving the clinical and histologic picture, and at preventing HCC from developing, by losing markers of HBV replication in serum and liver like HBV DNA, HBeAg, and HBcAg. Normalization of ALT activity, resolution of hepatic inflammation and the improvement of a patients symptoms usually a ccompany these virological changes.[11-12] There are two main classes of treatment : antiviral: amid at suppressing or destroying HBV by interfering with replication.[12-16] and immune modulators: aimed at helping the human immune system to mount a defense against the virus.[16]Currently chronic hepatitis B is treated with interferon's.[11-12-13-16] the only approved ones are interferon-α-2a and interferon-α-2b. Interferon's display avarity of properties that include antiviral,

immunomodulatory, and antiplorliferative effects. They enhance T-cell helper activity, cause maturation of B lymphocytes, inhibit T-cell suppressors, and enhance HLA type 1 expression. To be eligible for interferon therapy, patients should have infection documented for at least six months, elevated liver enzymes (ALT and AST) and an actively dividing virus in their blood (HBeAg, and/or HBV DNA positive enzymes). Patient with acute infection, end stage cirrhosis or other major medical problems should not be treated. Interferon-α-2b produces a long-term sustained remission of the disease in 35% of those with chronic hepatitis B, with normalization of liver enzymes and loss of the three markers for an active infection (HBeAg, HBV DNA and HBsAg). Complete elimination of the virus is achieved in some carefully selected patients.[11-12-16] Anew treatment introduced recently for chronic hepatitis B in adults with evidence of HBV viral replication and active liver inflammation is EPIVIR®- HBV (lamivudine, Glaxo welcome). The recommended 100 mg once-daily oral dose in form of tablets is easy to take and generally well tolerated, although safety and effectiveness of treatment beyond 1 year have not been established.[12-16] lamivudine is a 2,3-dideoxy cytosine analogue that has strong inhibitory effects on the HBV polymerase and therefore on HBV replication in vitro and in vivo. Lamiviudine is well tolerated and suppresses HBV replication in HBsAg carriers, but the effect is reversible, if therapy is stopped.[16]

2.1.3 Hepatitis C:

Is an infectious disease affecting primarily the liver, caused by the hepatitis C virus (HCV).The infection is often asymptomatic, but chronic infection can lead to scarring of the liver and ultimately to cirrhosis, which is generally apparent after many years. In some cases, those with

cirrhosis will go on to develop liver failure, liver cancer or life-threatening esophageal and gastric varices.[14] The hepatitis C virus is an enveloped single stranded positive sense RNA virus with a diameter of about 50 nm, classified as a separate genus (Hepacivirus) within the Flaviviridae family. The genomic organization and sequence of HCV resembles that of the pestiviruses and flaviviruses [14-15-17] The reservoir of HCV is man, but the virus has been transmitted experimentally to chimpanzees.[15-17] The genome of HCV is highly mutable. Because HCV is an RNA virus and lacks efficient proofreading ability as it replicates, virions infecting humans undergo evolution with time, giving rise to the notion that HCV persists as a collection of virus quasispieces. By constant mutation, HCV may be able to escape host immunologic detection and elimination.[17] Currently there is no vaccination against hepatitis C. One reason being that the virus comes in many forms and constantly mutates leading to swarms of closely related viral genomic sequences (referred to as quasi-species).[15]There are major challenges to the future development of a hepatitis C vaccine primary infection of champanzees does not protect against subsequent challenge by either the identical viral strain or a heterologus strains protective or neutralizing antibodies have not been found.[17] HCV is inactivated by: Exposure to lipid solvents or detergents, heating at 60°C for 10 h or 100°C for 2 min in aqueous solution, formaldehyde (1:2000) at 37°C for 72 h, ß-propriolactone and UV irradiation. HCV is relatively unstable to:
Storage at room temperature, repeated freezing and thawing [15]

2.1.3.1 Signs and symptoms:

2.1.3.1.1Acute infection:

The incubation period for acute hepatitis C averages 6 to 10 weeks. Most persons about 80% who develop acute hepatitis C have no symptoms. [15]

The onset of disease is usually insidious with anorexia, vague abdominal discomfort, nausea and vomiting, fever and fatigue, progressing to jaundice in about 25% of patients, less frequently than hepatitis B.[18] probably as many as 70 -90% of infected people fail to clear the virus during the acute phase of the disease and become chronic carriers. The course of acute hepatitis C is variable, although elevations in serum ALT levels, often in a fluctuating pattern, are its most characteristic feature. Normalization of ALT levels might occur and suggests full recovery, but this is frequently followed by ALT elevations that indicate progression to chronic disease [15]

2.1.3.1.2 Chronic infection:

Chronic infection is often not symptomatic, until evidence of liver failure becomes clinically apparent. The rate of progression to cirrhosis is usually slow with 20 or more years elapsing between infection and the development of serious complications. Most persons (60-80%) who have chronic hepatitis C have no symptoms.[15]chronic HCV infection appears to be associated with the development of hepatocellular carcinoma (HCC) in 1 -5% of persons with chronic hepatitis C.[15-17] chronic HCV infection develops in 75 -85% of persons with persistent or fluctuating ALT elevations indicating active liver disease developing in 60-70% of chronically infected persons. No clinical or epidemiologic features among liver disease. An important clinical feature of infection with HCV is the high rate of chronic hepatitis and slowly progressive lifelong infection, which may lead to cirrhosis and liver failure in about 10 -20% of persons with chronic hepatitis C.[15]HCV associated cirrhosis leads to liver failure and death in about 20 -25% of cirrhotic case. HCV associated cirrhosis now represents a leading indication for liver transplantation.[15-17] Chronic

HCV infection appears to be associated with the development of hepatocellular carcinoma (HCC) in 1%-5% of persons with chronic hepatitis C.[15]

2.1.3.1.3 Extra hepatic manifestations:

Damage to the bile ducts, lymphoid aggregates or follicles, and micro vesicular steatosis are some characteristic findings associated with HCV infection.[15] Manifestations of HCV infection are primarily non hepatic, and include membranoproliferative glomerulonephritis and necrotizing vasculitis of the Skin.[17-15] Hepatitis C may be associated with autoimmune diseases such as Sjögren's syndrome and sialadenitis, idiopathic pulmonary fibrosis, polyarteritis nodosa , porphyria cutanea-tarda, and a variant of autoimmune hepatitis associated with the presence of anti-kidney and liver microsomal auto antibodies.[15-17]Anti-viral treatment should be considered for hepatitis C patients manifesting extra-hepatic complications.[15]

2.1.3.2The lifecycle:

Very little is known about the replication cycle of HCV, because there is no in vitro cell culture system that is permissive for virus replication [15] however, progress has been made [15-17] HCV probably follows the replication strategy of other positive-strand RNA viruses. The virus enters the cell and is uncoated in the cytoplasm. The viral genome is transcribed to form a complementary negative-sense RNA molecule, which, in turn, serves as a template for the synthesis of progeny positive-strand RNA molecules. The newly translated polyprotein is cleared by a host-cell signalize as well as virus-specific non-structural proteins, NS-2 and NS-3. The enzyme capable of performing both steps of RNA synthesis is the virally encoded RNA-dependent RNA polymerase NS5b. HCV replicates by a negative-strand RNA intermediate and has no reverse transcriptase

activity.HCV replicates by a negative-strand RNA intermediate and has no reverse transcriptase activity.[15]

2.1.3.3 Genotypes:

HCV is classified into eleven major genotypes (designated 1-11), many subtypes (designated a, b, c etc.), and about 100 different strains (numbered 1,2,3, etc.) based on the genomic sequence heterogeneity. The genes coding for the envelope E1 and E2 glycoproteins are the most variable. Amino acid changes may alter the antigenic properties of the proteins, thus allowing the virus to escape neutralizing antibodies. .[15]Genotypes 1-3 have a worldwide distribution. Types 1a and 1b are the most common, accounting for about 60% of global infections. They predominate in Northern Europe and North America, and in Southern and Eastern Europe and Japan, respectively. Type 2 is less frequently represented than type 1. Type 3 is endemic in south-east Asia and is variably distributed in different countries. Genotype 4 is principally found in the Middle East, Egypt, and central Africa. Type 5 is almost exclusively found in South Africa, and genotypes 6-11 are distributed in Asia.[15-17] The influence of viral genotype in the pathogenesis of liver disease is still controversial. Environmental genetic and immunological factors may contribute to the differences in disease progression, so characteristic of HCV infection, observed among patients. The variability is distributed throughout the genome. However, the non-coding regions at either end of the genome (5'-UTR and 3'-UTR; UTR-untranslated region) are more conserved and suitable for virus detection by PCR. The determination of the infecting genotype is however important for the prediction of response to anti-viral treatment genotype 1 is generally associated with a poor response to interferon alone whereas genotypes 2 and 3 are associated with more favourable response.[15] The current gold

standard of therapy - pegylated interferon- α in combination with ribavirin – significantly improves response for all genotypes.[39]

2.1.3.4 Transmission:

Transmission occurs by percutaneous exposure to contaminated blood and plasma derivatives, contaminated needles and syringes are most important vehicles of spread, especially among injecting drug users. In over 40% of cases the risk factors cannot be identified. [15] The cause of transmission remains unknown in 20% of cases;[40] however many of these are believed to be accounted for IDU.[41]

