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College of Pharmacy

prevalence of Hepatitis C Virus infection among blood donors in Al-Diwaniyah governorate

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بسم الله الرحمن الرحيم

{يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَ الَّذِينَ أُوتُوا الْعِلْمَ دَرَجَات}

صدق الله العلي العظيم

(سورة المجادلة- اية11)

DEDICATION

To those suffering from these diseases

To our beloved families

To everyone who encouraged us especially our friends who aided us in every possible way to make this work see the light…

HussamThamer *Hossain Mohammed*

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Owing to the blessing of GOD , this work has come to light
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Abstract

Hepatitis C virus (HCV) is a leading cause of chronic liver disease, cirrhosis, and hepatocellular carcinoma, as well as the most common indication for liver transplantation in many countries.

This study wasconducted to detect the prevalence of hepatitis C virus (HCV) infection in blood donors in Al-Diwaiyahgovermente–Iraq during the period between Jan. 2016 and dec. 2017. Oute of 23800 serum samples of blood donors of age ranging from 20 to more than 60 year were 25 gave positive results by using enzyme linked immunosorbent assay (ELISA)

The results of the study showed that the highest rate of infection was in age group of (28-55) years old

seroprevalence of HCV infection in relation to the location of different geographical regions showed that highest infection rate was in rurals rather than centeralprvinces

ELISA was performed for direct and rapid detection of hepatitis C virus infection. The results of ELISA showed that there are 25 seropositive samples,Phylogenetic analysis of virus for detection its source

The epidemiological risk factor play a role in the distribution of disease in different groups of study

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CHAPTER ONE

INTRODUCTION

Introduction

The pathogenetic mechanisms of hepatitis C virus (HCV) infection are poorly known. An understanding of HCV biology and the potential clinical impact of HCV genetic variability is essential to managing, treating, and preventing HCV infections (Pawlotsky JM, Bastie A). Hepatitis C virus (HCV) belongs to the *Flaviviridae*family and it is the only member of the *Hepacivirus* genus (Dustin and Rice, 2015).

Most cases of viral hepatitis are caused by one of the following agents : Hepatitis A virus (HAV); Hepatitis B virus (HBV) ; Hepatitis C virus (HCV) ; Hepatitis D virus(HDV) ; Hepatitis E virus (HEV) ; Hepatitis G and F are newly discovered viruses, some viruses causing hepatitis as a part of general infection examples by Rubella virus , mumps virus, cytomegalic virus and Epstein-Barr virus and very rare by Herpes Simplex virus and yellow fever virus (Zuckerman *et al.,*2014)

Hepatitis C virus (HCV) is a major global healthcare problem. HCV prevalence is highest in Egypt at >10% of the general population and China has the most people with HCV (29.8 million)(Grebely, J. & Dore, G. J.) .The WHO estimates that up to 3% of the world's population has been infected with the virus, equating to more than 170 million carriers of HCV worldwide. Within this broad estimate, there is considerable variability in the prevalence of infection (Semin Liver Dis2012).

HCV is the agent that causes Hepatitis C, a disease that affects around 135 million people worldwide, primary infection with HCV often shows only mild symptoms, but in the majority of patients, the infection becomes chronic and leads to liver cirrhosis, which often results in liver cancer. HCV infection is currently one of the major reasons for liver transplantation in Europe and in the USA (Shepard *et al.*, 2005).

individuals exposed to HCV, 80% become persistently infected, and up to 30% of these develop progressive liver disease, including cirrhosis and hepatocellular carcinoma (HCC)(Poynard T, Bedossa P, Opolon P *et al).*

Differences in past HCV incidence and current HCV prevalence, together with the generally protracted nature of HCV disease

progression, has led to considerable diversity in the burden of advanced liver disease in different countries.

Natural history of liver fibrosis progression in patients with chronic hepatitis C.(Lancet 2011), The prevalence varies markedly from one geographical area to another and within the population assessed, in Western Europe, HCV prevalence rates range from 0.5% to 4%. It is higher in Eastern Europe, the Middle East and in other countries such as Egypt (15%), Romania (6%), Pakistan (4.7%) and in Ukraine (4.0%) (Esteban *et al.,*2008)HCV infection of the hepatocyte begins with a complex interaction of the virion with a series of cellular entry factors (Budkowska,2013)

The hepatitis C virus is a bloodborne virus. It is most commonly transmitted through:

- injecting drug use through the sharing of injection equipment;
- the reuse or inadequate sterilization of medical equipment, especially syringes and needles in healthcare settings; and
- the transfusion of unscreened blood and blood products. (WHO2017)

The investigation of HCV diagnosis starts with serological assays for detecting antibodies to HCV followed by molecular assays for detecting HCV RNA (. Initial diagnosis of HCV infection is classically done by serologic methods either by determining anti HCV antibody by EIAs or by immunoblot assays and by determining the presence of HCV RNA. The advent of simple rapid immunoassays has significantly reduced the risk of HCV transmission, but concern remains for patients in high risk groups(Clemens2013) Studies have shown that false negative results in rapid tests might arise in patients who are severely immunocompromised such as those co-infected with HIV in patients on hemodialysis, IDUs, thalassaemic. In these patient groups molecular detection by reverse transcription polymerase chain reaction (RT-PCR) remains the best method for detection.((Pawlotsky 2013).

