بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Prevalence of Hepatitis C Virus among Prisoners in Khartoum State

معدل إنتشار فيروس إلتهاب الكبد (ج) لدى المساجين بولاية الخرطوم

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

BY

Hagar Abdalmhmoud Abdalla Ibrahim

BSc. in Medical Laboratory Science, Sudan University of Science and Technology (2014)

Supervisor

Prof. Humodi Ahmed Saeed

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بسم الله الرحمن الرحيم

الآية

قال تعالى:

(هُوَ الَّذِيَ أَنزَلَ السَّكِينَةَ فِي قُلُوبِ الْمُؤْمِنِينَ لِيَزْدَادُوَاْ إِيمَاناً مَّعَ إِيمَانِهِمْ

وَلِلَّهِ جُنُودُ السَّمَاوَاتِ وَالأَرْضِ وَكَانَ اللَّهُ عَلِيماً حَكِيماً)

صدق الله العظيم

سورة الفتح الآية (4)

Dedication

To my beloved parents,

My wonderful brothers, sisters

and my supporting teachers

Acknowledgement

Firstly, thank my god for supporting my strength and will to achieve this study.

My great sincere thanks to my supervisor prof. Humodi A. Saeed for his constructive guidance and support.

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ABSTRACT

The objective of this study to determine the prevalence of Hepatitis C virus (HCV) among prisoners in Khartoum state. This study was achieved during the period between February and May 2017.

Total of ninety (90) blood samples were collected randomly from females inmates in Khartoum prison. 5 ml of the blood were collected and centrifugated to obtain the plasma. The Plasma was tested for anti-HCV via Enzyme Linked Immuno-sorbent Assay (ELISA).

result revealed that 2 (2.22%) out of 90 of the blood samples were positive for anti-HCV test and 88 (97.78%) plasma samples were negative.

The frequency of infection was (3.2%) in prisoner with age between (31- 45) and (6.2%) in prisoners with age between (46-60). Out of total samples 54 were married and 2 were infected 3.7% and 36 prisoner were single and with no HCV infection 0 %. Out of 90 investigated blood sample 47 prisoners were Muslims, and 43 were christians and two (4.6%) christians were anti-HCV positive. Both infected prisoners (2.8%) had spent 1-6 month in the prison.

Study concludes that married inmates are more infected than single, All those whom positive to HCV-serological test were had previous infected with hepatitis. And probably they acquired the infection before being in the prison.

For more accurate description of the risk factors of HCV infection the study should be done in large number of prisoner, all prisoners should be instructed to the right behavior and start the treatment for infected prisoners and screening test should take place in the prison from time to time.

المستخلص

تهدف هذه الدراسة الى تحديد مدى انتشار فيروس التهاب الكبد الوبائي سي بين السجناء في ولاية الخرطوم. وقد تحققت هذه الدراسة خلال الفترة ما بين فبراير و مايو 2017.

تم جمع 90 عينة دم عشوائيا من السجناء النساء في سجن الخرطوم. تم جمع 5 مل من الدم وطردها للحصول على البلازما. تم اختبار البلازما لمضاد التهاب الكبد الوبائي سي عن طريق مقايسة الممتز المناعي المرتبط بالإنزيم (= إليزا) .

وكشفت النتائج أن 2 (2.22٪) من أصل 90 من عينات الدم كانت إيجابية للاختبار المضادة لالتهاب الكبد الوبائي سي و 88 (97.78٪) من عينات البلازما كانت سلبية.

وكان معدل العدوى (3.2٪) في السجناء الذين تتراوح أعمارهم بين (31-45) و (6.2٪) في السجناء الذين تتراوح أعمارهم بين (46-60). من مجموع العينة 54 كانوا متزوجين و 2 أصيبوا 3.7٪ و 36 سجين كانوا عازبين و بدون عدوى بفيروس التهاب الكبد الوبائي 0٪.

من أصل 90 عينة دم كان 47 سجينا مسلمين، و 43 كانوا من المسيحيين واثنان (4.6٪) من المسيحيين كانوا مصابين بفيروس التهاب الكبد الوبائي سي. وقد قضى كل من السجناء المصابين (2.8٪) فتره ما بين 1-6 أشهر في السجن.

وخلصت الدراسة إلى أن السجناء المتزوجين أكثر إصابة من غيرهم، وكان جميع الذين اعطو نتيجه إيجابيه للاختبار المصلي لديهم عدوى سابقة بالتهاب الكبد الوبائي. وغالبا انهم قد حصلوا على العدوى قبل أن يدخلو السجن.

للحصول على وصف أكثر دقة لعوامل الخطر للاصابه بفيروس التهاب الكبد الوبائي سي يجب أن تتم الدراسة في عدد اكبر من السجناء، ينبغي توجيه جميع السجناء إلى السلوك السليم, بدء العلاج للسجناء المصابين و الفحص المسحي ينبغي أن يجري في السجن من وقت لآخر زمن.

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INTRODUCTION AND OBJECTIVES

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

Hepatitis B Virus and Hepatitis C Virus are the most common types of viruses that cause viral hepatitis which is an inflammation of liver (Birku *et al*., 2015)

In united state of America (USA) and worldwide Hepatitis C virus (HCV) is the most common blood borne infection (Chak *et al*., 2011).

Infection acquired mainly by blood and sharing of intravenous paraphernalia during illicit drug use (Granato *et al*., 2001; Weild *et al*., 2000; Amiri *et al*., 2007) and intranasal drug use (Amiri *et al*., 2007).

Hepatitis C virus infection progresses to chronic HCV in around 75% of cases (Dore *et al*., 2014).

Hepatitis C virus is major cause of liver failure and end stage liver disease (Amiri *et al*.,2007; Mudawi, 2008), hepatic fibrosis, cirrhosis worldwide About 27% of cirrhosis was attributed to HCV and hepatocellular carcinoma (HCC) and is the prime cause of liver grafting worldwide (Chak *et al*., 2011).

The epidemiology of HCV infection is not well defined in the Middle East area and the propagation of anti-HCV is 2.2–3% in Sudan (Fallahian and Najafi, 2011)

Seroprevalence range from 2.2% _ 4.8% in Gazira State, and 4.4% in asymptomatic blood donors and 23.7% in end stage renal disease (Mudawi, 2008).

In many western countries prisoners have a higher prevalence of antibodies (HCV) viruses than the general population (Weild *et al*., 2000).

Also martial personnel high risk people for antenatal and sexually transmitted disease such as HCV (Birku *et al*., 2015).

The relation between incarceration and the high transmission of HCV, in prisons has been known for several years, and injecting drug use is the most commonly reported risk factor (Andrew *et al*., 2006).

Survey to study the risk factors for transmission of blood borne viruses in prisons by measuring the anti-HCV antibodies and the frequency of risk behaviors among the prisoners (Weild *et al*., 2000).

Rationale

High propagation rates have been described among specific groups considered to be at higher risk for HCV infection, including prison inmates (Granato *et al*., 2001)

There is increasing guides that HCV infections have actually been transmitted to individuals while they were in prison, even though there is also evidence that some had the infection before they were sent to prison. (Andrew *et al*., 2006).

Few studies have been released analyzing Hepatitis c virus propagation in slammer. Some of these studies detect that HCV is a considerable problem in prisons (Granato *et al*., 2001)

Hepatitis C virus sero-prevalence was 19.2% in Ghana prisoner (Andrew *et al*., 2006), and was 39.8% in inmates in a Canadian federal penitentiary for women (Ford *et al*., 1995), 41% were positive for anti-HCV in Brazilian prisoner, while in Norway and Australia it was 57% and 54%, respectively, among prisoners who reported intravenous drug use (Granato *et al*., 2001).

Objectives

1. General objective

To investigate Hepatitis C Virus (HCV) among prisoners in Khartoum State.

2. Specific objectives

1. To detect Hepatitis C Virus among prisoners.
2. To determine the frequency of Hepatitis C Virus (HCV) in Khartoum Female Prison.
3. To determine prevalence of infection and the age risk group
4. To detect risk factor as sharing of intravenous paraphernalia, razor and blood transfusion

CHAPTER TWO

LITERATURE REVIEW

CHAPTER TWO
LITERATURE REVIEW

2.1. Hepatitis

Hepatitis is an inflammatory condition of the liver; it can be due to alcohol, medications, other toxic chemicals (Taylor *et al*., 2011; Franciscus, 2016) and autoimmune disorders (Taylor *et al*., 2011).

also can be caused by viral infection this called viral hepatitis (Birku *et al*., 2015) which caused by one of the five hepatitis viruses, referred to as types A, B, C, D and E (WHO, 2012).

