Prevalence of Hepatitis C Virus among Prisoners in Khartoum State

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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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الآية

قال تعالى:

(هُوَ الَّذِي أَنْزَلَ السَّلَآيَةَ فِيهَا قَلُوبَ الْمُؤْمِنِينَ لِيَهْدُوهَا إِبَاحًا مَعَ إِبَاحَتِهِ)

وَلَهُ جَنَوْدُ السَّفَاوَاتِ وَالأَرْضُ وَجَانِبَاللهِ عَليماً مَخْيِساً

حمد الله العظيم

سورة الفاتحة 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 إلى تحديد مدى انتشار فيروس التهاب الكبد الوبائي سي بين السجناء في ولاية الخرطوم. وقد تحقق هذه الدراسة خلال الفترة ما بين فبراير و مايو 2017.

تم جمع 90 عينة دم عشوائية من السجناء النساء في سجن الخرطوم. تم جمع 5 مل من الدم وطردها للحصول على البلازما. تم اختبار البلازما لمضادات التهاب الكبد الوبائي سي عن طريق مقايصة الممتز المناعي المرتبط بالإنتيزم (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 فيروس التهاب الكبد الوبائي 0%.

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

وخلصت الدراسة إلى أن السجناء المتزوجين أكثر إصابة من غيرهم، وكان جميع الذين اعطوا نتيجة إيجابية للاختبار المتصلي لديهم عدوى سابقة بالتهاب الكبد الوبائي. وغالبًا ماهم قد حصلوا على العدوى قبل أن يدخلوا السجن.

للحصول على وصف أكثر دقة لعوامل الخطر للاصابات بفيروس التهاب الكبد الوبائي سي يجب أن تتم الدراسة في عدد أكبر من السجناء، ينبغي توجيه جميع السجناء إلى السلوك السليم، بدء العلاج للسجناء المصابين والفحص المسحي ينبغي أن يجري في السجن من وقت لآخر زمن.
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CHAPTER ONE

INTRODUCTION AND OBJECTIVES
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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 States 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
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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 30 nm 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 10^8 International Units/mL (Corey _et al._, 2004).

2.3.8.4. Genotype Tests

Genotype tests 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 1 infections 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
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3.1. Study design

3.1.1. Type the study

A descriptive cross-sectional study conducted to detect hepatitis C viral 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 specificity 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 (4th 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

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>Percent</th>
<th>Age Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid no</td>
<td>88</td>
<td>97.8</td>
<td>33.94</td>
</tr>
<tr>
<td>yes</td>
<td>2</td>
<td>2.2</td>
<td>50.00</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>100.0</td>
<td>34.30</td>
</tr>
</tbody>
</table>

Table 4.2. Frequency of HCV infection in different age group and the correlation between the age and the infection

<table>
<thead>
<tr>
<th>Age- groups</th>
<th>Frequency of infection</th>
<th>Percent of infection</th>
<th>Asymptotic Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-30</td>
<td>0/41</td>
<td>0%</td>
<td>Pearson Chi-Square .510</td>
</tr>
<tr>
<td>31-45</td>
<td>1/31</td>
<td>3.2%</td>
<td></td>
</tr>
<tr>
<td>46-60</td>
<td>1/16</td>
<td>6.2%</td>
<td></td>
</tr>
<tr>
<td>61-75</td>
<td>0/2</td>
<td>0%</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>HCV test result</th>
<th>Muslim</th>
<th>Christian</th>
<th>Asymptotic Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>no</td>
<td>47</td>
<td>41</td>
<td>Pearson</td>
</tr>
<tr>
<td>yes</td>
<td>0</td>
<td>2</td>
<td>Chi-Square</td>
</tr>
<tr>
<td>Percentage</td>
<td>0%</td>
<td>4.6%</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>HCV test result</th>
<th>yes</th>
<th>no</th>
<th>Yes</th>
<th>no</th>
<th>Work Asymptotic Significance</th>
<th>Previous inf. Asymptotic significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV test result</td>
<td>yes</td>
<td>yes</td>
<td>2</td>
<td>1</td>
<td>Pearson Chi-Square</td>
<td>.873</td>
</tr>
<tr>
<td>HCV test result</td>
<td>no</td>
<td>39</td>
<td>32</td>
<td>56</td>
<td></td>
<td>.066</td>
</tr>
<tr>
<td>Percentage</td>
<td>2.5%</td>
<td>2%</td>
<td>5.8%</td>
<td>0%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Blood transfusion</th>
<th>had a surgery</th>
<th>Blood trans. Asymptotic Significance</th>
<th>Surgery Asymptotic Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>yes</td>
<td>no</td>
<td>yes</td>
<td>72</td>
</tr>
<tr>
<td>HCV test result</td>
<td>yes</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>HCV test result</td>
<td>no</td>
<td>10</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>80</td>
<td>16</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>HCV test result</th>
<th>Hemodialysis</th>
<th>Share sharp object</th>
<th>Hemodialysis Asymptotic Significance</th>
<th>Sharing object Asymptotic Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>no</td>
<td>yes</td>
<td>3</td>
<td>no</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>23</td>
<td>65</td>
<td>87</td>
</tr>
<tr>
<td>yes</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>87</td>
<td>23</td>
<td>67</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>HCV test result</th>
<th>marital status</th>
<th>Asymptotic Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>no</td>
<td>married</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>single</td>
<td>36</td>
</tr>
<tr>
<td>yes</td>
<td>married</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>single</td>
<td>0</td>
</tr>
<tr>
<td>Percentage p</td>
<td></td>
<td>3.7%</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>HCV test result</th>
<th>time in prisons</th>
<th>Asymptotic Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-6 month</td>
<td>7-12 month</td>
</tr>
<tr>
<td>no</td>
<td>68</td>
<td>10</td>
</tr>
<tr>
<td>yes</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Percentage</td>
<td>2.8%</td>
<td>0.0%</td>
</tr>
</tbody>
</table>