2.1.3.4.1 Intravenous drug use:

IDU is a major risk factor for hepatitis C in many parts of the world. Of 77 countries reviewed 25 (including the United States) were found to have prevalence of hepatitis C in the intravenous drug user population of between 60% and 80%.[42-43] Twelve countries had rates greater than 80%. It is believed that ten million intravenous drug users are infected with hepatitis C; China (1.6 million), the United States (1.5 million), and Russia (1.3 million) have the highest absolute totals.[43] most new infections are the consequence of high risk drug behavior (60%) or unsafe injection practices.[15] the prevalence of anti-HCV is highest in injecting drug users and hemophilia patients up to 98%.[39]

2.1.3.4.2 Healthcare exposure:

Blood transfusion, transfusion of blood products, or organ transplantation without HCV screening carry significant risk of infection.[44] Some countries do not screen for hepatitis C due to the cost.[45] Those who have experienced a needle stick injury from someone who was HCV positive

have about a 1.8% chance of subsequently contracting the disease themselves.[44] The risk is greater if the needle in question is hollow and the puncture wound is deep. There is a risk from mucosal exposures to blood; but this risk is low, and there is no risk if blood exposure occurs on intact skin. Hospital equipment has also been documented as a method of transmission of hepatitis C including: reuse of needles and syringes, multiple-use medication vials, infusion bags, and improperly sterilized surgical equipment, among others.[45]

2.1.3.4.3 Sexual intercourse:

The major risk factor for HCV infection is parental exposure primarily through blood products and needle sharing among injecting drug users. [15] Whether hepatitis C can be transmitted through sexual activity is controversial.[46] While there is an association between high-risk sexual activity and hepatitis C, it is not known whether transmission of the disease is due to drug use that has not been admitted to or sex as a risk factor.[44] The majority of evidence supports there being no risk for monogamous heterosexual couples. Sexual practices that involve higher levels of trauma to the anogenital mucosa, such as anal penetrative sex, or that occur when there is a concurrent sexually transmitted infection, including HIV or genital ulceration, do present a risk.[46] Transmission by household contact and sexual activity appears to be low.[15]

2.1.3.4.4 Body modification:

Tattooing is associated with two to three fold increased risk of hepatitis C. This can be due to either improperly sterilized equipment or contamination of the dyes being used. Tattoos or piercings performed either before the mid-1980s, underground, or nonprofessionally are of particular concern, since sterile techniques in such settings may be

lacking. The risk also appears to be greater for larger tattoos. It is estimated that nearly half of prison inmates share unsterilized tattooing equipment.[47]

2.1.3.4.5 Shared personal items:

Personal-care items such as razors, toothbrushes, and manicuring or pedicuring equipment can be contaminated with blood. Sharing such items can potentially lead to exposure to HCV.[48] neither is it transmitted through food or water.[49]

2.1.3.4.6 Vertical transmission:

Vertical transmission of hepatitis C from an infected mother to her child occurs in less than 10% of pregnancies. There are no measures that alter this risk.[50] it is not clear when during pregnancy transmission occurs but it may occur both during gestation and at delivery.[40] A long labor is associated with a greater risk of transmission.[45] there is no evidence that breast-feeding spreads HCV: however, to be cautions, an infected mother is advised to avoid breastfeeding if her nipples are cracked and bleeding.[51] or her viral loads are high.[40] The risk of mother to infant transmission of HCV increases dramatically if the mother is co-infected with HIV possibly due to an increase in HCV titer as a result of immune-suppression.[15-17]

2.1.3.5 Diagnosis:

There are a number of diagnostic tests for hepatitis C including HCV antibody enzyme immunoassay or ELISA, recombinant immunoblot assay, and quantitative HCV RNA polymerase chain reaction (PCR).[44] HCV RNA can be detected by PCR typically one to two weeks after

infection, while antibodies can take substantially longer to form and thus be detected.[52] Chronic hepatitis C is defined as infection with the hepatitis C virus persisting for more than six months based on the presence of its RNA. Chronic infections are typically asymptomatic during the first few decades,[41] and thus are most commonly discovered following the investigation of elevated liver enzyme levels or during a routine screening of high risk individuals. Testing is not able to distinguish between acute and chronic infections.[45] Diagnosis of hepatitis is made by biochemical assessment of liver function. Initial laboratory evaluation should include: total and direct bilirubin, ALT, AST, alkaline phosphatase, prothrombin time, total Protein, albumin, globulin, complete blood count, and coagulation studies.[15]

2.1.3.5.1 Serology:

Hepatitis C testing typically begins with blood testing to detect the presence of antibodies to the HCV using an enzyme immunoassay. If this test is positive, a confirmatory test is then performed to verify the immunoassay and to determine the viral load. A recombinant immunoblot assay is used to verify the immunoassay and the viral load is determined by a HCV RNA polymerase chain reaction. If there is no RNA and the immunoblot is positive it means that the person had a previous infection but cleared it either with treatment or spontaneously; if the immunoblot is negative, it means that the immunoassay was wrong.[44] It takes about 6–8 weeks following infection before the immunoassay will test positive.[53] Liver enzymes are variable during the initial part of the infection [41] and on average begin to rise at seven weeks after infection. Liver enzymes are poorly correlated with disease severity.[53]

2.1.3.5.2Biopsy:

Liver biopsies are used to determine the degree of liver damage present; however, there are risks from the procedure. The typical changes seen are lymphocytes within the parenchyma, lymphoid follicles in portal triad, and changes to the bile ducts. There are a number of blood tests available that try to determine the degree of hepatic fibrosis and alleviate the need for biopsy.[54]

2.1.3.5.3 Screening:

It is believed only 5–50% of those infected in the United States and Canada become aware of their status. Testing is recommended in those at high risk, which includes those with tattoos.[47] Screening is also recommended in those with elevated liver enzymes as this is frequently the only sign of chronic hepatitis.[55] Routine screening is not currently recommended in the United States.[44] However, in 2012, the U.S. Center for Disease Control and Prevention (CDC) recommended a single screening test for those born between 1945 and 1965.[56]

2.1.3.6 Treatment:

Combination therapy results in better treatment responses than monotherapy; the highest response rates have been achieved with pegylated interferon in combination with ribavirin. Genotype determinations influence treatment decisions. Currently the best indicator of effective treatment is a sustained viral response, defined by the absence of detectable HCV RNA in the serum as shown by a qualitative HCV RNA assay with lower limit of detection of 50 IU/mL or less at 24 weeks after the end of treatment.[15] Interferon has been shown to normalize

liver tests, improve hepatic inflammation and reduce viral replication in chronic hepatitis C and is considered the standard therapy for chronic hepatitis C. Currently it is recommended for patients with compensated chronic hepatitis C (anti-HCV positivity, HCV RNA detection, abnormal ALT levels over at least 6 months, fibrosis shown by liver biopsy).[15-57] Interferon-a is given subcutaneously at doses of 3 million units 3 times a week for 24 months. Patients with a reduced ALT activity or HCV RNA level within the first month of treatment are more likely to have a sustained response than patients in whom these changes do not occur. About 50% of patients respond to interferon-a by normalizing ALT at the end of therapy, but half of these relapse within the 6 months of follow-up after IFN withdrawal. The long-term biochemical response falls then to 20-25%. Only a minority of these have a persistent disappearance of HCV RNA from serum.[15-17]The duration of therapy depends on the genotype and level of viremia. In patients with genotype 2 or 3 the duration is 24 weeks while patient with genotype 1 need 48 weeks for treatment.[39]Combination therapy, approved in many countries, increases the proportion of patients who have a sustained viral response (SVR), reaching 40 -50%, compared with response rates of 15-25% with Interferon alone.[15]

2.2.1 Epidemiology of HB viral infection:

The world can be divided into three areas where the prevalence of chronic HBV infection is high (>8%). Intermediate (2-8%), and low (<2%).[12-16] The WHO American region is comprised of the countries of North America, Central America and South America. Hepatitis B prevalence in the American region needs to be considered in the context of 2 distinct subgroups:

(1) The United States and Canada (about 0.5-0.1%), which have low prevalence rates, and