Hepatitis A is form of hepatitis with the shorter incubation period, originally termed catarrhal or infectious hepatitis (Collier and Oxford, 2006), transmitted by the fecal-oral route (WHO, 2012).

Vaccine has been licensed in the United States since 1995 and no chronic infection develops after hepatitis A (CDC, 2005), HAV proved to be an RNA virus, a member of the Picornaviridae (Collier and Oxford, 2006).

Hepatitis B acute and chronic infections are a major cause of morbidity and mortality in the United States (CDC, 2005), HBV was the more dangerous and tended to become chronic, sometimes giving rise to cirrhosis and liver cancer (Collier and Oxford, 2006).

It’s a partially double-stranded DNA virus belongs to hepadnaviridae family (Colvin and Mitchell, 2010).

Transmitted through exposure to infectious blood, semen, and other body fluids (WHO , 2012), and from mother to infants during or after birth (Collier and Oxford, 2006).

Delta virus, or HDV, it's a novel feature of HBV associated agent (Collier and Oxford, 2006).

Prior or concurrent HBV infection necessary, can cause worsening of hepatitis B can cross the placenta (Nester *et al*.,2009) termed a satellite, as it depends on HBV to provide its envelope protein (WHO, 2012).

Delta agent is thus an incomplete virus, reminiscent of the Dependoviruses (CDC, 2005).

Hepatitis E caused by HEV Non-enveloped, single-stranded RNA calcivirus ,spread via enteric rout Similar to hepatitis A, except severe disease in pregnant women, and no vaccine (Nester *et al*.,2009).

Hepatitis C caused by a virus like HBV, is transmitted by exposure to blood and body fluids. (Collier and Oxford, 2006)Hepatitis C virus (HCV) is a primarily hepatotropic virus but hepatocytes are not the only localization of its replication (Inglot *et al*., 2013), HCV is an RNA single stranded, enveloped flavivirus (Cheesbrough, 2006).

2.1. Liver

Is the largest internal organ, located behind the ribcage on the right side of the abdomen and occupies upper part of abdomen (Franciscus, 2016; Snell, 2003).

liver perform many important function like bile secretion, synthesize heparin (Snell, 2003) neutralize and remove the toxin from the body, Controlling the rate of production and elimination of fat, cholesterol, hormones, Storing sugars, vitamins ,minerals, fighting infection and removing bacteria from the blood stream (Taylor *et al*., 2011).

Human liver composed of small hexagonal structures term as liver lobule. Change in liver by HCV may lead to cirrhosis gradually which is an irreversible replacement of liver lobule with permanent type of non-functional connective tissue (regenerative nodule) (Yamaguchi and Hachiya, 2015).

2.3. Hepatitis C

2.3.1. Clinical features

HCV is the infectious agent in the majority of non-A, non-B hepatitis (Ruiz *et al*., 1999).

Hepatitis C virus can cause swelling and fibrosis (scarring) of the liver (Taylor *et al*., 2011).

The incubation period is about 6 to 7 weeks About 65% of infected individuals have no symptoms relating to the acute infection, whereas only about 25% have jaundice (Nester *et al*., 2009, Cheesbrough, 2006).

Only 10–20 percent or so of those infected have symptoms like anorexia, nausea and increasing Alanine aminotransferase (Collier and Oxford, 2006; Nester *et al*., 2009) and frank jaundice is uncommon (Collier and Oxford, 2006).

2.3.2. The infection

After exposure to HCV, the window period usually lasts 2–26 weeks. The initial phase of hepatitis C is called acute infection (Franciscus, 2016).

However, up to 80 percent become chronically infected (Strauss and Strauss, 2008, Franciscus, 2016; Abreha *et al*., 2011), During chronic infection, up to 1012 viruses are produced each day and turn over with a half-life of about 3 hours, and the more or less constant viral load in the blood is 103–107 per ml (Strauss and Strauss, 2008).

WHO estimates that worldwide there are about 170 million chronic carriers of HCV at risk of developing liver cirrhosis and liver cancer (Cheesbrough, 2006; Abreha *et al*., 2011).

Chronic hepatitis C eventually leads to the development of hepatocellular carcinoma (HCC) in 0.4–2.5% of infected persons (Abreha *et al*., 2011) a progressive liver damage or cancer about 10% to 20% of chronic carriers develop (Nester *et al*.,2009).

HCV infection was significantly associated with chronic lymphoproliferative disorder (CLD) in unadjusted analyses and with non-hodgkin lymphoma (NHL) in an adjusted analysis (Chaabna *et al*., 2016).

As a Complications minority of HCV infections liver disease is accompanied by glomerulonephritis and various forms of vasculitis, of which some are caused by deposition of immune complexes (Collier and Oxford, 2006).

2.3.3. Transmission and groups at risk

Most cases of hepatitis C are Transmitted by blood from an infected person (Nester *et al*.,2009) in drug users by sharing of contaminated needles, syringes, or other injection drug equipment (Hellard *et al*., 2004).

Tattooing sharing tooth- brushes, razors, and towels can be responsible (Nester *et al*., 2009), and circumcision have also been implicated. Sexual transmission and congenital infections are less important (Collier and Oxford, 2006).

The transmission route for up to 10% of individuals infected with HCV cannot be identified. HCV is not transmitted by casual contact such as sneezing, coughing, hugging, or sharing eating utensils and drinking glasses (Franciscus, 2016).

2.3.4. Pathogenesis and Immune response:

Few details are known about the pathogenesis of hepatitis C, most instances are asymptomatic hepatitis, with persistent viraemia (Collier and Oxford, 2006, Nester *et al*., 2009).

Most people with acute infection, more than 80% develop chronic infections (Nester *et al*., 2009; Cheesbrough, 2006).

About 170 million chronic carriers of HCV at risk of developing liver cirrhosis and liver cancer (Cheesbrough, 2006).

The virus infects the liver and incites inflammatory and immune responses. The disease process in the liver waxes and wanes, then weeks or months later high inflammation is develop. Months or years later, cirrhosis and liver cancer develop in 10% to 20% of patients (Nester *et al*., 2009).

Structurally, it's difficult to distinguish HCV infections from other forms of viral hepatitis, except for the presence of lymphoid follicles within the portal tracts. There is also intense per portal infiltration with lymphocytes, and damage to the lining of the bile ducts. The 'ground glass' is absent. HCC seems to be a direct consequence of the cirrhosis (Collier and Oxford, 2006).

First line of defense against viral infection is the production of type 1 interferon (IFN) α and β, components of the innate immune system (Strauss and Strauss, 2008).

The cell-mediated response is more prominent than humoral immunity, but active proliferation of T-helper and cytotoxic lymphocytes seems insufficient either to clear the infection or to prevent re-infection. There is some evidence that HCV can mutate in vivo, thus escaping immune surveillance (Collier and Oxford, 2006).

2.3.5. Epidemiology

About 150 million people are chronically infected with HCV. More than 350000 people are estimated to die from HCV- related liver diseases each year (WHO, 2012).

WHO estimates 3 percent of the world's population, are infected with HCV. (Collier and Oxford, 2006) .

Infected individuals in the Arab world by such virus reached 25 million with an average prevalence rate of 3.5% (Daw and Dau, 2012).

The highest incidence of acute hepatitis C is found among persons 20-39 years (CDC , 2005).

All countries in the African Region consider viral hepatitis an urgent public health issue. . The prevalence of HCV is even higher in some areas, reaching levels of up to 10% (WHO, 2012).

2.3.6. Properties of hepatitis C virus

HCV is a primarily hepatotropic virus, but also Extrahepatic replication in peripheral blood mononuclear cells such as T-cell, B-cell lines and monocytes/macrophages (Radkowski *et al*., 2005).

Some studies also found replication of HCV in per hepatic lymph nodes, salivary glands, oral epithelial cells, pancreas, adrenal glands, thyroid and brain (Abreha *et al*., 2011).

HCV is a single stranded positive-sense RNA virus, enveloped flavivirus (Abreha *et al*., 2011, Cheesbrough, 2006), genome is 9.6 kb in size, The virions are about 50 nm, The core is about 30nm in diameter and its genus is Hepacivirus (Abreha *et al*., 2011).