P value: 0.747
No correlation between the infection and time in prison
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
REFERENCES
REFERENCES


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
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 enveloped, 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 carcinoma. Since the introduction in 1990 of anti-HCV screening of blood donors, 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 N54 (c100-3) region of the HCV genome as antigens. Earlier generation tests, which included recombinant / synthetic antigens from the Core (c22) and nonstructural regions N53 (c33c, c100-3) and N54 (c100-3, c200) resulted in a remarkable improvement in sensitivity and specificity, Clinical studies showed 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 N5 region of the viral genome in addition to N53 (c200), N54 (c33c) 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, highlyimmunoreactive 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 Tertamethylbenzidine (TMB) and urea peroxidase 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 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] → [Ag(p)+Ab(s)+ENZ] + –blue–yellow (+)
Incubation 1: Incubation 2: Immobilized Complex Colouring results
30min. 30min. 15min
Ag(p)-core-coated HCV antigens(core, N53; N54, N55; 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 step holder. The plate is sealed in aluminium pouch with desiccant.
8+12+12+8+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 together with the desiccant and return to 2-8°C.
NEGATIVE CONTROLS 5 vials
Blue-coloured liquid filled in a vial with white screw cap.
1ml per vial.

Procedure:

1. Sample Preparation:

   - **STORAGE AT 2-8°C**
   - **DIAGNOSTICS**
   - **.getProductCode()**
   - **BXS**
   - **STORAGE AT 2-8°C**

   **Antibody Detection**

   - **STORAGE AT 2-8°C**
   - **MICROWELL PLATE**
   - **STORAGE AT 2-8°C**
   - **STORAGE AT 2-8°C**

   **Sample Collection:** 8×12/12×8 well strips per plate.

   **Assay Protocol**

   1. **Ready to use as supplied.**
   2. **STORAGE AT 2-8°C**
   3. **STORAGE AT 2-8°C**

   **Colorimetric Analysis**

   - **STORAGE AT 2-8°C**
   - **STORAGE AT 2-8°C**

   **Comparison of Optical Density Results**

   - **STORAGE AT 2-8°C**
   - **STORAGE AT 2-8°C**

   **Interpretation**

   - **STORAGE AT 2-8°C**
   - **STORAGE AT 2-8°C**

   **Conclusion**

   - **STORAGE AT 2-8°C**
   - **STORAGE AT 2-8°C**

   **Note**

   - **STORAGE AT 2-8°C**
   - **STORAGE AT 2-8°C**

   **Specifications**

   - **STORAGE AT 2-8°C**
   - **STORAGE AT 2-8°C**

   **Additional Materials and Instruments Required But Not Provided:**

   - Freshly distilled or deionized water.
   - Microwell plate reader, single wavelength 450nm or dual wavelength 450nm and 630nm.
   - Disposable pipette tips.
   - Colorimetric plates.
   - Polyethylene gloves.

   **Important Details**

   - Store the kits at 2-8°C.
   - Avoid freezing or thawing the kits.
   - Do not use the kits if the expiry date has passed.

   **Packaging Information**

   - **STORAGE AT 2-8°C**
   - **STORAGE AT 2-8°C**

   **Manufacturer**

   - **STORAGE AT 2-8°C**
   - **STORAGE AT 2-8°C**

   **Quality Control**

   - **STORAGE AT 2-8°C**
   - **STORAGE AT 2-8°C**

   **Stability**

   - **STORAGE AT 2-8°C**
   - **STORAGE AT 2-8°C**

   **Safety**

   - **STORAGE AT 2-8°C**
   - **STORAGE AT 2-8°C**

   **Transport and Storage**

   - **STORAGE AT 2-8°C**
   - **STORAGE AT 2-8°C**

   **Specifications**

   - **STORAGE AT 2-8°C**
   - **STORAGE AT 2-8°C**

   **Manufacturer**

   - **STORAGE AT 2-8°C**
   - **STORAGE AT 2-8°C**
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, or 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 5 cycles, 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 autoclave for tran.
    15. The concentrated Washing solution should be diluted 100x 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:
15. The components of the kit will remain stable through the expiration date indicated on the label and packaging when stored between 2-8°C, do not freeze.
20. To assure maximum performance of this anti-HCV ELISA kit, during storage protect the reagents from contamination with microorganism or chemicals.