(2) Mexico, Central America and South America, which have areas of significantly higher prevalence. In Mexico, Central America and South America. Hepatitis B is considered to be highly prevalent in this region, but there is variability among and within each country. The estimated prevalence ranges from 0.5-8.0%, with the total number of carriers approaching 11 million. [58-59-60-] Mexico and most of Central America have low-intermediate endemicity ranging from 1. 0-2.0%, except for Honduras, the Dominican Republic and Haiti where there is a higher prevalence of 3-4%. In South America, the rate of HBsAg carriage is as high as 8% in the native population of the Western Amazon Basin (Brazil, Colombia, Peru and Venezuela). With increasing distance from this area, the HBsAg prevalence decreases in Chile, Uruguay, Paraguay and Argentina having the lowest HBsAg prevalence ranging from 0.5-1.0%.[58] the prevalence of HBV in Europe is heterogeneous with rates ranging from <0.1% to as high as 12%.[58-60-61] Europe can be divided into three types of epidemiological patterns. The first pattern occurs in Northern Europe (Scandinavian countries and the United Kingdom) and is generally characterized by a low HBsAg carrier rate of<0.1%. the second pattern exists in most Western European countries, where the carrier rate ranges between 0.1 to 0.5% the final pattern can be found in Southern Europe (countries bordering the Mediterranean sea) and Eastern Europe where the carrier rates in some parts can be greater than 8%. [58] Eastern Mediterranean region extends from the countries of North Africa through the Middle East to Pakistan. The WHO estimates that the HBsAg prevalence in this region ranges from 1-10%, making it a region of intermediated to high endemicity.HBV in Africa this region covers all of Sub-Saharan Africa and Algeria. Africa has the second largest number of

chronic carriers after Asia and is considered a region of high endmicity.[58-60] The exact burden of hepatitis B in Africa is difficult to assess due to inaccurate records and under-reporting, but between 70 and 95% of the adult population show evidence of past exposure to HBV infection and the estimated HBsAg seroprevalence ranges from 6-20%.[58] The prevalence rate in Gambia and Senegal are about 15%, with age-specific prevalence as high as 20% years old. Not surprisingly, this region also has one of the world's highest rates of HCC. In Gambia, HCC is the most common cancer among men and the second most common cancer among women.[58-62] HBV in South-East Asia This region is comprised of Bangladesh, Bhutan, North Korea, India, Indonesia, Maldives, Myanmar, Nepal, Sri Lanka, Thailand and Timor-Leste. It is considered to be of intermediate to high endemicity with prevalence rates ranging from 1-10% while in India, the national prevalence rate has been estimated to be 4% with approximately 36 million carriers overall.[58] An extensive review by the Indian National Association for the study of liver Diseases estimated the average national prevalence rate to be 4.7%.[63] However, as with other countries that cover a large geographic area, the prevalence of hepatitis B is variable throughout the country with a gradient of generally increasing prevalence from north to south. The lowest prevalence is 2.3% in a large cohort of 20,000 blood donors in northern India. The highest reported rate is 5.7% in a community based study in almost 2000 people from southern India.[64-58] HBV in the Western Pacific region includes: Australia, Brunei Darussalam, Cambodia, China, Cook Islands, Fiji, Japan, Kiribati, Lao people's Democratic Republic, Malaysia, Marshall Islands, Mongolia, Nauru, New Zealand, Niue, Palau, Papua New Guinea, Philippines, South Korea, Samoa, Singapore, Solomon Islands, Tonga, Tuvalu, Vanuatu and Vietnam. The Western Pacific and South-East Asia regions are the largest and most populous of the six WHO

regions and have the highest rates of hepatitis B in the world, comprising more than 75 % of the worlds estimated 350 million carriers.[65] The prevalence of HBsAg in the Western Pacific region ranges from <1% to 30%. The prevalence is lowest, about 0.1%, in the non-Aboriginal populations of Australia and New Zealand. The Northern and Central Asian countries have an HBsAg prevalence rate ranging between 7 and 12%. Rates are highest in the small South Pacific island nations, reaching up to 30%.[58] prevalence of HBV among population of Qassim region in Saudi Arabia between 2008 and 2010 in 2008 HBsAg were detected 0.7% in 2009 HBV positive 1.5% in 2010 HBV was 2.04% .[66] In teaching hospital Nigeria among blood donor 7.5% were positive for HBsAg there was a gradual decline in the prevalence rate of HBsAg from 9.20% in 2006 to 8.37% in 2007 and 6.25% in 2008 with arise in the first half of 2009 to 6.32% .[68] blood donor in Aden city, Yemen 5.1% were positive for HBsAg.[67] In Iran 3.5% positive for HBsAg.[69] Sudan is classified among countries with a high hepatitis B surface antigen (HBsAg) endemicity of more than 8%. Exposure to HBV infection ranges from 47% to 78% with a hepatitis B surface antigen (HBsAg) seroprevalence ranging from as low as 6.8% in central Sudan to as high as 26% in southern Sudan.[9] In Gezire state of central Sudan among population HBsAg were detected 6.9% [73] also Study was conducted at Kassala, Eastern Sudan to determine the seroprevalence and epidemiological risk factors of hepatitis B virus among healthy people visited Kassala teaching hospital were detected HBsAg 8.2%.[70] In shendi city blood donor in ElmakNimirHospital HBV was detected 5.1% .[74]

2.2.2 Epidemiology of HC viral infection:

Hepatitis C virus (HCV) continues to be a major disease burden on the world. In 1999, the WHO estimated a worldwide prevalence of about 3% with the virus affecting 170 million people worldwide. Among central and south America, a recent community based study in San Juan, Puerto Rico, showed that estimated prevalence of HCV in 2001-2002 was 6.3%. In Mexico, the prevalence reported was about 1.2%. Among blood donors in Chile and Brazil, prevalence of HCV Ab was low -0.3%,1.14% respectively[72].Wide variation of prevalence worldwide i.e. in northern Europe, USA 1%- South-east Asia including India(1.5%), Malaysia(2.3%) ,Philippines(2.3%),Japan(1.2%) and north Africa(9.6--13.5)% and Egypt (14.5%).[75]. In the United Kingdom, at least 200,000 adults carry HCV. In Northern Italy, prevalence of HCV Ab was 3.2%. Three studies in Central and Southern Italy showed a higher rate of HCV (8.4%-22.4%), especially in the older population. Among patients of general practitioners in Lyon, France, the prevalence of HCV was estimated to be 1.3%, very similar to the French general population. Within the Russian army, frequency of anti-HCV was 1.5% among servicemen and donors with increased prevalence in the North Caucasus, Far East and Siberia (3.1--3.8%) compared to the Trans Baikal region (0.7%).[72-78] Low rates were found in Hungary (0.73% of 15,864 blood donors.).[72] In the middle east in Bahrain among multitransfused patients with hemolytic anemia 40% were seropositive for HCV antibody and among HD patients was 9.24% while in Iraq the prevalence of anti HCV antibodies is 7.1% in general population, 26.5% of 102 sera from Omani patients on HD, 13.4%of 82 sera from kidney transplant patients and 1% of 103 sera from non dialyzed.[76] Egypt has a very high prevalence of HCV and a high morbidity and mortality from chronic liver disease,

cirrhosis and hepatocellular carcinoma. Approximately 20% of Egyptian blood donors are anti-HCV positive. Egypt has higher rates of HCV than neigh boring countries as well as other countries in the world with comparable socioeconomic conditions and hygienic standards for invasive medical.[79] In Qatar 6.3% incidence rate of HCV infections was reported in general population and 44.6% was reported in dialysis patients, Saudi Arabia the prevalence of HCV infections is low in general population while between 18-46% in regular HD patients. In United State Emirates was 23%. The overall prevalence of Anti-HCV among HD patients was 48.9%, 60.5% among intravenous drug abusers, 1.96% among prostitutes group and 95% among blood donors and among health care workers was 3% and general population 1%. In Yemen HCV was 1.7% in healthy volunteers, 2.7% in blood donors, 33.8% in patients on regular HD and 33.75% in patients with chronic liver disease.[76] The few studies on HCV infection in Sudan demonstrated a low seroprevalence ranging from 2.2% in the Gezira state, an area endemic with schistosomiasis to 4.8% in patients with schistosomal periportal fibroses. Genotype 4 was the commonest isolated genotype.[9]In Egypt seroprevalence of hepatitis C virus in the urban blood donor population was 14.5%, while the sero prevalence was 70.4% in HD patients, 7.7% in health care workers, and 75.6% in thalassemic children. Moreover, HCV was found in 12.1% of rural primary school children, 18.1% of residents in rural villages, 22.1% of army recruits, 16.4% of children with hepatosplenomegaly, 54.9% of hospitalized multi-transfused children, 46.2% of adults on HD, and 47.2% of adults with chronic liver disease or hepatoma.[76] HCV seroprevalence was noted in other African countries such Ethiopia (2%), Central African Republic (5%), and Libya (7.9%). Genotype 4 was also the commonest genotype isolated in Cameron, Nigeria, Egypt, and the Central African Republic. [9] Among male blood

donors in Karachi, Pakistan, the seroprevalence of HCV was 1.8%There has been very high prevalence rates of HCV reported in Egypt in the past (28%). This was confirmed among 90 blood donors in Cairo, where 14.4% were anti-HCV positive by RIBA test. Then 26.6% among 188 blood donors and 22% among 163 donors were positive with both studies done in Cairo, while Rates were lower in Saudi Arabia (1.8%) and Yemen (2.1%). [72] blood donor in Aden city, Yemen 1.3% was positive for anti HCV.[67] in Iran 35.8% positive for anti-HCV.[69] prevalence of HCV among population of Qassim region in Saudi Arabia between 2008 to 2010 in 2008 Anti HCV were detected 0.1% in 2009 HCV 3% in 2010 HCV was found 0.83%.[66]