The infected cells were reported to contain HCV RNA–negative strand, which is a viral replicative intermediate (Radkowski *et al*., 2005).

The virus receptor is CD81 or the low density lipoprotein receptor of the cell (LDLR), and replication is cytoplasm. The genome acts directly as an mRNA and a single polyprotein is translated and cleaved by both viral and cellular proteases (Collier and Oxford, 2006).

2.3.7. Variants of hepatitis C virus

There are six major genotypes, or clades (phylogenetic variants) with a large number of subtypes Clades are differ from each other by about 20 percent at the nucleotide level (Collier and Oxford, 2006).

Epidemiological difference, age distribution of major types, and the risk factors associated with particular genotypes (Zuckerman *et al*., 2004)

Different studies have shown that HCV genotypes effect on the response to therapy (Abreha *et al*., 2011) for example, genotype 1b is worse in the response to treatment than other genotypes (Collier and Oxford, 2006) and Genotype 1 and 3 are the most prevalent in most parts of the world, Type 2 with its sub-types is known to be one of the dominant types in Asia and Europe, genotype 5 and 6 are common only in South Africa (Abreha *et al*., 2011).

several clinical investigations have documented severe and progressive liver disease after infection with each of the well-characterized genotypes (1a, 1b, 2a, 2b, 3a, 4a, 5 and 6), so there is little evidence so far for variants of HCV that are completely non-pathogenic(Zuckerman *et al*., 2004).

2.3.8. Diagnosis

2.3.8.1. Rapid HCV Antibody Test

A point of care test that collects and processes a sample and gives results after 20 minutes, A finger prick and whole blood draw has been approved and a CLIA waiver rapid test issued by the Food and Drug Administration (FDA) (Franciscus, 2016).

2.3.8.2. The HCV ELISA or EIA

The HCV ELISA or EIA is a simple blood test that can detect HCV antibodies (Amiri *et al*.,2007; Weild *et al*., 2000; Franciscus, 2016).

ELISA positive or intermediate results were further tested using the third- generation HCV EIA (enzyme immunoassay) or the Chiron RIBA HCV 3.0 assay (Ford *et al*., 1995; Mudawi *et al*., 2007).

A positive HCV antibody test means that a person has been infected at one time and so HCV RNA or viral load test must be performed to find out if the person is currently infected with the hepatitis C virus (Franciscus, 2016; Mudawi *et al*., 2007).

Anti-HCV positive by EIA, verified by an additional more specific assay (e.g., RIBA for anti-HCV or nucleic acid testing for HCV RNA) (Colvin and Mitchell, 2010).

immunoenzymatic assay test uses synthetic peptides for the core, NS3, NS4, and NS5 regions. The samples with undetermined results were submitted to an immunoblot assay (Granato *et al*., 2001).

2.3.8.3. HCV RNA or Viral Load Tests

The HCV RNA test is used to confirm positive HCV serological tests and to detect the presence of HCV RNA in the blood. The positive result indicates active replication of the virus in the liver and possible liver damage (Corey *et al*., 2004).

Viral load tests measure the amount of HCV circulating in the blood. There are three different types of viral load test: HCV RNA PCR, branched-chain DNA (bDNA), and transcription mediated amplification, or TMA (Franciscus, 2016).

Tests for genome by quantitative RNA PCR provide valuable confirmatory evidence of infection and allow sensitivity to 100 RNA genome copies/ml (Collier and Oxford, 2006).

The HCV assay is now performed by the RT-PCR method and sensitivity for the assay is <60 International Units/ mL. The RT-PCR assay is also linear from 60 to 1 x 108 International Units/mL (Corey *et al*., 2004).

2.3.8.4. Genotype Tests

Genotype test are used to determine what type (‘strain’) of HCV you have (Franciscus, 2016).

The test should be done prior to the initiation of anti-viral therapy when the virus. Numerous studies have documented differences in response to therapy based on the HCV genotype present. HCV genotype 1infections are less responsive to therapy than other genotype require 12 months therapy to obtain a significant response. However, with

genotypes 2 or 3, optimal therapeutic responses usually require only 6 months of combination therapy (Corey *et al*., 2004).

2.3.8.5. Liver Function Tests

Most persons with chronic HBV or HCV infection are asymptomatic. Thus, testing programs for persons with risk factors for liver enzymes alanine aminotransferase and aspartate aminotransferase (ALT, AST) (CDC, 2005).

ALT and AST are released into the blood when the liver is damaged. They are often elevated in people with HCV infection. Other measurements include alkaline phosphatase (ALP), gamma- glutamyl transpeptidase (GGT), prothrombin time and bilirubin levels (Franciscus, 2016).

2.3.8.6. Liver Biopsy and fibroscan

Biopsies are done to measure the severity of inflammation, and the general health of the liver.

The Fibroscan is a diagnostic tool that is used to evaluate liver health. Fibroscan based on a technology using a machine that sends a vibration wave through the liver to detect and analyze any fibrosis.(Franciscus, 2016).

2.3.9. Treatment

The current standard of care (SOC) includes administration of pegylated interferons (IFN) and combination therapy with ribavirin improved considerably the virologic response rates (Inglot *et al*., 2013).

Treatment for acute hepatitis C is best initiated early; a commonly used dosage is 6mU of IFN-α three times weekly for 16–24 weeks. Liver transplantation has proved of short or medium term benefit in some cases of cirrhosis or HPC, but re-infection of the graft probably always occurs (Collier and Oxford, 2006).

Sustained virological response (SVR) is assessed 24 weeks after finishing treatment and is defined as negative HCV RNA in the serum, The presence of HCV RNA negative strand in PBMCs at the end of treatment as a factor determining higher risk of viral relapse has been confirmed in some studies (Inglot *et al*., 2013).

Treatment is not only expensive but relatively toxic and many patients tolerate it poorly (Strauss and Strauss, 2008).

2.3.10. Immunization and protection

No vaccine is available for preventing hepatitis C although new methods for producing viral proteins have raised hopes for vaccine development (Nester *et al*.,2009).

Because there is no effective vaccine or post-exposure prophylaxis, reducing HCV burden infection and HCV-related disease requires implementation of primary prevention activities to reduce the risk of contracting the infection and secondary prevention activities to reduce the risk of liver disease (CDC, 2005).

The detection of HCV by screening of blood donors and blood products has greatly diminished the chances of infection from these sources; thus control of transmission between intravenous drug abusers remains the most important target for public health measures (Collier and Oxford, 2006) and Avoidance of alcoholic beverages is recommended because of the toxic effect of alcohol on the already damaged liver (Nester *et al*., 2009)

Do not share needles or any other drug paraphernalia, razors, toothbrushes, clippers, nail files, or any items that may come in contact with blood (Franciscus, 2016)

2.3.11. Previous studies

In Sudan previous studies the prevalence of anti-HCV is 2.2–3% (Fallahian and Najafi, 2011; Mudawi *et al*., 2007).

Sudan In high-risk populations, most studies reported high HCV prevalence in the range of 4.5%–34.9%, High HCV prevalence was reported in clinical populations including HD patients (between 8.5% and 34.9%) and haemophilia patients (13%) (Fallahian and najafi, 2011; Chaabna *et al*., 2016).

The lowest prevalence was observed in a study among blood donors at 0% and in a study among pregnant women at 0.6% (Chaabna *et al*., 2016).

Previous studies of HCV epidemiology in Sudan showed different prevalence, from as high as 23% to as low as 0.6% for HCV (El-Amin *et al*., 2007; Elsheikh *et al*., 2007; McCarthy *et al*., 1994).

However, most of the reported prevalence in Khartoum State was among pregnant women, haemodialysis or patients with liver diseases (Osman *et al*., 2012, Chaabna *et al*., 2016).

Most descriptions of HCV epidemiology relay mainly upon HCV sero-prevalence studies. These studies are typically cross-sectional in design and are done in selected populations (Osman *et al*., 2012).

Although the high prevalence of blood-borne viral infections in correctional facilities has been well documented globally, such data are sparse from Africa, and there has been no such data from Sudan, one of documented studies in Ghana prisons in which HCV seroprevalence in Ghana prisons was 19.2% (Weild *et al*., 2000).

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

3.1.1. Type the study

A descriptive cross-sectional study conducted to detect hepatitis C virual infection among female prisoners in Khartoum state.

3.1.2. Study area

The study was conducted at Omdurman Female Prison (Dar EL-taipat) in Khartoum State, the samples collected in prison, processed and examined in Sudan University of Science and Technology.