Aims of study

1-This study aimed to evaluate the prevalence rate of HCV among blood donors in Al-diwaniya governorate and to study some epidemiological features of this disease by using ELISA test.

2-To assess the role of age , gender and location on the infection rate of the disease

CHAPTER TWO

LITERATURES REVIEW

2-1-History of hepatitis

1960s: Hepatitis B Is Accidentally Discovered

Baruch Blumberg was researching genetic links to disease susceptibility. During this time, he accidentally discovered the hepatitis B (HBV) virus in the blood sample of an Australian Aborigine. This discovery led to the development of a test to screen people for HBV. This also led to an effective vaccine for the disease. In 1976, Blumberg was awarded the Nobel Prize for his work.(Afdhal, 2004).

1973: Hepatitis A Is Discovered

Led by Steven Feinstone, scientists at the National Institutes of Health identified the virus responsible for hepatitis A (HAV). The virus was discovered in fecal samples from prisoner volunteers. Noted microbiologist Maurice Hilleman developed the first effective vaccine for HAV in 1981.(AL-Badry(2011).

1975: A Previously Unrecognized Hepatitis Is Found

American and British researchers identified a type of hepatitis that didn't test positive for the proteins found with HAV or HBV. Both teams conclude that a previously unrecognized human hepatitis virus is the likely cause.(Dustin and Rice, 2007).

1989: Hepatitis C Virus Is Identified

The Centers for Disease Control and Prevention and Chiron came together to identify the hepatitis C (HCV) virus. There isn't a vaccine for HCV at this time.(D'Souza and Foster, 2004).

Choo *et.al.,* (1989) found that Hepatitis C Virus (HCV) was shown to be the cause of most cases of NANB, also this Virus is a blood-born pathogen that poses a significant threat to public health worldwide. A significant number of parentally transmitted viral hepatitis cases in 1980's could not be ascribed to any of the know hepatitis viruses (hepatitis A virus, hepatitis B virus and delta virus) (Feinstone*et al.,* 1975). Some Researchers transmitted the virus from patients of transfusion associated hepatitis to chimpanzees, demonstrating that the disease resulted from a transmissible agent. Portions of HCV genome were isolated, by screening complentaryDeoxy Ribonucleic Acid (cDNA) expression libraries mode from Ribo Nucleic Acid (RNA) and Deoxy Ribonucleic Acid (DNA) of chimpanzees, infected with serum of NANB patient, the virus was given the new name hepatitis C virus, another breakthrough came in 1989 with the cloning of genome (Choo *et al.,* 1989) .

2-2-Etiology

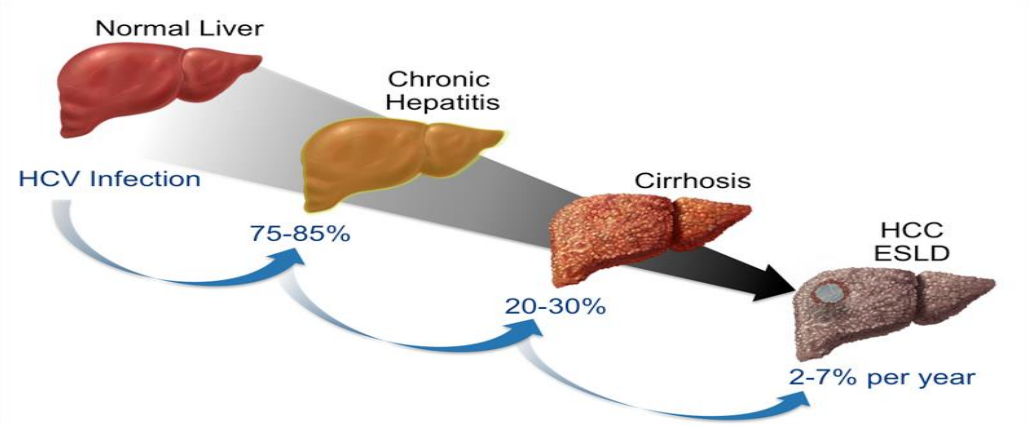
Hepatitis C virus (HCV) belongs to the *Flavivirus* family (Collier, 2000). It is a single-stranded, enveloped RNA virus with a genome about 10,000 nucleotides in length.

The virus is transmitted by percutaneous blood exposure. Unsafe healthcare procedures (including unsafe injection practices) and injection drug use were the leading causes of new infections in 2015. (Lavie *et.al*., 2012).Less frequently it is spread through sexual activity, perinatally, intranasal drug use, or after accidental blood contact (e.g., hemodialysis). Blood and blood products not screened for HCV have also been sources of infection. About 10% of people with HCV infection have no recognized risk factor(Lindenbach*et. al*., 2007).

The perinatal (vertical) transmission rate is 2.4%, while the horizontal transmission rate is <1% (Lindendach and Rice, 2001).