2.2.3 Epidemiology of HB and HC viral infection among health workers:

In the western Brazilian Amazon region, the previous studies among health care workers indicate the seroprevalence of HCV as 4.8 % and other control HCV as 3.7%.[33] while study was conducted at Ain Shams University Hospital, Cairo among Health care workers showed prevalence of Anti-HCV was 7.2%.[80] In the representative Tripoli central hospital in Libya among health care workers the prevalence of HBsAg as 1.1%.[81] In Indian Gandhi Medical College Shimla HBsAg was positive in 5% among health care workers while seropositivity among control group was 3.5 % and HCV antibody was not detected.[82] others previous studies among health care workers at El-minia university hospitals HBV antigen were detected 7.5% in male and 6.0 % in female compared to 2.6 % in male controls and 2.8% in female controls while HCV in health care workers showed 3.7 % in male and 5% in female compared to 1.3 % in male controls and 1.9% in female controls.[5] prevalence of HBV and HCV among laboratory workers of seven health units of Karbala province(Iraq) non of laboratory workers was showed a

positive result.[83] health care workers in southern region of Gaza strip .HBsAg was detected in 2.8% and antiHCV 1.3%.[84] in Gondar Town(Northwest Ethiopia) showed that the prevalence of HBsAg were detected among medical waste handlers 6.0% and non medical waste handlers 1.0% while HCV among medical waste handlers 1.0% and non medical waste handlers 0.0%.[85] In Cameroon among health care workers HBsAg were detected in 6.3% while anti-HCV were detected in 1.7%[7] and in three puplic hospitals in Addis Ababa city Ethiopia HBsAg was detected 35.8% and anti HCV antibody 22.5%.[86] In Eastern Libya among HCWs HBsAg was detected in 1.8% while anti-HCV was detected in 2.0%[87] seroprevalence rate of hepatitis B virus infection among the health workers in some of Wad Medani Hospitals was detected 4.2%[71] and Sudan was conducted in Omdurman Hospital among health care workers were detected HBsAg 2.4% while anti-HCV 0%[10]

2.2.4 Previous studies:

2.2.4.1 Previous study in Sudan

Study was conducted during November 2007 to determine seroprevalence of HB and C viruses and their risk factors among (211) participants(HCWs) done by Nail Abdelsalam,etals whose reported seroprevalence of HBsAg was 2.4% while anti-HCV was 0%, Age and past history of jaundice were significantly associated with HBsAg infection. The categories of HCWs with higher risk of occupational transmission for HBsAg were nurses and non- professional staff.[10]

2.2.4.2 Others Previous study in Sudan

This study was conducted during the period from 2009 to 2010. Atotal of 450 participants, done by Gasm N Elseed,etals whose reported the main serologic marker for hepatitis B virus (HBV) infection was detected among 4.2% (19/450) of the health care workers.[71]

2.2.4.3previous study in Gaza

Across sectional study of 399 healthcare personnel was conducted in govermmental healthcare setting of the southern region of Gaza strip. study done by Astal Z and Dhair M whose reported the prevalence of HBsAg was 2.8% while anti-HCV was 1.3% , None of the samples was found to be positive for both HBsAg and anti-HCV, needle injection showed a highly significant, high prevalenceof HBsAg and anti-HCV among categories age groups from41-50 and ≥50 years, recorded all positive cases among married participants and recorded the highest prevalence of HBsAg among nursing staffs while anti-HCV highest prevalence among nursing and physicians.[84]

2.2.4.4previous study in Egypt

study was conducted among HCWs at El-Minia University Hosipitals over a 24 months period starting from Jan.2008, done by T M Refat,etals whose recorded prevalence of HBsAg among HCWs was 13.5% while in control groups was 5.4%, whoever prevalence of anti-HCV among HCWs was 8.7% while in control groups was 3.2%, HCV showed higher incidence of seropositivity in medical staff working in dialysis units and operative rooms.[5]

2.2.4.5 Previous study in Europe

This study was conducted among 520 participants (HCWs) done by M Rybacki ,etals whose reported low rate of prevalence HBsAg was 1.2% while anti-HCV was 0.8%[88]

2.2.4.6 Previous study in Cameroon

237 participants among hospital workers in southwest Cameroon,study done by C Fritzsche ,etals whose recorded prevalence of HBsAg was 6.3% while prevalence of anti-HCV was 1.7% , duration of service significant in short years.[7]

2.2.4.7 Previous study in Saudi Arabia

Across- sectional study was conducted among 300 health college students and 300 health care workers in Najran Region of south-western Saudi Arabia, this study done by Alqahtani JM, etals whose reported prevalence of HBV among HCWs was 8.7% and in health students 1.7% while prevalence of anti-HCV among HCWs was 0.3% and in health students was 0%[89]

2.2.4.8 Previous study in Libya

This study conducted during period from July 2008 to June 2009 among 601 participants (HCWs), done by Abdel-Nasser E,etals whose recorded prevalence of HBsAg was 1.8% while anti-HCV was 2%, Nurses and

nurse-aides had the highest rates of both HBsAg and Anti-HCV, There was no significant difference between HBsAg status and the work period of HCWs. [87]

Chapter three

3. Material and methods

3.1 Study design:

This was hospital base cross sectional study.

3.2 Study area:

The study was done in Elmak Nimir University Hospital Shendi Sudan.

3.3 Duration of study: Des 2013 t0 Des 2015.

3.4 Study Population:

Study population two group were involved in this study ,health care workers in Emak Nimir University Hospital who considered as study group such as(Physicians, Nurses, laboratory and X-ray –technologist and Sudanese and non-Sudanese workers), and blood donor who considered as control group.

3.5 Inclusion criteria:

Any one of the hospital staff agree to be involved in this study, either medical or non medical regardless being infected or not infected with HB or HC viruses before began working at hospital.

3.6 Data collection tools:

Data and clinical information such as (sex, nationality and others) was collected via questionnaire in appendix number five.

3.7 Ethical considerations:

The present study was appeared from the ethical criteria , faculty of graduate study Shendi University, the participant were informed about the purpose of study before sample collection, the confided were insured

3.8 Sampling:

3.8.1 Sampling Size:

The study was included two hundred individual was included in the study and two hundred control blood donor.

3.8.2 Sampling Collection:

Under strict sterile conditions, 5 ml of whole venous blood samples was draw from each participant. Serum, aseptically, separated after clot retraction by centrifugation at 2000 rpm for 5 minutes, and stored at -8°C until tested (not more than a month).

3.8.3 Sampling Technique (Procedure and method):

3.8.3.1 Detection of HBsAg by AiD™ HBsAg ELISA:

3.8.3.1.1 Principle of the test:

For detection of HBsAg . AiD™ HBsAg ELISA uses antibody sandwich ELISA method in which polystyrene micro well strips are pre-coated with monoclonal antibodies specific to HBsAg. Patients serum or plasma sample is added to the micro wells, during incubation the specific immunocomplex formed in case of presence of HBsAg in the sample is captured on the solid phase then the second antibody conjugated the enzyme horse radish peroxidase (the HRP- conjugate) directed against a different epitope of HBsAg is added into the wells during the second incubation step these HRP-conjugated antibodies will be bound to any anti-HBs.HBsAg complexs previously formed during the first incubation and the unbound HRP-conjugate is then removed by washing chromogen solution containing tetramethyl-benzidine (TMB) and urea peroxide are added to the wells in presence of the antibody-antigen antibody(HRP) sandwich immuncomplex the colorless chromogens are hydrolyzed by the bound HRP-conjugate to a blue-colored product the blue color turns yellow after stopping the reaction with sulfuric acid the amount of color intensity can be measured and it is proportional to the amount of antigen captured in the wells and to its amount in the sample respectively wells containing samples negative for HBsAg remain colorless.

According to manufacture use this method AiD™ HBsAg ELISA to detected HBsAg all step of procedure was found in appendix number one

3.8.3.2 Detection of anti-HCV infection by AiD™ antiHCV ELISA:

3.8.3.2.1 Principle of the test:

AiD™ antiHCV ELISA employs solid phase. Indirect ELASA method for detection of antibodies to HCV in two-step incubation procedure polystyrene microwell strips are pre-coated with recombinant highly immunoreactive antigens corresponding to the core and the nonstructural regions of HCV (third generation HCV ELISA). During the first incubation step, anti-HCV specific antibodies if present will be bound to the solid phase pre-coated HCV antigens. The wells are washed to remove unbound serum proteins. And rabbit anti-human IgG antibodies (anti-IgG) conjugated to the enzyme horseradish peroxidase (HRP-Conjugate) are added. During the second incubation step these HRP-conjugated antibodies will be washing. Chromogen solutions containing tetrmethylbenzidine (TMB) and urea peroxide are added to the wells and in presence of the antigen-antibody-anti-IgG (HRP) immunocomplex, the colorless chromogens are hydrolyzed by the bound HRP conjugate to a blue-colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity can be measured and it is proportional to the amount of antibody captured in the wells. And to the amount of antibody in the sample respectively wells containing samples negative for anti-HCV remain colorless. According to manufacture use this method AiD™ antiHCV ELISA to detected anti-HCV all step of procedure was found in appendix number two.

3.8.3.3 Aspartate Aminotransferase (AST) GOT

3.8.3.3.1 measurement of AST by (IFCC method) mindray .

UV-assay according to IFCC(international federation of clinical chemistry and laboratory medicine) without pyridoxate activation. AST reagent is intended for quantitative determination of AST activity in serum on photometric systems.