3.1.3. Study duration

The study was run through January 2017 to April.

3.1.4. Study population

Female intimate in Khartoum State examined for HCV

3.1.5. Sample size

Ninety prisoner (n= 90) were voluntary enrolled in this study and 5 ml of blood sample were collected.

3.2. Ethical consideration

Ethical approval was obtained from the College Ethical Committees of Sudan University of Science and Technology.

3.3. Data collection

Socio-characteristics and associated risk factors for HCV infections were collected using structured questionnaire, The study variables included in this study were age, marital status, history of hospitalization, blood transfusion, surgical procedure, family history of liver disease, sharing of razor or nail cutter needles or other sharp object.

3.4. Sample collection

Five milliliters of venous blood was collected from each study participant by trained laboratory technologist, venipuncture technique was used for collection, skin was cleaned with 70%(v/v) ethanol, sterile 5 milliliters syringe was used for blood collection, the blood was dispended in sterile EDTA vacationer container finally the plasma was separated by centrifugation.

3.5. Laboratory analyzing

3.5.1. Preparation of specimens

All collected blood samples were centrifuged at 3000 rounds per minute for 5 minutes to obtain the plasma, and then plasma was transfered into another sterile tube and store at -20˚C until the serological examination took place.

3.5.2. Sample analysis

Plasma were screened by standard enzyme-linked immune-sorbent assay (ELISA) techniques with commercial kits from fortress diagnostic company in United Kingdom anti-HCV fourth generation the assay for qualitative detection of antibodies to HCV in human serum or plasma , the serological test performed following the manufacturer instruction and control positive and negative included in the assay.

According to the information included in the kits insert, the specifity of this anti-HCV ELISA kit was 99.55%, the sensitivity was 99.79% and no cross reaction with sample from patients infected with HAV, HBV, HIV and CMV.

3.5.3. Principle of the assay

This kit employs solid phase, indirect ELISA 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 non-structural regions of HCV (4^{th} 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 patient, and rabbit anti-human IgG antibodies (Anti-IgG) conjugated to horseradish peroxidase (HRP-conjugate) is added. During the second incubation step, these HRP-conjugate antibodies will be bound to any antigen-antibody (IgG) complexes previously formed and the unbound HRP-conjugate is then removed by washing.

Chromogen solution containing tetramethylbenzidine (TMB) and urea peroxide are added to the wells and in presence of the antigen- antibodies-anti-IgG (HRP) immunocoplex; the colorless Chromogen was hydrolyzed by the bound HRP conjugate to a blue-color product. The blue color turns yellow after stopping the reaction with sulphuric acid. The amount color intensity can be measured and is proportional to the amount of antibody captured in the wells and to the sample reactivity. Wells contain sample negative for anti-HCV remain colorless.

3.5.4. Procedure

All reagents and samples allowed to reach the room temperature (18-30˚C), and the stock buffer was diluted 1 to 20 with distilled water, 100 µl of specimen diluents were added to each well except the blank, then 10µl of positive control (two positive control E1, F1),

negative control (three negative control B1, C1, D1) and samples were added into their respective wells.

Plate was covered and incubated for 30 minutes at 37˚C, after the incubation the plate cover was removed and each well was washed 5 times with diluted buffer. After washing 100µl of HRP-conjugate was added to each well except the blank, the plate covered and incubated at 37˚C for 30 minutes.

After the incubation the cover was removed and each well was washed 5 times with diluted wash buffer, then 50µl of Chromogen A and 50µl Chromogen B solutions were added to each well including the blank and incubated for 15 minutes at 37˚C. After incubation 50µl of stop solution was added to each well, intensive yellow color was developed in positive control and anti-HCV positive samples. Absorbance read within 5 minutes in 450nm.

3.5.5. Quality control and calculation

Standards, reagents and control were checked for storage, stability and preparation before starting the assay, each micro plate were considered separately when calculating and interpreting results of the assay.

The test results were validated by verified the quality control criteria, the OD value of the blank was less than 0.080, the OD value of the positive controls were greater than 0.800 , and the OD of the negative controls were less than 0.100.

The results were calculated by relating each samples optical density (OD) to the cut off value (C.O) of the plate, the blank well OD was not subtracted from the printed report values of samples and controls.

Calculation of cut- off value (C.O) =* Nc + 0.12

* Nc = the mean absorbance value for the three negative controls

CHAPTER FOUR

RESULTS

CHAPTER FOUR

THE RESULTS

During the period between February and April 2017, total of 90 samples were collected from female prisoners in Sudan out of 90 there is two prisoners were hepatitis c virus positive 2.2% and 87 (97.8%) prisoner were negative (figure 4.1) the mean age of infected prisoners were 50 years and the mean age of non-infected personnel were 33.9 (table 4.1).

The incidence of infection among the different age group was (3.2%) in prisoner with age between 31- 45, (6.2%) in prisoner with age between (46-60), other group show (0.0%) or no infection, and there is no relation between the age group and HCV infection (Table 4.2) and (figure 4.2).

Out of 90 investigated blood sample 47 prisoners were Muslims, and 43 were Christians and 2 (4.6%) Christians were HCV positive but there is no relation between the infection and religion (table 4.3).

The relationship between HCV infection and other factor had been studied by using administrated self questionnaire data, first relation between HCV infection and work and previous infection and HCV infection and both show no relation to the infection, both patients have previous hepatitis infection (5.8%), and one prisoner work (2.5%) (Table 4.4)

Infected personal show no history of blood transfusion or surgery and there is no significant relation between the infection and blood transfusion or surgery (table 4.5), also the study show no relation between hemodialysis and sharing sharp object (razor, nail clump ….etc) (table 4.6)

Out of total sample 54 were married and 2 were infected 3.7% and 36 prisoner were single and with no HCV infection 0 %, and there is no relation between marital status and infection (table 4.7).

70 prisoner were recently send to prison spend only 1-6 month, and 2 were infected (2.8%), 10 personel spend 7-12 month in prison and 10 spend over one year in prison and both group show no infection (0%) (Table 4.8)

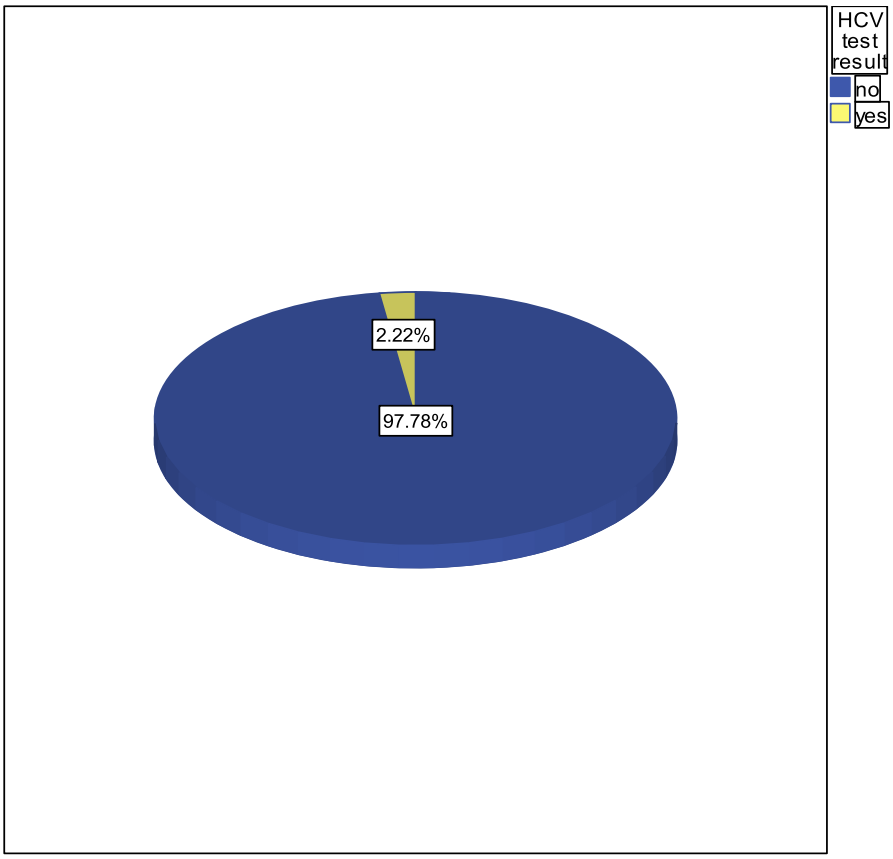

Figure 4.1: Incidence of Hepatitis C Virus among prisoners in Khartoum state.

Table 4.1: Frequency of HCV test result and age mean of infected prisoner

		Frequency	Percent	Age Mean
Valid	no	88	97.8	33.94
	yes	2	2.2	50.00
	Total	90	100.0	34.30

Table4.2. Frequency of HCV infection in different age group and the correlation between the age and the infection

Age- groups	Frequency of infection	Percent of infection		Asymptotic Significance
15-30	0/41	0%	Pearson Chi-Square	.510
31-45	1/31	3.2%		.
46-60	1/16	6.2%		
61-75	0/ 2	0%		

P value: 0.510

There is no relation between the age and the infection

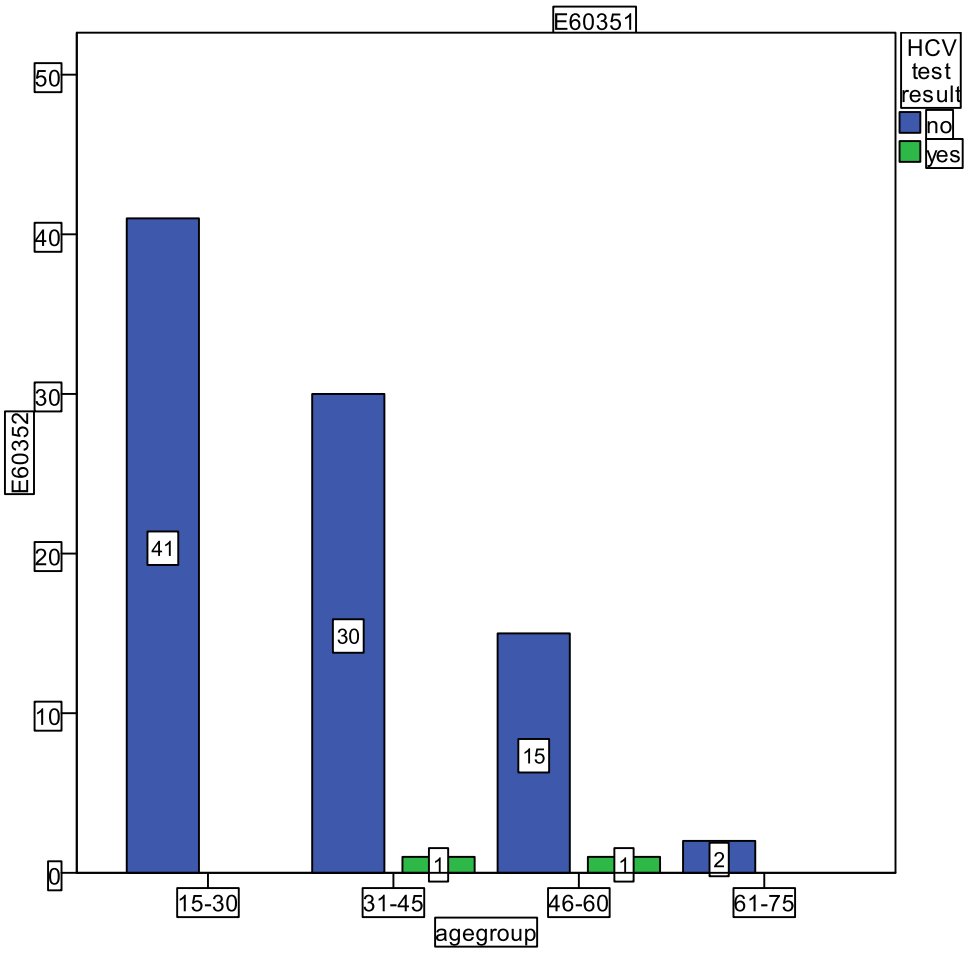

Figure 4.2: HCV infection in different age group

Table 4.3: Frequency of HCV infection and correlation to the religion

		religion			Asymptotic Significance
		Muslim	Christian		
HCV test result	no	47	41	Pearson	.135
	yes	0	2	Chi-Square	
Percentage		0%	4.6%		

P value: 0.135

There is no relation between the religion and HCV infection

Table 4.4: correlation between HCV infection and previous infection and work

		work		previous infection with hepatitis			Work Asymptotic Significance	Previous inf. Asymptotic significance
		yes	no	Yes	no			
HCV test result	no	39	49	32	56	Pearson Chi-Square	.873	.066
	yes	1	1	2	0			
Percentage		2.5%	2%	5.8%	0%			

P value > 0.05

There is no correlation between the work, previous infection and HCV infection

Table 4.5: Cross tabulation of HCV test result and blood transfusion and surgery.

		blood transfusion		had a surgery			Blood trans. Asymptotic Significance	Surgery Asymptotic Significance
		yes	no	yes	no			
HCV test result	no	10	78	16	72	Pearson Chi-Square	.613	.506
	yes	0	2	0	2			
Total		10	80	16	74			

P value > 0.05

There is no correlation between blood transfusion, surgery and HCV infection

Table 4.6: correlation between HCV result and hemodialysis and sharp object sharing

		hemodialysis		Share sharp object			Hemodialysis Asymptotic Significance	Sharing object Asymptotic Significance
		yes	no	yes	no			
HCV test result	no	3	85	23	65	Pearson Chi-Square	.791	.402
	yes	0	2	0	2			
Total		3	87	23	67			

P values > 0.05

No correlation between HCV infection and both hemodialysis and sharp object sharing

Table 4.7: frequency and correlation between HCV infection among married and single prisoner and the correlation

		marital status			Asymptotic Significance
		married	single		
HCV test result	no	52	36	Pearson Chi-Square	.243
	yes	2	0		
Percentage p		3.7%	0.0%		

P value: 0.243

No correlation between the infection and marital status

Table 4.8: **correlation between HCV infection and time in prison, and the incidence of the infection**

		time in prisons				Asymptotic Significance
		1-6 month	7-12 month	over an year		
HCV test result	no	68	10	10	Pearson	.747
	yes	2	0	0	Chi-Square	
Percentage		2.8%	0.0%	0.0%		

P value: 0.747

No correlation between the infection and time in prison

CHAPTER FIVE

DISCUSSION

CHAPTER FIVE

DISCUSSION

The main objective of this study was to find out the incidence of Hepatitis C viral infection among the prisoners.

90 samples were collected, prepared and adopted different standardized tool and method for realization of the problem through serological examination and data collection.

This study revealed that 2.2% of the prisoner were infected and disagreed with (Weild *et al*; 2000 and Kazi *et al*., 2010) whom found HCV seroprevalence was 7% and 15.1% in Pakistan respectively, but Agree with (Taylor *et al*., 2013 and Fallhian and Najafi; 2011) whom reported 3% are sero-positive for HCV among people with no injecting history in Scotlanda and 2.2–3% in Sudan respectively

This study showed that Hepatitis C Viral Infection is common among Married inmate rather than single prisoner; similar to what reported by (Amiri *et al*, 2007) who show that 63.4% of prisoner were married.

Time in the prisons rival that most infected personals spend between 1-6 months disagreed with (Amiri *et al*; 2007) who found HCV infection is more common in duration 2-5 years in prison.

History with blood transfusion and infection with HCV were 0% that disagreed with the report of (Amiri *et al*; 2007) in which there is about 2.2% infected personal with a history of blood transfusion, and also no association between the hemodialysis and the infection disagree with (Daw and Dau, 2012).

The percentage of HCV infection among Muslims is 0%, and 4.6% in Christians

Conclusion

1. Married inmate more infected than single, and the most infected age groups are 13-45 an 46-60 years.
2. All those whom positive to HCV-serological test were previously infected with hepatitis.
3. Sharing sharp objects, hemodialysis, surgery and blood transfusion are risk factors to acquire the infection although in this study there is no relation between those factors and HCV infection.
4. 1-6 months is the time which all infected personal spend in prison and probably they acquired the infection before being in prison.

Recommendations

1. For more accurate description of the risk factor of HCV infection the study should be done in large number of prisoners.
2. All prisoners with HCV should start the treatment and instructed to the right behavior to control the infection inside the prison.
3. This study should take place in male prisoner also.
4. The health care facilities in the prison should do screening test from time to time in order to control the HCV infection
5. Prisoner should tested for anti-HCV before enter slammer to prevent infection spreading to other prisoner

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APPENDICES

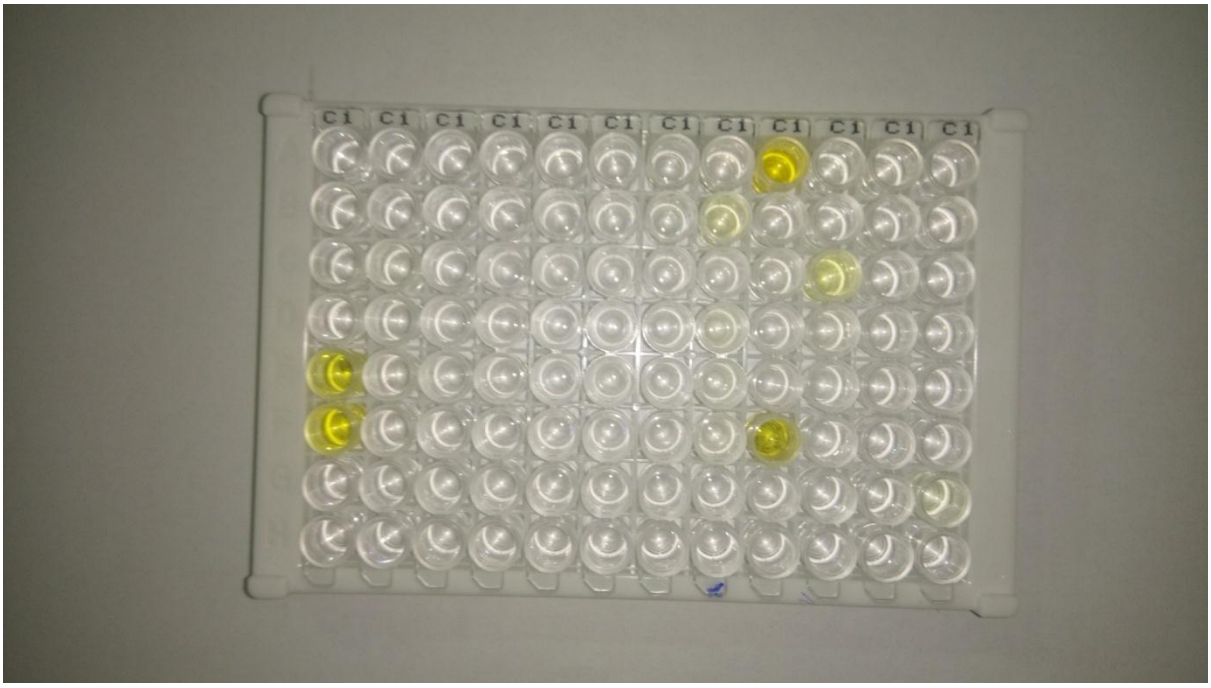

Figure: Characteristic of samples that show a positive reaction on ELISA micro wells

Questionnaire

Prevalence of Hepatitis C Virus infection among Prisoners in Khartoum State

- Name:……..
- Age : ……...
- Sex : ……....
- Tribe : …….
- Education: ……..…..
- Occupation : ……..……
- Serial No.:……
- Marital status:……..
- Date of arrested: …….
- Any Symptoms: ……..
- Needle sticks, Sharing Razor and razor plade and shaving machine

1) Yes 2) No

- History of blood transfusion and Surgery:

1) Yes 2) No

- Family History with hepatitis

1) Yes 2) No

- Self injection with I.V drugs:

1) Yes 2) No

- Haemodialysis :

1) Yes 2) No

- History of jaundice :

1) Yes 2) No

fortress diagnostics

ISO 13485 accredited company

BXE0781C

480 TESTS

STORE AT 2-8°C

FOR IN-VIRTO DIAGNOSTICS USE ONLY

ANTI-HCV (4th Generation)

ELISA

Intended Use:
Fortress HCV kit is an enzyme-linked immunosorbent assay for qualitative detection of antibodies to hepatitis C virus in human serum or plasma. It is intended for screening blood donors and diagnosing patients related to infection with hepatitis C virus.

Summary:
Hepatitis C virus (HCV) is an envelope, single stranded positive sense RNA (9.5 kb) virus belonging to the family of Flaviviridae. Six major genotypes and series of subtypes of HCV have been identified. Isolated in 1989, HCV is now recognized as the major cause for transfusion associated non-A, non-B hepatitis. The disease is characterized with acute and chronic form although more than 50% of the infected individuals develop severe, life threatening chronic hepatitis with liver cirrhosis and hepatocellular carcinomas. Since the introduction in 1990 of anti-HCV screening of blood donations, the incidence of this infection in transfusion recipients has been significantly reduced. The first generation of HCV ELISAs showed limited sensitivity and specificity and was produced using recombinant proteins complementary to the NS4 (c100-3) region of the HCV genome as antigens. Earlier generation tests, which included recombinant / synthetic antigens from the Core (c22) and nonstructural regions NS3 (c33c, c100-3) and NS4 (c100-3, c200) resulted in a remarked improvement in sensitivity and specificity. Clinical studies show that significant amount of HCV infected individuals develop antibodies to NS5 non-structural protein of the virus. For this, the forth generation tests include antigens from the NS5 region of the viral genome in addition to NS3 (c200), NS4 (c200) and the Core (c22). Forth generation tests have improved sensitivity and shorten the time between infection with HCV and the appearance of detectable antibodies.

Principle:
This kit employs solid phase, indirect ELISA 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 non-structural regions of HCV (Fourth 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 horseradish peroxidase (HRP-Conjugate) are added. During the second incubation step, these HRP-conjugated antibodies will be bound to any antigen-antibody(IgG) complexes previously formed and the unbound HRP-conjugate is then removed by washing. Chromogen solutions containing Tetramethylbenzidine (TMB) and urea peroxide are added to the wells and in presence of the antigen-antibody-anti-IgG (HRP) immunocomplex, the colourless Chromogens are hydrolyzed by the bound HRP conjugate to a blue-coloured product. The blue colour turns yellow after stopping the reaction with sulfuric acid. The amount of colour intensity can be measured and is proportional to the amount of antibody captured in the wells, and to the sample respectively. Wells containing samples negative for anti-HCV remain colourless.

Assay principle scheme: Indirect ELISA
Ag(p)+Ab(s)→[Ag(p)–Ab(s)+ENZ] → [Ag(p)–Ab(s)–ENZ] →blue→yellow (+)
Ag(p)+ → [Ag(p) +ENZ] → [Ag(p)]→ no colour (-)
Incubation1 Incubation 2 Immobilized Complex Colouring results
30min. 30min. 15min
Ag(p)–pre-coated HCV antigens(core, NS3/4,NS5);
Ab(s)–HCV antibodies in sample (IgG);
ENZ–HRP conjugated rabbit anti-human IgG;

Kit Contents:
MICROWELL PLATE 480 Tests: 5 plates
Blank microwell strips fixed on white strip holder. The plate is sealed in aluminium pouch with desiccant.
8×12/12×8-well strips per plate.
Each well contains recombinant HCV antigens. The microwell strips can be broken to be used separately.
Place unused wells or strips in the plastic sealable storage bag together with the desiccant and return to 2~8°C.

- **NEGATIVE CONTROL** 5 vial

Blue-coloured liquid filled in a vial with white screw cap.
1ml per vial.
Protein-stabilized buffer tested non-reactive for HCV antibodies. Preservatives: 0.1% ProClin 300.
Ready to use as supplied.
Once open, stable for one month at 2-8°C.

- **POSITIVE CONTROL SERUM** 5 vial

Red-coloured liquid filled in a vial with white screw cap.
1ml per vial.
anti-HCV antibodies diluted in protein-stabilized buffer
Preservatives: 0.1% ProClin 300.
Ready to use as supplied.
Once open, stable for one month at 2-8°C.

- **SPECIMEN DILUENT** 1 bottle

Blue liquid filled in a white bottle with white screw cap.
65ml per bottle.
Protein-stabilized buffer, casein, and sucrose solution.
Ready to use as supplied.
Once open, stable for one month at 2-8°C.

- **HRP-CONJUGATE REAGENT** 1 bottle

Red-coloured liquid filled in a white vial with white screw cap.
65ml per bottle.
Horseradish peroxidase-conjugated rabbit anti-human IgG antibodies.
Ready to use as supplied.
Once open, stable for one month at 2-8°C.