2-3-Pathophysiology

The pathophysiology and treatment of hepatitis C infection, formerly known as non-A, non-B hepatitis, are discussed. The worldwide prevalence is approximately 1%. The majority of patients infected with hepatitis C virus will develop chronic infection, leading to cirrhosis in a significant percentage. (***Hepatitis***C.Ann Intern Med.. 1996) Transmission of hepatitis C is primarily through parenteral routes. Those who use intravenous drugs or received blood transfusions prior to 1992 comprise the major risk groups for the infection. The progression of chronic hepatitis C infection is insidious with possible progression to an inflammatory hepatitis developing within 5–10 years, cirrhosis in 10–20 years, and hepatocellular carcinoma in 20–30 years. Combination therapy with interferon alpha-2b and ribavirin is currently the treatment of choice. Therapy for hepatitis C continues to evolve with newer forms of interferon and HCV antivirals under development. (Lavie*et.al.*, 2007)



Fig(1):(Liver Pathology an Atlas and Concise Guide 2014)

2-4-Classification:-

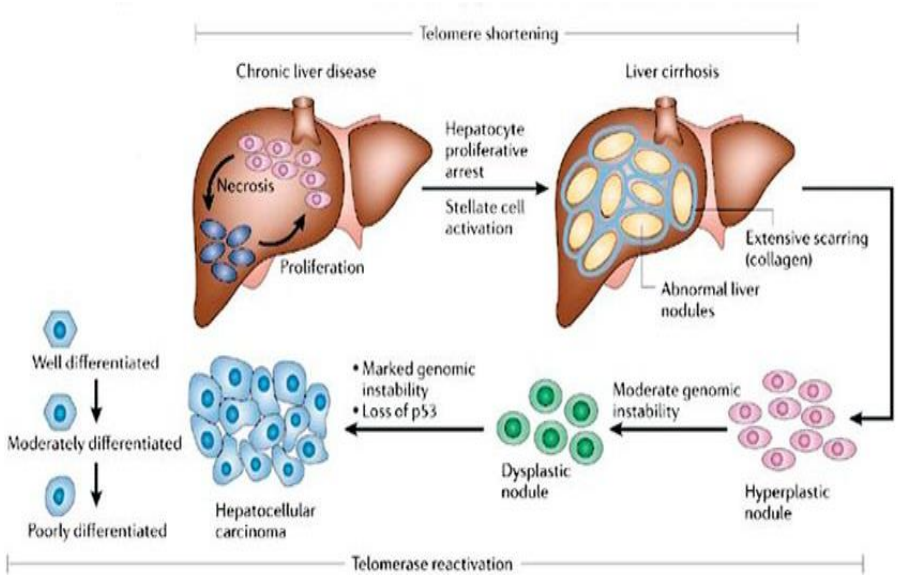
Classification of the hepatitis C virus in to:

Family: Flaviviridae.

Genus: Hepacivirus.Type species: Hepatitis C virus (Collier, 2006).