In the assay reaction the AST catalyzes the reversible trancamination of L-aspartate and a-oxoglutarate to oxalcetate and L-glutamate. The oxalcacetate is then reduced to malate in the presence of malate dehydrogenase with NADH being oxidized to NAD. Therate of the photometrically determined NADH decrease is directly propotional to the rate of formation of oxaloacetate and thus the AST activity. All step of procedure was found in appendix number three

3.8.3.3.2 Alanine aminotransferase (ALT) GPT

3.8.3.3.1 measurement of ALT by (IFCC method) mindray.

ALT reagent is intended for quantitative determination of alanine aminotransferase activity in serum or plasma on photometric systems.

Principle of method:

Alanine aminotransferase catalyzes the reversible transamination of L-alanine and a-oxoglutarate to pyruvate and L-glutamate. The pyruvate is then reduced to lactate in the presence of lactate dehydrogenase (LDH) with the concurrent oxidation of reduced ß-nicotinamide adenine dinucleotide (NADH) to ß-nicotinamide adenine dirucleotide (NAD). This change in absorbance is directly proportional to the activity of ALT in the sample. All step of procedure was found in appendix number four

2.8 data analysis:-

The obtained result was performed by the Statistical Package for Social Sciences(SPSS) , using Mann-Whitney test for compared prevalence between study groups and control groups, while used chi-square to others analysis .

Chapter Four

4. Results:

Table 4.1 table showed prevalence of HBV infection in study population.

Study population	HBsAg		Total
	Sero-positive	Sero-negative	
Study group	5(2.5%)	195(97.5%)	200(100%)
Control group	1 (0.5%)	199(99.5%)	200(100%)
Total	6 (1.5%)	394(98.5%)	400(100%)

P= 0.1 by Mann-Whitney test

The above table showed insignificant difference in the prevalence of HBV infection between study group and the control group.

Table 4.2 table Showed prevalence of HCV infection in study population.

Study population	Anti-HCV		Total
	Sero-positive	Sero-negative	
Study group	3(1.5%)	197(98.5%)	200(100%)
Control group`	1(0.5%)	199(99.5%)	200(100%)
Total	4(1.0%)	396(99%)	400(100%)

P=0.3 by Mann-Whitney test

The above table showed no statistical difference between the prevalence of HCV infection in study group and the control group.

Table 4.3 Showed prevalence of HBV infection in study groups in the relation to gender

Gender	HBsAg		
	Ser-positive	Sero-negative	Total
Male	1(1.4%)	72(98.6%)	73(100%)
Female	4(3.1 %)	123(96.9%)	127(100%)
Total	5(2.5%)	195(97.5%)	200(100%)

P=0.4 by chi-square

The above table showed no difference in prevalence of HBV infection in relation to sex in study group.

Table 4.4 Showed prevalence of HCV infection in study group in the relation to gender.

Gender	Anti-HCV		
	sero -positive	sero -negative	Total
Male	2(2.8%)	71(97.2%)	73(100%)
Female	1(0.8%)	126(99.2%)	127(100%)
Total	3(1.5%)	197(98.5%)	200(100%)

P=0.3

The above table showed no statistical difference in HCV infections in relation to sex.

Table 4.5 Showed distribution of HBV infection in the relation to age group.

Age group	HBsAg		
	Sero- positive	Sero-negative	Total
< 25years	0 (0%)	38 (100%)	38(100%)
(25-35) years	2(1.9%)	104(98.1%)	106(100%)
(36-45)years	2(5.3%)	36(94.7%)	38(100%)
>45years	1(5.6%)	17(94.4%)	18(100%)
Total	5(2.5%)	195(97.5%)	200(100%)

P=0.3

The above table showed the age was not factor for contracting the infection of HBV in hospital**.**

Table 4.6 Showed distribution of HCV infection in the relation to age group.

Age group	Anti-HCV		
	sero-positive	sero-negative	Total
< 25years	0(0%)	38(100%)	38(100%)
(25-35) years	0(0%)	106(100%)	106(100%)
(36-45)years	3(7.9%)	35(92.1%)	38(100%)
>45years	0(0%)	18(100%)	18(100%)
Total	3(1.5%)	197(98.5%)	200(100%)

P=0.05

The above table showed all of infected individual with HCV in the age group 36-45 years

Table 4.7 Showed prevalence of HBV infection in study groups in the relation to Nationality.

Nationality	HBsAg		
	Sero-positive	Sero-negative	Total
Sudanese	5(2.6%)	184(97.4%)	189(100%)
Foreign	0(0%)	11(100%)	11(100%)
Total	5(2.5%)	195(97.5%)	200(100%)

P=0.5

The above tables showed the infected Individual with HBV were Sudanese in study group.

Table 4.8 Showed prevalence of HCV infection in study groups in the relation to Nationality

Nationality	Anti-HCV		
	Sero-positive	Sero-negative	Total
Sudanese	3(1.6%)	186(98.4%)	189(100%)
Foreign	0(0%)	11(100%)	11(100%)
Total	3(1/5%)	197(98.5%)	200(100%)

P=0.6

The above tables showed the infected Individual with HCV were Sudanese in study groups

Table 4.9 Showed prevalence of HBV infection in study groups in the relation to marital status.

Marital status	HBsAg		
	Sero-positive	Sero-negative	Total
Marriage	5(4.2%)	113(95.8%)	118(100%)
Single	0(0%)	82(100%)	82(100%)
Total	5(2.5%)	195(97.5%)	200(100%)

P=0.05

The above table showed all the infected individuals are married, and showed statistical significant relation marital status may be a factor in the spread of the virus.

Table 4.10 Showed prevalence of HCV infection in study groups in the relation to marital status.

Marital status	Anti-HCV		
	Sero-positive	Sero-negative	Total
Marriage	3(2.5%)	115(97.5%)	118(100%)
Single	0 (0%)	82(100%)	82(100%)
Total	3(1.5%)	197(98.5%)	200(100%)

P=0.1

The above table showed the marital status does not increase the risk for infection with HCV

Table 4.11 Showed prevalence of HBV infection in study groups in relation to Education.

Education status	HBsAg		
	Sero-positive	Sero-negative	Total
Graduate	1(0.8%)	129(99.2%)	130(100%)
Un graduate	4(5.7%)	66(94.3%)	70(100%)
Total	5(2.5%)	195(97.5%)	200(100%)

P=0.03

The above table showed most of infection with HBV in un graduated individuals
Graduated [had minimum higher secondary school]
Un graduated [had maximum higher secondary school]

Table 4.12 Showed prevalence of HCV infection in study groups in relation to Education status.

Education status	Anti-HCV		
	Sero-positive	Sero-negative	Total
Graduate	1(0.8%)	129(99.2%)	130(100%)
Un graduate	2(2.9%)	68(97.1%)	70(100%)
Total	3(1.5%)	197(98.5%)	200(100%)

P=0.2

The above table showed education does not factor in distribution of HCV.

Table 4.13 Showed HBV infection in relation to hospital specialty.

Occupation	HBsAg		
	Sero-positive	Sero-negative	Total
Doctor	1(3.1%)	31(96.9%)	32(100%)
Nurse	2(2.7%)	71(97.3%)	73(100%)
Lab-technicians	0(0%)	29(100%)	29(100%)
X-ray tech	0(0%)	4(100%)	4(100%)
Sanitary	2(5.1%)	37(94.9%)	39(100%)
Others	0(0%)	23(100%)	23(100%)
Total	5(2.5%)	195(97.5%)	200(100%)

P=0.7

The above table showed most seropositive individual were sanitary, this result was statistically not significant

Table 4.14 Showed HCV infection in the relation to Occupation.

Occupation	Anti-HCV		
	Sero-positive	Sero-negative	Total
Doctor	0(0%)	30(100%)	30(100%)
Nurse	1(1.3%)	72(98.6%)	73(100%)
Lab-technicians	0(0%)	29(100%)	29(100%)
X-ray tech	0(0%)	4(100%)	4(100%)
Sanitary	2(5.1%)	37(94.9%)	39(100%)
Others	0(0%)	23(100%)	23(100)
Total	3(1.5%)	197(98.5%)	200(100%)

P=0.4

The above table shows no difference in incidence of HCV infection in relation to Occupation in health care workers.

Table 4.15 Showed distribution of HBV infection in the relation to Occupation period.

Occupation period	HBsAg		
	Sero-positive	Sero-negative	Total
≤ 4 year	1(1.1%)	90(98.9%)	91(100%)
5 -9 year	0(0%)	61(100%)	61(100%)
≥ 10 year	4(8.3%)	44(91.7%)	48(100%)
Total	5(2.5%)	195(97.5%)	200(100%)

P=0.01

The above table showed most positive case of HBV was detected in health care workers period ≥ 10 year in the medical field the result was statistically highly significant

Table 4.16 Showed distribution of HCV infection in the relation to Occupation period.

Occupation period	Anti-HCV		
	Sero-positive	Sero-negative	Total
≤ 4 year	0(0%)	91(100%)	91(100%)
5 -9 year	2(3.3%)	59(96.7%)	61(100%)
≥ 10 year	1(2.1%)	47(97.9%)	48(100%)
Total	3(1.5%)	197(98.5%)	200(100%)

P=0.2

The above table showed no difference in prevalence of HCV infection in relation to Occupation period in health care workers.

Table 4.17 Showed HBV infection in the relation to Department.

Department	HBsAg		
	Sero-positive	Sero-negative	Total
Surgery	0(0%)	22(100%)	22(100%)
Medicine	1(2.4%)	40(97.6%)	41(100%)
Pediatric	0(0%)	15(100%)	15(100%)
Dialysis	2(8.7%)	21(91.3)	23(100%)
Gynecology	1(5.9%)	16(94.1%)	17(100%)
Oncology	0(0%)	6(100%)	6(100%)
Theater	0(0%)	12(100%)	12(100%)
Cardiology	1(12.5%)	7(87.5%)	8(100%)
Laboratory	0(0%)	28(100%)	28(100%)
Blood bank	0(0%)	5(100%)	5(100%)
X-ray	0(0%)	4(100%)	4(100%)
Refer	0(0%)	19(100%)	19(100%)
Total	5(2.5%)	195(97.5%)	200(100%)

P=0.4

The above table showed no difference in prevalence of HBV infection in relation to department in health care workers.

Table 4.18 Showed HCV infection in the relation to Department.

Department	Anti-HCV		
	Sero-positive	Sero-negative	Total
Surgery	1(4.6%)	21(95.4%)	22(100%)
Medicine	1(2.4%)	40(97.6%)	41(100%)
Pediatric	0(0%)	15(100%)	15(100%)
Dialysis	0(0%)	23(100%)	23(100%)
Gynecology	0(0%)	17(100%)	17(100%)
Oncology	0(0%)	6(100%)	6(100%)
Theater	0(0%)	12(100%)	12(100%)
Cardiology	1(12.5%)	7(87.5%)	8(100%)
Laboratory	0(0%)	28(100%)	28(100%)
Blood bank	0(0%)	5(100%)	5(100%)
X-ray	0(0%)	4(100%)	4(100%)
Refer	0(0%)	19(100%)	19(100%)
Total	3(1.5%)	197(98.5%)	200(100%)

P=0.4

The above table showed no difference in incidence of HCV infection in relation to department in health care workers.

Table 4.19 showed the serological result of HB infection according to vaccination among study group

vaccination	HBsAg		
	Sero-positive	Sero-negative	Total
Vaccinated	0(0%)	74(100%)	74(100%)
Not vaccinated	5(4%)	121(96%)	126(100%)
Total	5(2.5%)	195(97.5%)	200(100%)

P=0.08

The above tables showed most of the individuals in the study are not vaccinated against HBV

Table 4.20 showed serological result of HBV infection according to it is risk factor among study group.

N=200

Type of Individual exposure	HBsAg			
	Ser-Positive	Ser-negative	Total	P
history of jaundice	2(7.4%)	25(92.6%)	27(100%)	0.6
history of blood transfusion	2(16.7%)	10(83.3%)	12(100%)	0.8
history of injury	5(4.3%)	110(95.7%)	115(100%)	0.2
history of operation	4(6.9%)	54(93.1%)	58(100%)	0.3

These risk factors did not increase the probability for infection with HBV.

Table 4.21 showed serological result of HCV infection according to it is risk factor among study group

Type of Individual exposure	Anti-HCV			
	Sero-Positive	Sero-negative	Total	P
history of jaundice	3(11.1%)	24(88.9%)	27(100%)	0.7
history of blood transfusion	0(0%)	12(100%)	12(100%)	0.6
history of injury	2(1.7%)	113(98.3%)	115(100%)	0.6
history of operation	2(3.4%)	56(96.6%)	58(100%)	0.7

These risk factors did not increase the probability for infection with HCV.

5. Discussion:

This was descriptive Hospital based cross sectional study conducted in Elmak Nimir University Hospital, during the period from Des 2013 to Des 2015 to find out the prevalence of HBsAg and anti- HCV among Hospital workers. The prevalence of HBsAg was detected in (5/200) 2.5% HCWs compared to (1/200) 0.5% control group blood donor during the same period, this was shown in table (4.1), the prevalence of HBsAg among HCWs 2.5% was higher than the control group 0.5% this might be due to the higher risk of exposure, but this result statistically was insignificant (P=0.1).

Which the prevalence of anti-HCV was detected in (3/200) 1.5% of HCWs and in (1/200) 0.5% of control group participant shows in table (4. 2), the prevalence among HCWs was higher than control group due to high risk of exposure, but this result statistically was insignificant (P=0.3) None of the workers was found positive for both HBsAg and anti-HCV to gather. The infected individuals showed normal liver enzymes (AST and ALT) indicated inactive liver infections. These findings were relatively higher than that reported by M Rybcacki,etals in Central Europe among HCWs whose reported 1.2% prevalence of HBsAg and anti-HCV infection was 0.8 %[88], in USA among HCWs the KE Nelson and Willians reported prevalence of HBV infection was 2%[90], while AK. Agarwal ,who stated prevalence of anti-HCV among HCWs in USA was 1%[75] , regarding prevalence of HBsAg and anti- HCV among HCWs in Europe and in USA was lower than prevalence in this study that may be due to development of health system care and higher qualification of health workers, highly safety precautions, immunization programs and low rate of HBV infections among population, thus low rate as general. The prevalence of HBsAg in this study lower than prevalence among

HCWs in Africa, as one study done in Cameron by C Fritzsche, etals whose reported prevalence of HBsAg was 6.3%[7].

Increase prevalence of HBsAg among HCWs in Arab countries, study done in El-minia university hospital (Egypt) as TM Refat , etals whose reported prevalence of HBsAg was 13.5%[5] ,it may be due to high rate of infection among population, in Najran (South Western Saudi Arabia) as M Jobran Alqahtani , etals whose reported prevalence of HBsAg was 8.7%[89] , this result may be due to increase foreign workers in this country, while the prevalence was near to as Astal Z and Dhair M (in Southern Gaza) whose finding prevalence of HBsAg was 2.8% [84]. This study agreement with that reported done in Sudan by Abdelsalam N, etals who's reported that prevalence of HBsAg was 2.4%. And this study lower than study done in Wad Medani as, Gasm.N Elseed, etals whose reported prevalence of HBsAg was 4.2%[10-71]. Concerning anti-HCV in this study was near to prevalence of anti-HCV in Cameron by C Fritzsche, etals and in Southern Gaza by Astal Z and Dhair M were reported prevalence of anti-HCV were 1.7% and 1.3% respectively[7-84].While prevalence in this study was higher than prevalence of anti-HCV was done in Najran (Southwestern Saudi Arabia) as M JobranAlqahtani,etals and in Sudan as Abdelsalam N, etals, those were reported prevalence of anti-HCV were 0.3% ,0% respectively [89-10] Regarding all previous study prevalence of anti-HCV was low except in Egypt at El-minia university hospital prevalence of anti-HCV was 8.7%[5] , this might be suggest that the high prevalence of HCV antibodies among HCWs in Egypt reflected the community prevalence rather than exposures to contaminated blood from patients at the work site. Generally the prevalence is not high this may be attributed to low disease prevalence in general population, high coverage rate of vaccination

,Knowledge of bio-safety procedures and their application in the health setting .

High rate of workers were female (127/200), and most cases detected among female in HBsAg 4(3.1%) shows in table (4.3), but this result statistically insignificant (P=0.4), while anti-HCV high rate among male 2(2.8%) showing in table (4.4) also this result statistically insignificant (P=0.3) , in this study HBsAg were higher among female than male, while prevalence of anti-HCV among male is higher than female this is not agree with study done in El-minia Hospital asTM Refat, etals whose recorded that positive cases of HBsAg among male than female, while anti-HCV positive cases among female than male[5]. Other study not agree with study done in Omdurman and Wad Medani in which hepatitis prevalence was higher in male than female [10-71],It may be not representative because the sample size is small and sex not increase the risk for infection with HBV or HCV, also may be due to the fact that the majority of the study participants in Elmak Nimir Hospital study were female.

The main age of the categories from (25-35) as (106/200), and most cases of HBsAg were 1(5.6%) detected among categories age more than 45 years shows in table (4.5), this result statically insignificant (P=0.3), while all positive cases of anti-HCV were detected 3(7.9%) among category age from (36-45) shows in table (4.6) this result statically significant (P=0.05) this study agree in case of HBsAg and anti-HCV with Astal Z and Dhair M whose reported that high prevalence of HBsAg and anti-HCV among categories age groups from 41-50 and ≥50 years[84] This may be due to the increased number of service years. Thus greater possibility of risky exposure to blood born viruses through exposure to contaminated instruments in healthcare settings,

The most of study group were Sudanese nationality, Sudanese (189/200), all of positive cases of HBsAg Sudanese 5(2.6%) shows in table (4.7) this study insignificant (P=0.5), anti-HCV detected among study group were Sudanese, prevalence of anti-HCV was 3(1.6%) shows in table (4.8) this was statistically insignificant (P=0.6), because foreign worker must be tested for HBsAg and anti-HCV testes before working.

Most of study group were marriage (118/200) and all positive cases of HBsAg, were marriage prevalence was 5(4.2%), shows in table (4.9) this result statistically significant (P=0.05), anti-HCV were detected among marriage prevalence 3(2.5%) shown in table (4.10), this result statistically insignificant (P=0.1), these result in case of HBsAg agree with Abdelsalam N, etals whose detected all positive case among marriage [10]. And agree with Astal Z and Dhair M were record all reactive cases among married participants[84] ,This may explained by the sexual risk factor but this is not cut off point because coupled were not surveyed, and this out of the scope of this study.

Majority of study group were graduate (130/200), the most cases of HBsAg were detected among un graduate, prevalence was 4(5.7%) shows in table (4.11) ,this study was statistically significant (P=0.03), that means knowledge may decrease the exposure to infection.

Most case of anti –HCV prevalence 2(2.9%) were detected among un graduate shows in table (4.12) this result statistically insignificant (P=0.2).

High rate of workers were nurses (73/200), most of positive cases of HBsAg were detected in sanitary workers were 2(5.1%) shows in table (4.13), this result was statistically insignificant (P=0.7), also anti-HCV were detected among sanitary staff 2(5.1%),shows in table (4.14), this result was statistically insignificant (p=0.4), agree with Omdurman(Sudan) Nail Abdelsalam,etals whose reported that the high

exposure among nurses and cleaning staff[10] , disagree with Astal Z and Dhair M whose recorded the highest prevalence of HBsAg among nursing staffs, however for anti-HCV the highest prevalence among nursing staffs and physicians[84] , also disagree in Wad Medani Gasm. N Elseed ,etals whose reported to be highest among Anesthetists and surgical assistant in case of HBsAg[71]. This may be due to the frequent exposure to possible source of infection such as body fluids, needles and contaminated instruments, which leads to higher risk of infection. It appears that, no reactive cases were recorded in certain sections as laboratory, blood bank; this might be due to sample size low.

Most of hospital workers of this study working for four years or less (91/200), but most positive cases were detected among workers in case of HBsAg for period more or equal 10 years 4(8.3%) shows in table (4.15), this result statistically highly significant (P=0.01), while anti-HCV were detected among workers for period from (5-9) years 2(3.3%) shows in table (4.16) this result statistically insignificant(P=0.2),comparing with the Cameroon duration of service significantly in short years [7],while in Eastern Libya AN Elzouki,etal there was insignificant difference between HBsAg status and work period of HCWs[87] and this result statistically was highly significant in this study (P=0.01). This means that long period of working in the hospitals increase risk of exposure to infection. Most frequency volunteer in this study was medicine department was (41/200), the highest prevalence of HBsAg was 1(12.5%) detected in Cardiology shows in table (4.17), also the highest prevalence of anti-HCV was 1(12.5%) detected in cardiology shows in table (4.18). Disagree in Southern Gaza by Astal Z and Dhair M were reported that highest prevalence of HBsAg in the radiology section. While the highest prevalence of anti-HCV in operation, surgery and pediatric section[84], and T M Refat, etals were recorded that the high incidence of

occupational exposures to be in hemodialysis units, emergency departments and operating rooms[5], cardiology consider are sub department of medicine, statistically insignificant (P=0.4), Many invasive procedure, thus it is clearly a high-risk location for serious blood exposures.

Most frequency among study groups were not vaccinated was (126/200), and all of positive 5(4%) was detected among unvaccinated shown in table (4.19), (P=0.08) statistically there was insignificant difference in HBsAg positivity between the vaccinated and non-vaccinated study participants. This result agree with Abdelsalam N etal.[10] his is probably due to non-co-operation towards HBV vaccination since pre-employment screening and vaccination are available in Sudan, in other wares may be due to effective prevention of HBV infection by vaccination to unexposed HCW, however acceptance of vaccine should be promoted for such high risk categories.

Hospital workers of this study most of exposed to risk factor, history of injury (115/200), HBsAg positive cases were detected among exposure to blood transfusion 2(16.7%) shows in table (4.20), but this was statistically insignificant (P=0.2) while anti-HCV positive cases were detected among exposure to jaundice 3(11.1%) shows in table (4.21), also this result statistically insignificant (P=0.7), may be due to low sample size , these risk factors did not increase the probability for infection with HBsAg and HCV. This agreement with that reported by, Abdelsalam N,etals and Gasm N Elseed,etals[10-71]. While Astal Z and Dhair M were detected needle injection highly significant for HBsAg and antiHCV and previous blood transfusion associated for HBsAg[84]

5.1Conclusion:

This is cross sectional study was conducted to determine the seroprevalence rate of hepatitis B and C virus's infections among the health care workers in Elmak Nimir Hospital, Shendi River Nile State, Sudan. For that purpose A total of 200 blood samples collected and 200 sample of blood donors as control group screened for HB and C viral antigen (HBsAg) and antibodies (anti-HC) respectively .The prevalence of hepatitis B viral infection used HBsAg as marker was (5/200) 2.5 % compared with (1/200) 0.5% in the control group (P=0.1). While prevalence of HC viral infection was (3/200) 1.5% compared with (1/200) 0.5% prevalence in the control group (P=0.3). Using the Enzyme-Linked Immuno sorbent Assay (ELISA), and liver enzymes assay such as (AST and ALT) .The prevalence of HBsAg and anti-HCV were higher among HCWs than control group but these differences were not statistically significant. Prevalence of HBsAg and anti-HCV was higher in the study group compared with results done in central Europe and other in USA. Prevalence of HB virus in this study was less than results done in Cameron, Saudi Arabia and other in Egypt, Prevalence of hepatitis B virus in this study equal the other result done in Sudan, while prevalence of HC virus in this study was higher than results done in Gaza strips and Saudi Arabia. While Prevalence of HC virus in this study was lower than Egypt. There was statistical correlation between HBV infection and marital statuses, long period of occupational, and under graduation, also the correlation between HCV infection and old age. The highest percentage of infection of HB and HC viruses found among cleaning staff

5.2 Recommendation:

For control of HB and HC viral infection among Hospital workers this recommendation should be consider.

1) The sample size in the study is not enough to represent the situation; this is for financial aspect, so we need to do the same study in representative sample size.

2) All hospital personal should be vaccinated against HB.

3) Regular screaming for HB and HC viral infection should be done at regular interval especial in the high risk department for viral transmission.

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Appendix number (1):

Procedure to detection HBsAg:

Reagents preparation: allow the reagents to reach room temperature (18-30 ^{o}C) . Check the wash buffer concentrate for the presence of salt crystals. If crystals. If crystals have formed . Resolubilize by warming at 37 ^{o}C until crystals dissolve. Dilute the wash buffer (20x) as indicated in the instructions for washing. Use distilled or deionized water and only cleans vessels to dilute the buffer. All other . All other reagents are ready to as supplied.

Step1 preparation: mark three wells as negative control (e.g.B1,C1,D1). Two wells as positive control (e.g.E1,F1) and one blank (e.g.A1, neither samples nor HRP-conjugate should be added into the blank well). If the results will be determined by using dual wavelength plate reader. The requirement for use of blank well could be omitted. Use only number of strips requirement for the test.

Step2 Adding diluents: add 20µl of specimen diluents into each well except the blank

Step3 Adding sample: add 100µlof positive control, negative control, and specimen into their respective wells except the blank. Note: use a separate disposal tip for each specimen, negative control, and positive control to avoid cross-contamination. Mix by tapping the plate gently.

Step 4 Incubating: cover the plate with the plate cover and incubate for 60minutes at 37 ^{o}C.

Step 5 Adding HRP-conjugate: at the end of the incubation, remove and discard the plate cover. Add 50µl HRP-conjugate into well except the blank. An mix by tapping the plate gently.

Step 6 incubating: cover the plate with the plate cover and incubate for 30minutes at 37 ^{o}C..

Step 7 washing: at the end of the incubation remove and discard the plate cover. Wash each well 5 times with diluents washing buffer. Each time allow the micro wells to soak for 30-60 second. After the final washing cycle, turn down the plates onto blotting paper or clean towel and tap it to remove any remainders.

Step 8 coloring: add 50 µl of chromogen A and 50 µl chromogen B solution into each well including the blank. Incubate the plate at 37 ^{o}C for 30 minutes avoiding light. The enzymatic reaction between the chromogen solutions and HRP-conjugate produces blue color in positive control and HBsAg positive sample wells.

Step 9 stopping reaction: using a multichannel pipette or manually. Add 50 µl stop solution into each well and mix gently. Intensive yellow color develops in positive control and HBsAg positive sample wells. **Step10** Measuring the absorbance: calibrate the plate reader with the blank well and read the absorbance at 450nm. If a dual filter instrument is used set the referen wavelength at 630nm. Calculate the cut-off value and evaluate the result.(Note: read the absorbance within 10 minutes after stopping the reaction).

Quality control and calculation of the results:

Each micro plate should be considered separately when calculating and interpreting the result of the assay regardless of the number of plates concurrently processed. The results are calculated by relating each specimen absorbance (A) value to the cut-off value (C.O) of the plate. If the cut –off reading is based on single filter plate reader.

The results should be calculated by subtracting the blank well a value from the print report values of specimens and controls. Calculation of the cut-off vaue (C.O) = NC + 0.06 (NC = the mean absorbance value for three negative controls).