- **STOCK WASH BUFFER** 5 bottles

DILUTE BEFORE USE
Colourless liquid filled in a white bottle with white screw cap.
50 ml per bottle.
PH 7.4, 20 × PBS (Contains Tween-20 as a detergent)
The concentrate must be diluted **1 to 20** with distilled/deionized water before use. Once diluted, stable for one week at room temperature, or for two weeks at 2-8°C.

- **CHROMOGEN SOLUTION A** 5 bottle

Colourless liquid filled in a white vial with green screw cap.
7ml per bottle.
Urea peroxide solution.
Ready to use as supplied.
Once open, stable for one month at 2-8°C.

- **CHROMOGEN SOLUTION B** 5 bottle

Colourless liquid filled in a black vial with black screw cap.
7ml per bottle.
TMB solution(Tetramethyl benzidine dissolved in citric acid).
Ready to use as supplied.
Once open, stable for one month at 2-8°C.

- **STOP SOLUTION** 5 bottle

Colourless liquid filled in a white vial with yellow screw cap.
7 ml per bottle.
Diluted sulfuric acid solution (2.0M H_2SO_4).
Ready to use as supplied.

- **PLASTIC SEALABLE BAG** **5** units

For enclosing the strips not in use.

- **CARDBOARD PLATE COVER** 5 sheets

To cover the plates during incubation and prevent evaporation
or contamination of the wells.

- **PACKAGE INSERTS** 1copy

Additional Materials And Instruments Required But Not Provided:

- Freshly distilled or deionized water.
- Disposable gloves and timer.
- Appropriate waste containers for potentially contaminated materials.
- Disposable V-shaped troughs.
- Dispensing system and/or pipette (single or multichannel), disposable pipette tips.
- Absorbent tissue or clean towel.
- Dry incubator or water bath, 37±0.5°C.
- Microshaker for dissolving and mixing conjugate with samples.
- Microwell plate reader, single wavelength 450nm or dual wavelength 450nm and 630nm.
- Microwell aspiration/wash system.

Specimen Collection, Transportation And Storage:

1. **Sample Collection: Eit**her fresh serum or plasma samples can be used for this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible as to avoid haemolysis of the RBC. Care should be taken to ensure that the serum samples are clear and not contaminated by microorganisms. Any visible particulate matters in the sample should be removed by centrifugation at 3000 RPM for at least 20 minutes at room temperature, or by filtration on 0.22u filters. Plasma samples collected into EDTA, sodium citrate or heparin may be tested, but highly lipaemic, icteric, or haemolized samples should not be used as they could give erroneous results in the assay. Do not heat inactivate samples. This can cause sample deterioration.
2. **Transportation and Storage:** Store samples at 2-8°C. Samples not required for assaying within 3 days should be stored frozen (-20°C or lower).Multiple freeze-thaw cycles should be avoided. For shipment, samples should be packaged and labelled in accordance with the existing local and international regulations

for transport of clinical samples and ethological agents.

Special Instructions For Washing:

1. A good washing procedure is essential to obtain correct and precise analytical data.
2. It is therefore recommended to use a good quality ELISA microplate washer, maintained at the best level of washing performances. In general, no less than 5 automatic washing cycles with dispensing of 350-400µl/well, are sufficient to avoid false positive reactions and high background (all wells turn yellow).
3. To avoid cross-contaminations of the plate with sample or HRP-conjugate, after incubation do not discard the content of the wells, but allow the plate washer to aspirate it automatically.
4. Anyway, we recommend calibrating the washing system on the kit itself in order to match the declared analytical performances. Assure that the microplate washer's liquid dispensing channels are not blocked or contaminated, and sufficient volume of Wash buffer is dispensed each time into the wells.
5. In case of manual washing, we suggest to perform at least 5cycles, dispensing 350-400µl/well and aspirating the liquid for 5times. If poor results (high background) are observed, increase the washing cycles or soaking time per well.
6. In any case, the liquid aspirated out the strips should be treated with a sodium hypochlorite solution(final concentration of 2.5%) for 24 hours, before liquids are disposed in an appropriate way.
7. The concentrated Washing solution should be diluted **1 to 20** before use. For one plate, mix 50 ml of the concentrate with 950ml of water for a final volume of 1000ml diluted Wash Buffer. If less than a whole plate is used, prepare the proportional volume of solution.

Storage And Stability:

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8°C, **do not freeze**. To assure maximum performance of this anti-HCV ELISA kit, during storage protect the reagents from contamination with microorganism or chemicals.

Precautions And Safety:

The ELISA assay is a time and temperature sensitive method. To avoid incorrect result, strictly follow the test procedure steps and do not modify them.

1. Do not exchange reagents from different lots, or use reagents from other commercially available kits. The components of the kit are precisely matched as to achieve optimal performance during testing.
2. Make sure that all reagents are within the validity indicated on the kit box and are of the same lot. Never use reagents beyond the expiry date stated on reagents labels or on the kit box.
3. **CAUTION - CRITICAL STEP:** Allow the reagents and samples to stabilize at room temperature (18-30°C) before use. Shake reagent gently before, and return to 2-8°C immediately after use.
4. Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so, may cause in low sensitivity of the assay.
5. Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with microwell reading.
6. When reading the results, ensure that the plate bottom is dry and there are no air-bubbles inside the wells.
7. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air-bubbles when adding the reagents.
8. Avoid assay steps long time interruptions. Assure same working conditions for all wells.
9. Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Always use different disposal pipette tips for each specimen and reagents as to avoid cross-contaminations. Never pipette solutions by mouth.
10. The use of automatic pipettes is recommended.
11. Assure that the incubation temperature is 37°C inside the incubator.
12. When adding samples, avoid touching the well's bottom with the pipette tip.
13. When reading the results with a plate reader, it is recommended to determine the absorbance at 450nm or at 450nm with reference at 630nm.
14. All specimens from human origin should be considered as potentially infectious.
15. Materials from human origin may have been used in the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to HIV ½, HCV, TP and HBsAg. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety. Never eat, drink, smoke, or apply cosmetics in the assay laboratory.
16. Bovine derived sera may have been used in this kit. Bovine serum albumin (BSA) and faetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.
17. The pipette tips, vials, strips and sample containers should be collected and autoclaved for 1hour at 121°C or treated with 10% sodium hypochlorite for 30minutes to decontaminate before any further steps for disposal.
18. The Stop solution (2M H_2SO_4) is a strong acid. Corrosive. Use it with appropriate care. Wipe up spills immediately or wash with water if come into contact with the skin or eyes. ProClin 300 used as a preservative can cause sensation of the skin.
19. The enzymatic activity of the HRP-conjugate might be affected from dust, reactive chemical, and substances like sodium hypochlorite, acids, alkalines etc. Do not perform the assay in the presence of such substances.
20. Materials Safety Data Sheet (MSDS) available upon request.
21. If using fully automated microplate processing system, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.

Assay Procedure:

Step1 Reagents preparation: Allow the reagents and samples to reach room temperature **(18-30°C)** for at least 15-30minutes. Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37°C until crystals dissolve. Dilute the stock wash Buffer **1 to 20** with distilled or deionized water. Use only clean vessels to dilute the Wash buffer.

Step2 Numbering Wells: Set the strips needed in strip-holder and number sufficient number of wells including three Negative control **(e.g. B1, C1, D1)**, two Positive control **(e.g. E1, F1)** and one Blank (**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.

Step3 Adding Diluent: Add **100µl** Specimen Diluent into each well except the blank.

Step4 Adding Sample: Add **10µl** of Positive control, Negative control, and Specimen into their respective wells. **Note: Use a separate disposal pipette tip for each specimen, Negative and Positive Control as to avoid cross-contamination.** Mix by tapping the plate gently.

Step5 Incubating (1): Cover the plate with the plate cover and incubate for **30minutes at 37°C**. It is recommended to use thermostat-controlled water tank to assure the temperature stability and humidity during the incubation. If dry incubator is used, do not open the door frequently.

Step6 Washing (1): After the end of the incubation, remove and discard the plate cover. Wash each well **5times** with diluted Wash buffer. Each time, allow the microwells to soak for 30-60seconds. After the final washing cycle, turn the strips plate onto blotting paper or clean towel, and tap it to remove any remainders.

Step7 Adding HRP-Conjugate: Add **100µl** HRP-Conjugate to each well except the Blank.

Step8 HRP-Conjugate Incubating(2): Cover the plate with the plate cover and incubate for **30minutes at 37**°C.

Step9 Washing(2): At the end of the incubation, remove and discard the plate cover. Wash each well **5**times with diluted Wash buffer as in **Step6.**

Step10 Colouring: Dispense **50µl** of Chromogen A and **50µl** Chromogen B solution into each well including the **Blank** and mix by tapping the plate gently. Incubate the plate at **37°C for 15minutes avoiding light**. The enzymatic reaction between the Chromogen A/B solutions produces blue colour in Positive control and anti-HCV positive sample wells.

Step11 Stopping Reaction: Using a multichannel pipette or manually, add **50µl** Stop Solution into each well and mix by tapping the plate gently. Intensive yellow colour develops in Positive control and anti-HCV positive sample wells.

Step12 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 **630nm**. Calculate the Cut-off value and evaluate the results (**Note:** read the absorbance within **5** minutes after stopping the reaction) .

Interpretation Of Results And Quality Control:

Each microplate should be considered separately when calculating and interpreting results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each sample's optical density (OD) 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 OD value from the print report values of samples and controls. In case the reading is based on dual filter plate reader, do not subtract the Blank well OD from the print report values of samples and controls.

1. **Calculation of Cut-off value (C.O.) = *Nc + 0.12**

*Nc = the mean absorbance value for three negative controls.

Important: If the mean OD value of the negative control is lower than 0.02, take it as 0.02. If higher than 0.02 see the Quality control range.

Example:
1. Calculation of Nc:

Well No	B1	C1	D1
Negative controls OD value	0.02	0.012	0.016

Nc = 0.016
(The mean value is lower than 0.02, so take it as 0.02).
2. Calculation of Cut-off (C.O.) = 0.02 + 0.12 = 0.140

If one of the Negative control values does not meet the Quality control range specifications, it should be discarded and the mean value is calculated again using the remaining two values. If more than one negative control OD value does not meet the Quality control range specifications, the test is invalid and must be repeated.

2. Quality control range:
The test results are valid if the Quality Control criteria are verified. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient sample being analyzed.

1. The OD value of the Blank well, which contains only Chromogens and Stop solution, is less than 0.080 at 450 nm.
2. The OD value of the Positive control must be equal to or greater than 0.800 at 450/630nm or at 450nm after blanking.
3. The OD value of the Negative control must be less than 0.100 at 450/630nm or at 450nm after blanking.

3. Interpretations of the results:
(S = the individual absorbance (OD) of each specimen)

Negative Results (S/C.O. <1): samples giving absorbance less than the Cut-off value are negative for this assay, which indicates that no antibodies to hepatitis C virus have been detected with this anti-HCV ELISA kit. Therefore, the patient is probably not infected with HCV.

Positive Results (S/C.O.≥1) : samples giving an absorbance greater than, or equal to the Cut-off value are considered initially reactive, which indicates that antibodies to hepatitis C virus have probably been detected using this anti-HCV ELISA kit. Retesting in duplicates of any initially reactive sample is recommended. Repeatedly reactive samples could be considered positive for antibodies to HCV and therefore the patient is probably infected with hepatitis C virus. Blood unit positive for HCV antibodies should be immediately discarded .

Borderline: Samples with absorbance **O.D.≤Cut-off ×2** are considered borderline and retesting of those samples in duplicates is recommended. Repeatedly positive samples could be considered positive for hepatitis C virus infection.

Follow-up and supplementary testing of any anti-HCV positive samples with other analytical system (e.g. RIBA, WB) is required to confirm the diagnosis.

Test Performance And Expected Results:

Clinical Specificity:
A blood donor population of 2948 individuals was tested with 3 different kits from different manufacturers. The specificity of this anti-HCV ELISA kit was 99.55%.

Clinical Sensitivity:
Among 480 clinical hepatitis C patients confirmed positive by RIBA 3.0, 479 were positive when tested with this anti-HCV EISA kit. The sensitivity was 99.79%.

Analytical Specificity:
1. No cross reactivity observed with samples from patients infected with HAV, HIV, HBV, CMV, and TP. No interference was observed from rheumatoid factors up to 2000U/ml.
2. This assay performance characteristics are unaffected from elevated concentrations of bilirubin, haemoglobin, and triolein.
3. Frozen specimens have been tested to check for interferences due to collection and storage.

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	BBI Ref. Data				Anti-HCV ELISA
	Western Blot	EIA Result, S/CO			
No.	RIBA 3.0	EIA 2.0	EIA 3.0	Lot 1	Lot 2
1	POS	2.2	>4.7	6.6	4.5
2	POS	>4.9	4.3	6.3	14.9
3	POS	1.3	4.5	6.7	5.4
4	POS	2.7	>4.7	4.2	4.7
5	POS	2.1	4.4	6.6	5.5
6	POS	2.1	3.3	4.8	6.0
7	IND	2.7	1.7	4.0	2.8
8	IND	3.8	2.9	3.6	3.3
9	IND	1.1	1.7	2.5	2.5
10	NEG	0.1	0.0	0.0	0.0
11	IND	2.3	>4.7	3.6	3.0
12	POS	2.5	4.4	2.4	2.0
13	IND	2.2	3.5	1.4	1.6
14	IND	2.7	3.7	5.6	4.2
15	POS	2.8	1.8	2.3	2.8

Performance of low and mixed titers anti-HCV panels

HCV Genotype Antibody Testing:
1. Non-repeatable positive result may occur due to the general biological characteristics of ELISA assays. The assay is design to achieve very high performance characteristics of sensitivity and specificity and the "indirect model" minimizes the unspecific reactions, which can occur due to interference between unknown meters in sample and the rabbit anti-human IgG used as a conjugate. Antibodies may be undetectable during the early stages of the disease and in some immunosuppresed individuals.

GENOTYPE	SAMPLES	POSITIVE
1a-b	15	15
2a-b	13	13
3a-b	10	10
4h	6	6
5	12	12
6	18	18
TOTAL	74	74

Reproducibility		Within run		Between run	
Sample	No	Mean OD	CV%	Mean OD	CV%
Weak positive	10	0.436	9.1%	0.401	9.5%
Moderate positive	10	0.946	7.0%	0.916	7.5%
Strong positive 1	10	1.917	4.4%	1.895	4.2%
Strong positive 2	10	2.372	3.8%	2.309	4.0%

2. Positive results must be confirmed with another available method. Any positive result must be interpreted together with the patient clinical information and other laboratory results.
3. Common sources for mistakes: kits beyond the expiry date, bad washing procedures,

contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add samples or reagents, equipment, timing, volumes, sample nature and quality.

4. If, after retesting of the initially reactive samples, the assay results are negative , these samples should be considered as non-repeatable (false positive) and interpreted as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are related but not limited to inadequate washing step. The prevalence of the marker will affect the assay's predictive values.
5. This kit is intended ONLY for testing of individual serum or plasma samples. Do not use it for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.

Indications Of Instability Or Deterioration Of The Reagents:

1. Values of the Positive or Negative controls ,which are out of the indicated Quality control range, are indicator of possible deterioration of the reagents and/or operator or equipment errors. In such case, the results should be considered as invalid and the samples must be retested. In case of constant erroneous results classified as due to deterioration or instability of the reagents, immediately substitute the reagents with new ones.
2. If after mixing of the Chromogen A and B solutions into the wells, the, the colour of the mixture turns blue within few minutes, do not continue carrying out the testing and replace the reagents with fresh ones.

Instruments And Applications:

Fortress Diagnostics' immunoassay products are designed to work in both manual and automated lab environments and are compatible with any open-ended instrumentation, including chemistry analyzers, microplate readers and microplate washers. There may or may not be an application developed for your particular instrument please contact info@fortressdiagnosticscom Fortress offers several instruments, including Plate Reader/Plate Washer. Please enquire by email.

Reference:

1. Alter HJ. (1978) You will wonder where the yellow went: A 15-year retrospective of posttransfusion hepatitis. In: Moore SB, ed. Transfusion-Transmitted Viral Diseases. Alington, VA. Am. Assoc. Blood Banks, pp. 53-38.
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3. Choo Q-L,Weiner AJ, Overby LR, Kuo G, Houghton M. (1990) Hepatitis C Virus: the major causative agent of viral non-A, non-B hepatitis. Br Med Bull 46: 423-441.
4. Engvall E, Perlmann P. (1971) Enzyme linked immunosorbent assay (ELISA): qualitative assay of IgG. Immunochemistry 8:871-874.