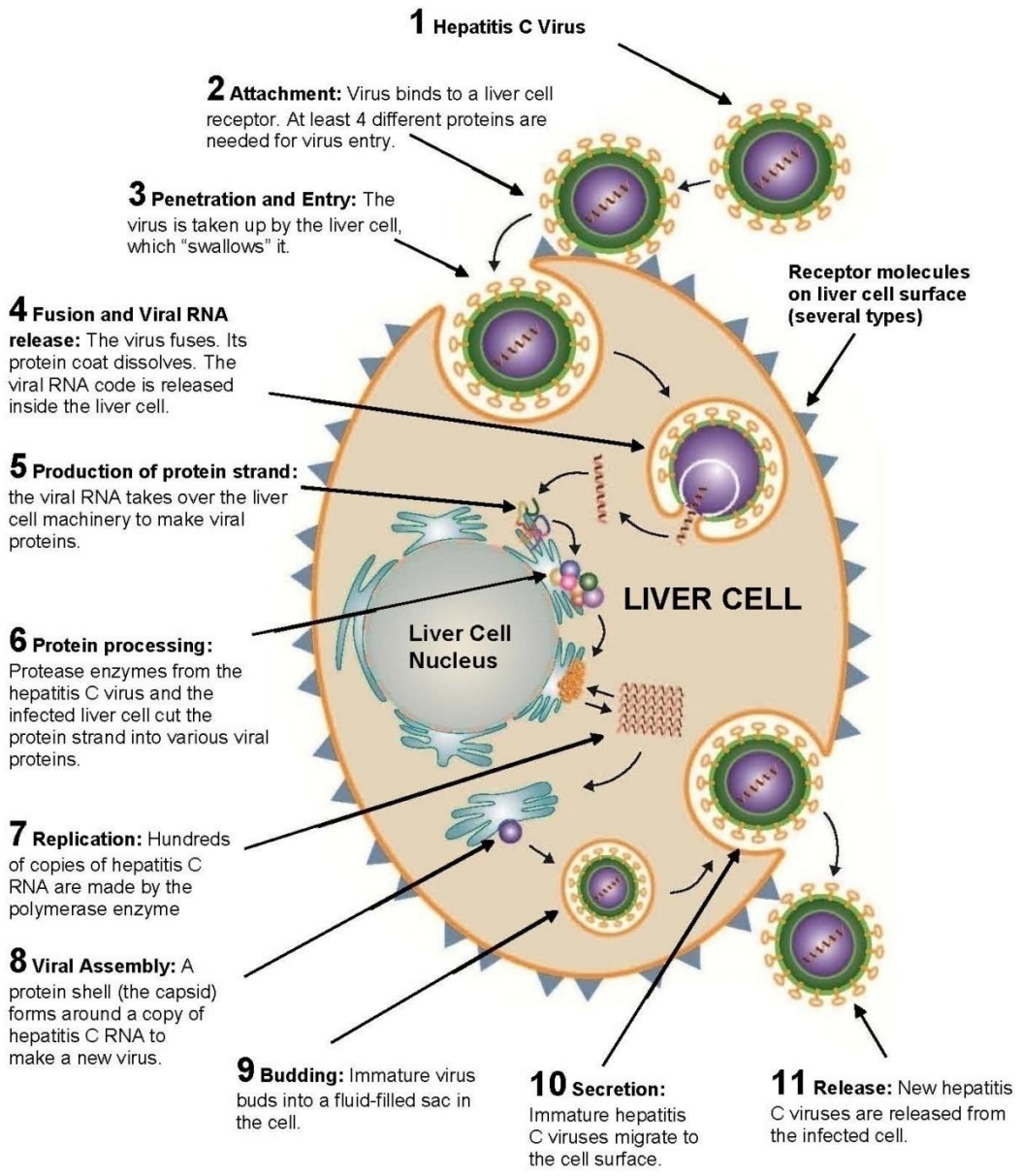
2-5-pathogenesis

The hepatitis C virus (HCV) is a single stranded RNA virus. In 60-80% of patients, it is able to escape innate and adaptive immune surveillance. Thus it establishes itself as an agent of chronic hepatitis. Cytotoxic lymphocytes then contribute to liver injury in an attempt to eradicate the virus. On the other hand, strong multispecific T-lymphocyte reaction against HCV proteins is associated with viral clearance. Both CD4+ and CD8+ lymphocyte functions are important to effect this outcome. In chronic infection, genetic and environmental factors determine the progression of inflammation and fibrosis in individual patients. Of these factors, age, gender, race and alcohol use are the most established ones. The development of hepatocellular carcinoma is mainly restricted to patients with cirrhosis.(Marcellin, 1999).



Fig(2):(Liver Pathology an Atlas and Concise Guide)

2-6-life cycle of *HCV*



Fig(3):(hepatitis c viruses genomes and molecular biology(2006)

2-7-Symptoms

Long-term infection with the hepatitis C virus (HCV) is known as chronic hepatitis C. Chronic hepatitis C is usually a "silent" infection for many

years, until the virus damages the liver enough to cause the signs and symptoms of liver disease. Among these signs and symptoms are:

Bleeding easily ,Bruising easily ,Fatigue ,Poor appetite ,jaundice Dark-colored urine ,Itchy skin ,Fluid buildup in your abdomen (ascites) ,Swelling in your legs ,Weight loss ,Confusion, drowsiness and slurred speech (hepatic encephalopathy)(Wasley*et al.,* 2008).

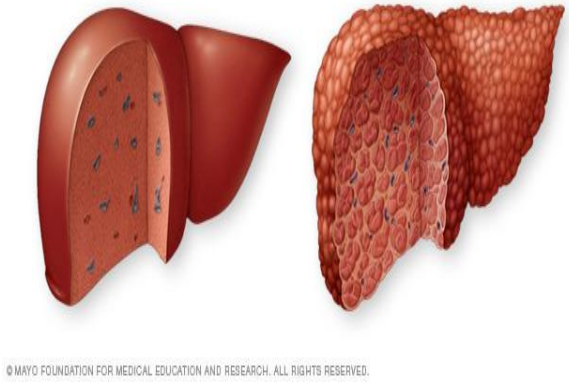
Every chronic hepatitis C infection starts with an acute phase. Acute hepatitis C usually goes undiagnosed because it rarely causes symptoms. When signs and symptoms are present, they may include jaundice, along with fatigue, nausea, fever and muscle aches. Acute symptoms appear one to three months after exposure to the virus and last two weeks to three months.(Tsang *et al.,* 2008).

Acute hepatitis C infection doesn't always become chronic. Some people clear HCV from their bodies after the acute phase, an outcome known as spontaneous viral clearance. In studies of people diagnosed with acute HCV, rates of spontaneous viral clearance have varied from 14 to 50 percent. Acute hepatitis C also responds well to antiviral therapy.

2-8-Complications

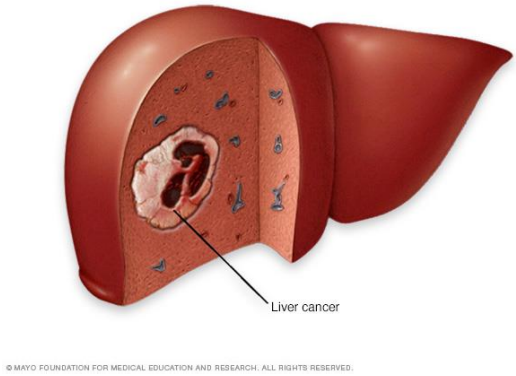
Hepatitis C infection that continues over many years can cause significant complications, such as:

- **Scarring of the liver (cirrhosis).** After 20 to 30 years of hepatitis C infection, cirrhosis may occur. Scarring in your liver makes it difficult for your liver to function.
- **Liver cancer.** A small number of people with hepatitis C infection may develop liver cancer. (Ngo *et al.,* 2006).
- **Liver failure.** Advanced cirrhosis may cause your liver to stop liver function(Ngo *et al.,* 2006).



Normal liver vs. liver cirrhosis

A normal liver (left) shows no signs of scarring. In cirrhosis (right), scar tissue replaces normal liver tissue. (Liver Pathology an Atlas and Concise Guide 2016)



Liver cancer

Liver cancer begins in the cells of the liver. The most common form of liver cancer begins in cells called hepatocytes and is called hepatocellular carcinoma.
(Liver Pathology an Atlas and Concise Guide 2016)

2-9-Prevalence:

World Health Organization (WHO) estimates that about 3% of the world's population has been infected with HCV and that some 170 million are chronic carriers at risk of developing liver cirrhosis and/or hepatocellular carcinoma (Wong and Lee, 2006). There are considerable regional differences: in some countries, e.g., Egypt, the

prevalence is as high as 22% (WHO 2011).While in Africa and the Western Pacific prevalence is significantly higher than in North America and Europe (CDC 2012) .Estimated that there are 2-5 million HCV-positive people in Europe. In Europe and the United States chronic hepatitis C is the most common chronic liver disease, the majority of liver transplants performed in these regions are for chronic HCV. It is difficult to determine the number of new HCV infections, as most acute cases are not noticed clinically. Recent numbers from Europe still show an ongoing epidemic of acute HCV especially among Intra venous drug user (IVDU) and men who have sex with men (MSM) (Rockstroh, 2012).There are six different major genotypes, and about 100 subtypes of HCV with varying presence in different parts of the world. Genotype 1-3 are spread worldwide, genotypes 4-5 are found mainly in Africa and genotype 6 is found in Asia (Schuppan *et.al.*,2003). WHO (1999) showed that in the Western world genotype 1 is predominate (60-90% of infected) and genotype 4 dominate in Egypt.

The prevalence of anti-HCV antibodies in Iraq is 7.1% in general population and 66.0% in Human immunodeficiency virus (HIV) infection hemophilia patients, for HCV genotypes, 1a, 1b, 4 and 4 mixed with 3a were detected, and HCV-1b was the most frequent genotype (Al-Kubaisy*et.al.,* 2002*)*. The anti-HCV seroprevalence in pregnant women was 3.21% and correlated with the number of miscarriages and HCV-1b genotype (Chironna*et.al.,* 2003*).* HIV, HBVand HCV the three most common chronic viral infections all over the world, share similar transmission routes including sexual, blood-blood contact and injecting drug usage (Saravanan *et.al.,* 2007). Co-infection with HIV and HCV and/or HBV is very common in certain population, such as intravenous drug users (IDUs) who often share the contaminated needles/syringes for intravenous drug injection (Koziel and Peters, 2007). It has been reported that the prevalence of HIV-HCV co-infection among IDUs can surpass 90% (Aceijas and Rhodes, 2007). Co-infection with HIV and hepatitis viruses has significantly increased morbidity and mortality of the HIV patients (Thio, 2009). Therefore, it is critical to investigate the prevalence of

co-infection with HIV and HCV and/or HBV, especially among the IDUs considered to be a high-risk population of coinfection (Rotman and Liang, 2009). HCV infection is associated with significant morbidity and mortality (Maier and Wu 2002). Worldwide, approximately 170 million people are infected with HCV, including 243,000 to 300,000 Canadians citizens (Zou et*.al.*, 2000). There is a considerable geographical variation in seroprevalence of anti-HCV throughout the world, with approximately 1.3% in developed countries and 2.6% in developing countries (Parkin, 2006). About 25% of infected individuals spontaneously clear infection and 75% become chronically infected (Hoofnagle, 2002;Seeff, 2002). Within 20 to 30 years of infection, approximately 10% to 40% of HCV-infected individuals will develop

Cirrhosis (Thein H-H *et.al.,* 2008).

2-10-Routes of Transmission

Blood Transfusion/Receipt of Blood Products

Early case-control studies of patients with newly acquired, symptomatic non-A, non-B hepatitis found a significant association between disease acquisition and a history six months prior to illness of blood transfusions, injection drug use, health care employment with frequent exposure to blood, personal contact with others who had hepatitis, multiple sexual partners or low socioeconomic status (Mortimer, 2005). Today, HCV is rarely transmitted by blood transfusion or transplantation of organs due to thorough screening of the blood supply for the presence of the virus and inactivation procedures that destroy bloodborne viruses. In the last several years, blood banks have instituted techniques that utilize nucleic acid amplification of the hepatitis C virus, which will detect the presence of virus even in newly-infected patients who are still hepatitis C antibody-negative. These techniques are estimated to have prevented 56 transfusion-associated HCV infections per year in the U.S. since 1999, and have lowered the current risk of acquiring HCV via transfused blood products to 1 in 2 million(Choo *et. al.,* 1989) .

Injection Drug Use

Injection drug use has been the principal mode of transmission of *HCV* since the 1970's. In comparison to other viral infections, *HCV* is more rapidly acquired after initiation of intravenous drug use(Heintges*et al.,* 1997*).*In addition, rates of *HCV* among young injecting drug-users are four times higher than HIV infection (Rè*et.al.,* 2008). Studies of injection drug users have demonstrated that the prevalence of HCV infection in them is extremely high, with up to 90% having been exposed (Idrees and Riazuddin, 2008). In addition, the incidence of new infections is also high, with seroconversion rates of 10-20 percent per year of injecting (Abou-Setta, 2004). Duration of injecting is the strongest single predictor of risk of *HCV* infection among injection drug users (Thompson *et.al.*, 2003).

Sexual Transmission

The topic of sexual transmission of *HCV* has been controversial. It is believed that *HCV* can be transmitted sexually, but that it is inefficient -- meaning, it is not easy or likely to pass the virus during sex. On the other hand, *HCV* infection is very efficient when it is passed from the blood of one person to the blood of another person, such as when people share needles for drug use. The frequency of *HCV* transmission between monogamous sex partners is very low according to most studies. However, the likelihood of sexual transmission of *HCV* is increased under any of the following circumstances:

- Having multiple lifetime sex partners
- Engaging in rough sex such as anal sex
- Having a history of a sexually transmitted disease
- Having HIV
- Having sex with a prostitute or intravenous drug user
- Having sex during menstruation or whenever blood is present

When counseling patients regarding sexual transmission, the following issues may be relevant:

For discordant couples, with one *HCV*-positive partner and one *HCV*-negative partner, the negative partner should be regularly screened for *HCV* infection.

For discordant couples in long-term monogamous relationships, a change in sexual practices is not necessary (e.g., if they have not been using condoms, they do not have to start using condoms).

For patients who have new or multiple partners, *HIV* infection, or high-risk sexual behaviors, it is recommended that they use condoms and exercise caution regarding potential blood exposure to help reduce the chance of *HCV* infection.

For *HCV*-negative patients who have a new *HCV*-positive partner or engage in high-risk behaviors with a partner of unknown *HCV* status, regular screening is recommended.

(Otho*et. al.*,1994).

Other Modes of Transmission

Occupational Exposures

Health care workers who have exposure to blood are at risk of infection with HCV and other bloodborne pathogens. The prevalence of HCV infection, however, is no greater in health care workers, including surgeons, than for the general population. According to the CDC, the average rate of anti-HCV seroconversion after unintentional needlesticks or sharps exposure from an HCV-positive source is 1.8% (range 0%-7%). An Italian study of 4,403 needlesticks among healthcare workers found 14 seroconversions (0.31%) (Alte, 1994).There is an emerging body of literature, however, that close follow-up of health care workers after a needlestick from a patient with chronic HCV, with early interferon and ribavirin therapy for the healthcare worker if they develop HCV viremia but fail to clear within 3-6 months, can be a beneficial management strategy (Rè2008).

No Identifiable Source of Infection

According to the Centers for Disease Control and Prevention, injection drug use accounts for approximately 60% of all HCV infections in the United States, while other known exposures account for 20-30% (Idrees and Riazuddin, 2008). Approximately 10% of patients in most epidemiological studies, however, have no identifiable source of infection (Mortimer, 1995). HCV exposure in these patients may be from a number of uncommon modes of transmission, including vertical transmission, and parenteral transmission from medical or dental procedures prior to the availability of HCV testing. There are no conclusive data to show that persons with a history of exposures such as intranasal cocaine use, tattooing or body piercing are at an increased risk for HCV infection based on these exposures solely. It is believed, however, that these are potential modes of HCV acquisition in the absence of adequate sterilization techniques(Heintges1997*).*

2 -11- Diagnosis:-

2-11-1-Liver function tests:-Laboratory liver tests are broadly defined as tests useful in the evaluation and treatment of patients with hepatic dysfunction, the liver carries out metabolism of carbohydrate, protein and fats, some of the enzymes and the end products of the metabolic pathway which are very sensitive for the occurred abnormality may be considered as biochemical marker of liver dysfunction, some of the biochemical markers are : serum bilirubin, alanine amino transferase, aspartate amino transferase, alkaline phosphatase and gamma glutamyl transferase. An isolated or conjugated alteration of biochemical markers of liver damage in patients can challenge the clinicians during the diagnosis of disease related to liver directly or with some other organs(Shivaraj*et. al.,* 2009).

2-11-2-Serological tests (Antibody tests):Diagnostic tests used for the detection of HCV infection include the HCV antibody enzyme immunoassay, recombinant immunoblot assay and quantitative HCV RNA polymerase chain reaction (PCR) (Ghany.,2009). A number of

immunoassays has been developed to detect anti-HCV IgG in serum or plasma specimens. First-generation assays were based on a yeast-expressed recombinant protein containing an epitope from the NS4 region (C100-3) of the HCV genome, although these assays identified anti-HCV IgG in approximately 80% of patients with post transfusion hepatitis and led to the substantial reduction in transfusion-associated HCV infections, they lacked sensitivity and specificity (Barrera 1991). Second- and third-generation assays used a multiantigen format and included antigens from the core, NS3, and NS4 regions these modifications markedly improved sensitivity and specificity (Alter , 1992).The difference between the second- and third-generation assays is the inclusion of an additional antigen from the NS5 region (Barrera 1995). These assays reduced the window period observed in first-generation assays by an average of 5 weeks and permitted anti-HCV to be detected as early as 10 weeks after exposure, the diagnostic specificity of third-generation assays is >99% (Colin 2001). The detection of anti-HCV antibodies in serum is based on the use of third-generation Enzyme Immunoassay (EIAs), that detect mixtures of antibodies directed against various HCV epitopes. Recombinant antigens are used to capture circulating anti-HCV antibodies onto the wells of microtiter plates, microbeads, or specific holders adapted to closed automated devices. The presence of anti-HCV antibodies is revealed by anti-antibodies labeled with an enzyme that catalyzes the transformation of a substrate into a colored compound (Pawlotsky, 1999). The optical density (OD) ratio of the reaction is proportional to the amount of antibodies in the serum sample, none the less, third-generation EIAs can yield false-negative results in patients who are undergoing hemodialysis or who are immunocompromised (Ghany2009).Third-generation EIAs detect mixed antibodies against HCV core, NS3, NS4 and NS5 antigens. The target antigens are coated on microtiter plates, microbeads or holders designed for “closed” automated devices, the specificity of current EIAs is greater than 99%, there is no gold standard, so sensitivity is more difficult to determine. In routine use, more than 99% of immunocompetent patients with detectable HCV RNA are

positive with current EIAs (Colin 2001). EIAs can be negative during hemodialysis and in profoundly immunodeficient patients despite ongoing HCV replication, but this is rare with the most recent tests (Thio*a,* 2000). In order to shorten the duration of the diagnosis of heaptitis C virus infection especially in preseroconversion period being capable of the detection of antibodies against NS 5 proteins means that a third generation reactive is very important for anti-HCV assays, because there remains a window period, estimated at 82 days with the second-generation assays, at 66 days with the third generation assays, between the infection and the detection of HCV antibodies (Courouce, 2000). NS5 enables the detection of HCV antibodies on an average of 26 days earlier in individuals with transfusion-transmittedHCV infection (Denoyel*.,* 2004).

2- 12-Treatment of HCV:-

The combination therapy with interferon-α and ribavirin, which is the most effective therapy known today, neutralizes the virus after 6 months in 40-50% of the infection cases with genotype 1 and in 80% of the infection cases with genotype 2 and 3(Schuppan*et al.,* 2003). Infections of HCV genotype 4 is, as genotype 1, relatively resistant to the interferon- α/ribavirin combination therapy (WHO, 1999).

The treatment with interferon-α and ribavirin has significant side effects and are quite expensive (Schuppan2003). The side effects mainly derive from ribavirin as cough, shortness of breath, insomnia and haemolyticanaemia, despite these drawbacks and the fact that ribavirin is teratogenic and requires frequent dose modifications, the combination therapy is at least twice as effective as the mono therapy, if we compare the sustained viral responses (SVR), even from the economic point of view the combination therapy is preferable preventing future costs, which else should

occur in connection with chronic liver diseases (Mchutchison, 2002). Still, the therapies known today are not optimal and new approaches are needed. The HCV virus has a high rate of mutations and for example can be noted a mutation rate in the RdRp region at $5x10^{3}$/site per year, this fact together with the high genetic diversity and different genotypes in different parts of the world make developing a worldwide useful antiviral agent a great challenge, to be successful against HCV you probable will have to use combinations therapies with antiviral agents designed to bind to and to disable the functionality of functional proteins with conserved genetic regions (Locarnini and Bartholomeusz, 2002).

CHAPTER THREE

MATERIALS AND METHODS

3-1- Materials:-

3-1-1- Equipments and Instruments:

Table (3-1): The equipments and instruments that used in this study with their companies and countries of origin:

No.	Equipment & instrument	Companyl/ Origin
1	High Speed centrifuge	Techne (USA)
2	Oven	Memmert/Germany
3	Micropipettes 5-50, 0.5-10, 100-1000µl	Cyan/ Belgium
4	Eppendorf tubes	Bioneer/ korea
5	Multichannel pipette	Cyan/ China
6	Exispin vortex centrifuge	Bioneer/ Korea
7	Elisa Reader	Bio kit(USA)
8	Elisa Washer	Bio Tek(USA)
9	Micrometer stage	England

3-1-2- The kits used in this study with their companies and countries of origin:- (www.fortressdiagnostics.com)

Table (3-2):ELISA (IgG) HCV Kit (fortress)

NO.	Components	Volume
1	Micro well plate	1 block (96 wells)
2	Positive control	1 Vial (0.2 ml, antibodies diluted in protein stabilized buffer containing preservatives: o.1% proclin300).
3	Negative control	1 Vial (0.2 ml, protein stabilized buffer containing preservatives: o.1% proclin300).
4	Enzyme conjugate	1 Vial (13ml, Horseradish peroxidase (HRP)-Conjugated rabbit anti-human IgG antibodies).
5	Substrate solution A	1 Vial (8ml of 3, 3', 5, 5'-Tetramethyl-benzidine (TMB) dissolved in citric acid).
6	Substrate solution B	1 Vial (8ml of urea peroxide solution)
7	Stop solution	1 Vial (0.5M sulphuric acid,8ml,readey to use)
8	Sample diluents	1 Vial (13ml,protein-stabilized buffer, casein and sucrose solution)
9	Wash buffer	1Vial, 50ml,PH7.4,PBS(Containing Tween 20 as a detergent, concentrate[20x])
10	Cardboard plate cover sheets	2

3-2- Methods:-

3-2-1- Patients samples :

This study was carried out from Jan. 2016 to Dec. 2017, a total of 51812 Individuals were donors in the central blood bank at Al-Diwaiyahgovermente

3-2-2- Blood samples collection:

A Sample of 5ml of fresh blood was drawn from individual and collected in a sterile plastic tube, left to clot at room temperature then centrifuged at 2000 rpm for 10 minutes, then serum was collected in sterile tube and examined by ELISA Assay to detect anti HCV

3-2-3-Investigation of anti HCV antibodies (IgG) in serum by ELISA test (fortress):- 3-2-3-1-Principle

Anti-HCV enzyme immunoassay kit was a qualitative determination of Abs to HCV (anti-HCV) in human serum samples, diluted patient's sample (serum) was added to microtiter wells precoated with purified antigen mimicking the core, NS3, NS4, NS5 gene segments of HCV genome, these peptides have been shown to react and bind with the predominant classes of anti-HCV Abs present in HCV positive serum.

After incubation, peroxidase- conjugated anti-human IgG Ab was added to form a detectable complex, and then, substrate was added to form a colored complex. The intensity of color was proportional to the amount of anti-HCV present in the sample, then, the reaction was stopped by the addition of acid and the resulting color intensity can be read spectrophotometrically at 450 nm.

For the detection of antibodies to HCV antigens, ELISA (fortress) was used as following of the manufacture.

3-2-3-2-Preparation of reagents (according to manufacturer's instructions):

3-2-3-3- ELISA Procedure (according to manufacturer's instructions):

We add 100 µl of S.Diluent with 10 µl of S,NC,PC then covering the plate , numbering of wells and incubate for 30 min at 37c , After incubation, The plate cover was removed and discarded and each well was washed 5times with diluted wash buffer. The wells allowed soaking for 30-60 seconds after washed cycle, the strips plate was turned onto paper or clean towel, and tapped it to remove any remainders.100µl of HRP-Conjugate was added to each well except the Blank. The plate was covered with the plate cover and incubated for 30 minutes at 37 ^{0}C.
At the end of the incubation, the plate cover was removed, each well was washed 5times with diluted wash buffer
50 µl of cromagen A and 50 µl of cromagen B added to each well except the Blank , the well covered and incubated for 15 min at 37c then the cover was removed and 50µl stop solution added to each well and absorbanse was measured

Cut off = negative control + 0.12

Quality control range:-

- Positive: ratio absorbance ≥ cut-off
- Negative: ratio absorbance < cut-off
- Blank must be > 0.080

CHAPTER FOUR

RESULTS & DISCUSSION

4-1- Seroprevalence of HCV infection :-

Hepatitis C virus infection is a worldwide health problem, causing chronic hepatitis in approximately 85% of the cases, with a frequent progress to severe forms of liver damage like cirrhosis and hepatocellular carcinoma(Levrero, 2006).Serologic tests for detection of HCV antibodies are important first-line tests in screening and diagnosis of HCV infection, the presence of anti-HCV antibody in serum and plasma reflects exposure to the virus and may indicate an acute or chronic infection (Mahy and Van Regenmortel, 2010).

The present study was the results of serological examination by indirect Enzyme Linked Immunsorbent Assay (ELISA) for detection of Antibodies of HCV in central blood bank in Al-Diwaiyahgovermenteshowed that 25 out of 23800examined donors were positive in percentage 0.106%(Table 4-1)

Table (4-1): Positive number and total percentage of infected donors.

Total No. of samples	No. of positive samples	Percent %
23800	25	0.106%

4-1-1- Seroprevalence of HCV Infection in relation with Age.

The results of seroprevalence of HCV infection by using indirect ELISA in relation to the different age groups 20-30 years , 31-40 years ,41-50 years and 51->60 years old were 24% ,36% ,32% and 8% respectively, the highest rate of the seropositivity was in age groups31-40 years 36% and 41-50 years 32% and the lowest rate of seropositivity was in age groups 51->60 years old8% .

Table (4-2): Seroprevalence of HCV infection in different Age of donors.

Age (years)	No. infection	% of infection
20-30	6	24
31-40	9	36
41-50	8	32
51->60	2	8
Total	25	100

4-1-3- Results of Seroprevalence infection of HCV in relation with months of year.

The results of seropositivity of HCV infection by using ELSA in relation with different months of year showed that the seropositivity in 2016. The highest seropositivity percent was in February (3 cases) while the lowest seropositivity percent was inMay,August,November (0 cases).**(Tab 4-3)**

Months-year (2016)	No. of donors	No. of infection
January	1821	1
February	1933	3
March	1724	1
April	2136	2
May	1290	0
June	2350	1
July	2278	1
August	2177	0
September	2751	1
October	2293	1
Nvember	2254	0
december	1974	1
Total	24981	12

The results of seropositivity of HCV infection by using ELSA in relation with different months of year showed that the seropositivity in 2017 The highest seropositivity percent was in May(3 cases) while the lowest seropositivity percent was in March,August,October (0 cases)

(Table 4-4).

Months-year (2017)	No. of donors	No. of infection
January	1965	1
February	2122	2
March	2117	0
April	2587	1
May	2929	3
June	2040	1
July	2478	1
August	2178	0
September	2261	1
October	1804	0
Nvember	2423	2
December	1927	1
Total	26831	13

Conclusion

1-*HCV* is important life threating disease and reported examined blood doners

2-Most of positive individual were asymptomatic that may refer to carriers

3-The epidemiological risk factor play a role in the distribution of disease in different groups of study

Recommendation

1-Further study to evaluate the prevalence of *HCV in AL-Diwannia*

2-Using molecular techniqes to confirm the serological methods

3-Phylogenetic analysis of virus for detection its source

Appendix


80-1 TABLE TOP LOW SPEED CENTRIFUGE
PILOT LAMP
TIME
POWER
SPEED
MADE IN CHINA


EL x 50
bioelisa Washer
biokit


fortress
CE

Reference

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