Interpretations of the results:

Negative results (A/C.O. < 1): specimens giving absorbance less than the cut-off value are negative for this assay. Which indicates that no hepatitis B virus surface antigen has been detected with AiD^{TM} HBsAg ELISA. Therefore the patient is probably not infected with HBV and the blood until do not contain hepatitis B virus surface antigen and could be transfused in case that other infectious diseases markers are also

Positive result (A/C.O ≥ 1): specimens giving an absorbance equal to or greater than the cut-off values are considered initially reactive with indicates that hepatitis B virus surface antigen have probably been detected using AiD^{TM} HBsAg ELISA. All initially reactive specimens should be retested in duplicates using AiD^{TM} HBsAg ELISA before the final assay result interpretation. Repeatedly reactive specimens can be considered positive for hepatitis B virus surface antigen with AiD^{TM} HBsAg ELISA

Borderline (A/C.O = 0.9-1.1): specimens with absorbance to cut-off ratio between 0.9 and 1.1 are considered borderline and retesting of these specimens in duplicated to confirm the initial result

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Appendix number (2)

Procedure to detection anti-HCV:

Reagents preparation: all the reagents to reach room temperature (18-30 ^{o}C). check the wash buffer concentrate for the presence of salt crystals. If crystals have formed resolubilize by warming at 37 ^{o}C until crystals dissolve. Dilute the wash buffer (20X) as indicated in the instructions for washing. Use distilled or deionized water and only clean vessels to dilute the buffer. All other reagents are ready to use as supplied. **Step1 preparation:** mark three wells as negative control (e.g.B1,C1,D1). Two wells as positive control(e.g.E1,F1) and one blank (e.g.A1, neither samples nor HRP-conjugate should be added into the blank well). If the results will be determined by using dual wavelength plate reader, the requirement for use of blank well could be omitted. Use only number of strips required for the test.

Step 2 Adding diluents: Add 100 µl of specimen diluents into each well except the blank.

Step 3 Adding sample: Add 10 µl of positive control, negative control, and specimen into their respective wells except the blank. **Note use a separate disposal pipette tip for each specimen, negative control, positive control to avoid cross-contamination. Mix by tapping the plate gently.**

Step 4 Incubating: Cover the plate with the plate cover and incubate for 30 minutes at 37 ^{o}C.

Step 5 washing: At the end of the incubation, remove and discharge the plate cover. Wash each well 5 times with diluted wash buffer. Each time allow the micro wells to soak for 30-60 seconds. After the final washing

cycle, turn down the plate onto blotting paper or clean towel, and tap it to remove any reminders.

Step 6 Adding HRP-conjugate: Add 100 µl HRP-conjugate into each well except the blank.

Step 7 Incubating: cover the plate with the plate cover and incubate for 30 minutes at 37 ^{o}C.

Step 8 washing: At the end of the incubation, remove and discharge the plate cover. Wash each well 5 times with diluted washing buffer. Each time allow the micro wells to soak for 30-60 second. After the final washing cycle turn down the plate onto blotting paper or clean towel and tap it to remove any remainders.

Step 9 coloring: Add 50 µl of chromogen A and chromogen B solution into each well including the blank. Incubate the plate at 37 ^{o}C for 50 minutes avoiding light. The enzymatic reaction between the chromogen solutions and the RHP-conjugate produce blue color in positive control and anti-HCV positive sample wells.

Step 10 stopping reaction: using a multichannel pipette or manually add 50 µl stop solution into each well and mix gently. Intensive yellow color develops in positive control and anti-HCV positive sample wells.

Step 11 measuring the absorbance: calibrate the plate reader with the blank well and read the absorbance at 450nm. If a dual filter instrument is used, set the reference wavelength at 360nm. Calculate the cut-off value and evaluate the result. (Note : read the absorbance within 10 minutes after stopping the reaction).

Quality control and calculation of the result:

Each microplate shoud be considered separately when calculating and interpreting the results of the assay regardless of the number of plates

concurrently processed. The results are calculated by relating each specimen absorbance (A) value to the cut-off value (C.O) of the plate. If the cut-off reading is based on single filter plate reader, the result shoudr3344i79800oio090 be calculated by subtracting the blank well A value from the print report values of specimens and controls. In case the reading is based on dual filter plate reader, do not subtract the blank well A value from the print report values of specimens and controls. Calculation of the cut-off value (C.O) = NC+0.12 (NC =the mean absorbance value for three negative controls). Important: If the mean A value of the negative controls is lower than 0.02 take it as 0.02. Quality control (assay validation): the test results are valid if the quality control criteria are fulfilled. It is recommended that each laboratory must establish appropriate quality control system with quality control system with quality control material similar to or identical with the patient sample being analyzed.

Interpretations of the results:

Negative result: (A/C.O. < 1): specimen giving absorbance less than the cut-off value are negative for this assay, which indicates that no anti-HCV antibodies have been detected with AiD^{TM} antiHCV ELISA, therefore the patient is probably not infected with HCV and the blood unit do not contain antibodies to HCV and could be transfused in case that other infectious diseases markers are also absent.
Positive result: (A/ C.O. ≥ 1): specimens giving an absorbance equal to or greater than the cut-off value are considered initially reactive which indicates that anti-HCV antibodies have probably been detected using AiD^{TM} antiHCV ELISA. All initially reactive specimens should be retested in duplicates using AiD^{TM} antiHCV ELISA, before the final assay results interpretation. Repeatedly reactive specimens can be

considered positive for antibodies to HCV with AiD™ antiHCV ELISA. **Borderline:** (A/ C.O = 0.9-1.1): specimens with absorbance to cut-off ratio between 0.9 and 1.1 are considered borderline and retesting of these specimens in duplicates is required to confirm the initial results.

Appendix number (3)

Procedure to measurement AST:

	Blank	Sample
Reagent 1 Dist-water Sample	1000 µL 100 µL -	1000 µL - 100 µL

Mix incubate for 5 min the add

Reagent 2	250 µL	250 µL

Mix thoroughly read the absorbance after 1 min and monitor time. Read the absorbance again for additional 3 min.

Quality control:

At least two levels of control material should be analyzed with each batch of sample. These controls should be run with each new calibration, each new reagent cartridge and after specific maintenance or troubleshooting procedures as detailed in the appropriate system manual. It is recommended to use the Human Assayed control from Mindray to verify the performance of the measurement procedure. Each laboratory should establish its own internal quality control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

Calculation:

The analyzer calculates the activity of each sample automatically with a specified valid calibration factor from calibration process conversion factor of traditional unit (U/L) into SI units (µkat/L):

1 U/L = $16.67X10^{3}$ µkat/L 1 µkat/L =60 U/L

Reference intervals:

Each laboratory should establish its own reference intervals based upon its patient population. The reference intervals measured at 37c listed below were taken from literature.

Gender	conventional units	SI.units
Male	≤ 35 U/L	≤ 0.58 µkat/L
Female	≤ 31 U/L	≤ 0.52 µkat/L

Appendix number (4)

Procedure to measurement ALT:

	Blank	Sample
Reagent 1	1000 µL	1000 µL
Dist-water	100 µL	-
Sample	-	100 µL

Mix incubate for 5 min the add

Reagent 2	250 µL	250 µL

Mix thoroughly read the absorbance after 1 min and monitor time. Read the absorbance again for additional 3 min.

Quality control:

At least two levels of control material should be analyzed with each batch of sample. These controls should be run with each new calibration, each new reagent cartridge and after specific maintenance or troubleshooting procedures as detailed in the appropriate system manual. It is recommended to use the Human Assayed control from Mindray to verify the performance of the measurement procedure. Each laboratory should establish its own internal quality control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

Calculation:

The analyzer calculates the activity of each sample automatically with specified valid calibration factor from calibration process conversion factor of traditional unit (U/L) into SI units (µkat/L):

1 U/L = $16.67X10^{3}$ µkat/L 1 µkat/L =60 U/L

Reference intervals:

Each laboratory should establish its own reference intervals based upon its patient population. The reference intervals measured at 37c listed below were taken from literature.

Gender	Conventional units	SI.units
Male	≤ 45 U/L	≤ 0.75 μkat/L
Female	≤ 34 U/L	≤ 0.57 μkat/L

Appendix number (5) : Questionnaire:

بسم الله الرحمن الرحيم

جامعة شندي كلية الدراسات العليا

Questionnaire about prevalence of HBV and HCV among health worker in ElmakNimirHospital .

[1] Serial NO:----------

[2]Age :----------

[3] Sex : Male [ ] Female [ ]

[4] Nationality: ----------

[5] Education state : [

[a] Graduate [ ][b]ungraduate[ ]

[6]Marital state: Married [ ] Single [ ]

[7] Occupational department: ----------

[8]Occupation: ----------

[9] Occupation period: ----------

[10] Vaccination : yes [ ] NO [ ]

[11] Past history of jaundice: yes [ ] NO [ ]

[12] Past history of blood transfusion: yes [ ] NO [ ]

[13] Past history of injury:

[a] sharp tools : yes[ ] No [ ]

[b]needle : yes[ ] No [ ]

[c] Others : yes[ ] No [ ]

[14]Past history of operation:

[a] surgical : yes[ ] No [ ]

[b] In dentist clinical: yes[ ] No [ ]

[c] Others : yes[ ] No [ ]

Appendix number (6)

Consent:

بسم الله الرحمن الرحيم

Consent

نحن الموقعون أدناه نوافق في الدخول لدراسة مسح فيروس الكبدالوبائي ب و ج في مستشفي المك نمر الجامعي