Precautions And Safety:
3. 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 lots. The components of the kit are precisely matched as to achieve optimal performance during testing.
2. Make sure that all reagents are within the valid expiration date 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 before the experiment to assure the accuracy of samples/reagents dispensing.
10. Always use different disposal pipette tips for each specimen and reagents as to avoid cross-contaminations. Never pipette solutions by mouth.
11. The use of automatic pipettes is recommended.
12. Ensure that the incubation temperature is 37°C inside the incubator.
13. When adding samples, avoid touching the well’s bottom with the pipette tip. 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 1/2, 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. Always adhere 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 serum (FCS) are used in the kit. If the kit is used 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 the incubator is used, do not open the door frequently.
17. The pipette tips, vials, strips and sample containers should be collected and autoclaved for one hour at 121°C or treated with 10% sodium hypochlorite for 30minutes to decontaminate before any further steps for disposal.
18. The Stop solution (2M H2SO4) 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 chemicals, and substances like sodium hypochlorite, acids, alkalines etc. Do not perform the assay in the presence of such substances.
20. Material and Safety Data Sheet (MSDS) available upon request.
21. If using fully automated microplate processing system, during incubation, do not cover the plate. The plates with the pipettes could wash out the reagents from the strips and the plate after washing, can also be omitted.

Assay Procedure:

Step 1 Reagents preparation: Allow the reagents and samples to reach room temperature (18-30°C) for at least 15-30minutes. Check the Wash Buffer concentrated 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 1:20 with distilled water. Use only clean vessels to dilute the Wash buffer.

Step 2 Numbering Wells: Set the strips needed in strip-reminders inside the plate after washing, and mix by tapping the plate gently. Incubate the plate at 37°C for 15minutes avoiding light. The enzymatic reaction between the Chromagen A/B solutions produces blue colour in Positive control and anti-HCV positive sample wells.

Step 3 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 stoppage).

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 value is exceeded on the strip, the results should be calculated by subtracting the Blank

Step 5 Incubating (1): Cover the plate with the plate cover and incubate for 30minutes at 37°C.

Step 6 Washing (1): After the end of the incubation, remove and discard the cover plate. 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 remains.

Step 7 Adding HRP-Conjugate: Add 100μl HRP-Conjugate to each well except the Blank.

Step 8 HRP-Conjugate Incubating(2): Cover the plate with the plate cover and incubate for 30minutes at 37°C.

Step 9 Washing(2): At the end of the incubation, remove and discard the plate cover. Wash each well 5times with diluted Wash buffer as in Step6.

Step 10 Colouring: Dispense 50μl of Chromogen A and 50μl of 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 Chromagen A/B solutions produces blue colour in Positive control and anti-HCV positive sample wells.

Step 11 Colouring Reaction: Using a multichannel pipette, dispense 50μl of the 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.

Step 12 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 stoppage).
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. Repeated 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 ELISA kit. The sensitivity was 99.79%.

Analytical Specificity:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Western Blot</th>
<th>EIA Result, S/CO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>POS</td>
<td>&gt;4.7</td>
</tr>
<tr>
<td>2</td>
<td>POS</td>
<td>&gt;4.9</td>
</tr>
<tr>
<td>3</td>
<td>POS</td>
<td>1.3</td>
</tr>
<tr>
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<tr>
<td>7</td>
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<td>8</td>
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<td>3.8</td>
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<tr>
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<tr>
<td>10</td>
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<tr>
<td>11</td>
<td>IND</td>
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<tr>
<td>15</td>
<td>POS</td>
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</tr>
</tbody>
</table>

NOTE 1: In the event that a negative control OD value is greater than the cut-off value (0.02), retest the control.

NOTE 2: If the absorbance of the patient sample is less than 0.100 at 450 nm or 0.450 nm after blanking, the sample is probably not infected with HCV.

Sample 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 less 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. OD value of the Blank well, which contains only Chromogens and Stop solution, is less than 0.060 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 afterblanking.
   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:

   Positive Results (S/C.O.≥1): samples giving an absorbance less than the Cut-off value are negative for this assay, which indicates that antibodies to hepatitis C virus have not been detected with this anti-HCV ELISA kit. Therefore, the patient is probably not infected with HCV.

   1. Calculation of C.O.:
      Western Blot | EIA Result, S/CO |
      Lot 1 | Lot 2 |
      1 POS | >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 |

   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.

   1. OD value of the Blank well, which contains only Chromogens and Stop solution, is less than 0.060 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. Interpreted of the results:

   Performance of low and mixed filters anti-HCV panels
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 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@fortressdiagnostics.com. Fortress offers several instruments, including Plate Reader/Plate Washer. Please enquire by email.

Reference:

