Sudan University of Science and Technology
College of Graduate Studies

Detection of Hepatitis C Virus among Healthcare Workers in Ad Douiem Locality

الكشف عن فيروس إلتهاب الكبد الوبائي (س) بين العاملين في مجال الرعاية الصحية في محلية الدويم

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

BY

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الآية

قال تعالى:

{۞اللَّهُ نُورُ السَّمَاوَاتِ وَالْأَرْضِ ۚ مَثَلُ نُورِهِ كَمِشْكَوٰةٍ فِيهَا مِصْبَاحٌ ۖ الْمِصْبَاحُ فِي زُجَاجَةٍ ۖ الزُّجَاجَةُ كَأَنَّهَا كَوْكَبٌ دُرِّيٌّ يُوقَدُ مِن شَجَرَةٍ مُّبَارَكَةٍ زَيْتُونَةٍ لَّا شَرْقِيَّةٍ وَلَا غَرْبِيَّةٍ يَكَادُ زَيْتُهَا يُضِيءُ وَلَوْ لَمْ تَمْسَسْهُ نَارٌ ۚ نُّورٌ عَلَىٰ نُورٍ ۗ يَهْدِي اللَّهُ لِنُورِهِ مَن يَشَاءُ ۚ وَيَضْرِبُ اللَّهُ الْأَمْثَالَ لِلنَّاسِ ۗ وَاللَّهُ بِكُلِّ شَيْءٍ عَلِيمٌ۞}.سورة النور الآية(35)

صدق الله العظيم

DEDECATION

To

My Mother, Father, brothers and sisters

AKNOWLEDGEMENT

Piously my gratitude and prayers to almighty *Allah* for the mercy which followed me during the long way of this research. I owe so much to my supervisor Dr. Ahmed Ibrahim Hashim for his immense efforts, advices and guidance throughout the research. Special thanks to Ustaz Mohamed Karrar Abdalla and Mr. Mysara for the assistance with the ELISA methods and machine. Many thanks to the health care staff at Ad Douiem locality for their patience kindness.

ABSTRACT

Hepatitis C virus (HCV) is a parenterally transmitted virus that poses an occupational hazard to the health care workers (HCWs). This was a descriptive cross sectional study designed to detect HCV among health care workers in Ad Douiem locality. This study involved Ninety two (n=92) healthcare workers (Nurses 33, laboratory specialists 22, laboratory assistants 18, laboratory attendant 16, and pharmacists 3). The participants consisted of 47/92 males (51.1%) and 45/92 females (48.9%), 24/92(26.1%) subjects had accidental injury during work while 68/92 (73.9%) subjects never get accidental injury during work. Blood samples were collected from each subject and the serum was separated in plain containers, and then the serum samples were screened by ELISA for the presence of anti-HCV antibodies. None of the Health care workers were positive for HCV antibodies. In conclusion hepatitis C virus was not detected among Health care workers in Ad Douiem Locality. Further study with larger sample size is required to confirm these results.

المستخلص

فيروس التهاب الكبد الوبائي (س) هو فيروس منقول عن طريق الحقن الذي يشكل خطرا مهنياً على العاملين في مجال الرعاية الصحية . صممت هذه الدراسة الوصفية المقطعية للكشف عن فيروس التهاب الكبد الوبائي(س) بين العاملين في مجال الرعاية الصحية في محلية الدويم. شملت هذه الدراسة اثنان وتسعون شخصاً من العاملين في مجال الرعاية الصحية (33 ممرض, 22 اختصاصي مختبر, 18 مساعد المختبر, 16 عامل نظافة , 3 صيدلي)حيث شارك في هذه الدراسة92/47 (51.1%) من الذكور بينما 92/45 (48.9%)من الاناث. عدد الذين تعرضوا للإصابة اثناء العمل92/24(26.1%), بينما 92/68(73.9%) لم يتعرضوا للإصابة اثناء العمل.

جمعت عينات الدم من كل عامل في مجال الرعاية الصحية وفصل مصل الدم في حاويات خالية من مضاد التجلط ثم تم فحص مصل الدم لكل مشارك بواسطة فحص (اليزا) للكشف عن وجود الأجسام المضادة لفيروس التهاب الكبد الوبائي (س). لم يكن أي من العاملين في مجال الرعاية الصحية إيجابيين للأجسام المضادة لفيروس التهاب الكبد الوبائي.خُلصت الدراسة إلي أن فيروس التهاب الكبد الوبائي (س) لا يوجد في العاملين في مجال الرعاية الصحية في محلية الدويم. يرجى في المستقبل إجراء دراسات بحجم عينة اكبر لتأكيد هذه النتائج.

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Abbreviations	Full name
CLDN1	Claudin1
CD81	Cluster of differentiation 81
Cdna	Complementary deoxyribo-nucleic acid
CO	Cut-Off
DNA	Deoxyribo-Nucleic Acid
ELISA	Enzyme Linked Immunosorbant assay
ER	Endoplasim R
EphA2	Ephrin A2
EGFR	Epidermal growth factor receptor
HCV	Hepatitis C virus
HCWs	Health Care Workers
HBV	Hepatitis B virus
HVR1	Hypervariable region 1
INF	Interferon
LDLR	Low density lipoprotein receptor
NS3	Non structural protein 3
NANB	Non hepatitis A non hepatitis B
NCR	Non coding region
NT	Nucleotide
NPC1L1	Niemamn-pick C1-like 1 cholesterol absorption receptors
PBMCs	Peripheral blood mononuclear cells
RTK	Receptor tyrosine kinase
RNA	Ribonucleic Acid
RdRp	RNA-dependent RNA polymerase
SRB1	Scavenger receptor class B type 1
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

CHAPTER ONE

INTRODUCTION

1.1 Introduction

Hepatitis C virus (HCV) was first identified in 1989 but has been prevalent for many decades (Thorburn *et al*., 2001). According to the WHO, approximately 150 million people in the world are chronically infected with HCV, and hepatitis C is the cause of 350 000 deaths annually (Thorburn *et al*., 2001). Low endemicity areas include North America, Western Europe and Australia, where anti HCV anti-bodies <1.5%. Areas with intermediate endemicity Include Mediterranean countries and Asia (Anti -HCV 1-2%), while the highest endemicity has been detected in Africa, South- Eastern Asia and Latin America (Anti-HCV >2%) (Westermann *et al*., 2015). Healthcare workers (HCWs) are those (e.g., doctors, nurses, technicians, students, ward boys/ aaya and sweepers) who come in contact with patients blood and other body fluids from patients in a healthcare set up. Hepatitis C is hepatotropic blood borne viruses that are important cause of liver related mortality and morbidity (Doddaiah *et al.,* 2013). Transmissions of over 20 different pathogens have been reported in HCWs because of occupational exposure (Yazdanpanah *et al*., 2006). Incidence of HCV seroconversion after accidental needle stick exposure is uncertain; with reports ranging from 0-10% (Thorburn *et al*., 2001).Three percent of HCV infection in HCWs is due to occupational exposures (Taher *et al*., 2016). This virus compared to a viral time bomb is leading hepatotropic virus and predominant cause of severe pathological consequences like acute hepatitis, chronic liver diseases and hepatocellular carcinoma (Umar *et al.*, 2010)

1.2 Rationale

Healthcare workers are at risk of contracting infection with HCV through close contact with patients' blood or other body fluids. Moreover, patients can get HCV infection from infected HCWs in any health care setting. The WHO stated that there is evidence that blood borne viruses can be transmitted from infected HCWs to patients during exposure prone procedures (EPPs) (WHO, 2014). The incidence of HCV seroconversion after accidental needle stick exposure is uncertain, with reports ranging from 0 to 10 % (Thorburn *et al*., 2015). There is no pre and post exposure prophylaxis for HCV and most hospitals in Sudan lack educational programs and training about possible risks and prevention of blood borne infections after occupational exposure. The paucity of information about HCV infection among Sudanese HCWs particularly in States other than Khartoum State encourages immediate research in this field. The aim of this study was to determine the prevalence of HCV among HCWs in Ad Douiem locality in White Nile State.

1.3 Objectives

1.3.1 General objective

To determine the seroprevalence of HCV infection among HCWs in Ad Douiem Locality

1.3.2 Specific objectives

1. To detect the seropositivity of Anti-HCV antibodies in HCWs by enzyme linked immunosorbant assay
2. To determine the possible risk factors associated with HCV infection among HCWs in Ad Douiem locality

CHAPTER TWO

LITERATURE REVIEW

CHAPTER TWO

LITERATURE REVIEW

2.1 Historical background

A series of hepatitis infections following blood transfusions was observed that could not be identified as either hepatitis A or hepatitis B, and were therefore designated as “non-A-non-B (NANB) hepatitis.”The discovery of the hepatitis C virus (HCV) by molecular biological means in1988 was an elegant piece of work: RNA was extracted from the plasma of an infected chimpanzee, from which cDNA was produced using reverse transcriptase. The cDNA was then cloned and the corresponding proteins expressed. About one million clones were tested for reactivity with sera from patients suffering from chronic NANB hepatitis. A protein was found by this method that reacted with antibodies to NANB, whereupon the corresponding cloned DNA was used as a probe to identify further overlapping gene segments (Kayser *et al.*, 2005). They identified a clone termed *5-1-1* that expressed an antigen that was shown to be specific for NANBH, The expression product of clone 5-1-1 became the basis for the first serologic assay for antibody to the NANBH agent, which was now termed Hepatitis C virus (HCV), They found that at least 80% of patients with post transfusion NANBH developed antibody to 5-1-1, and 58% of patients with chronic NANBH without a known exposure to blood had antibody. The 5-1-1 antigen remains a part of the commercial multi antigen test for HCV antibody in use today (Major *et al*., 2001).The hepatitis C virus belongs to the genus Hepacivirus a member of the family Flaviviridae. Until recently it was considered to be the only member of this genus. However a member of this genus has been discovered in dog's canine Hepacivirus (Kapoor *et al.*, 2011). Hepatitis C

virus is predominantly a blood borne virus, with very low risk of sexual or vertical transmission (Shepard *et al.,* 2005)

2.2 Epidemiology

Hepatitis C is found worldwide. The most affected regions are Central and East Asia and North Africa. The hepatitis C epidemic can be concentrated in certain high-risk populations (for example, among people who inject drugs); and/or in general populations. There are multiple strains (or genotypes) of the HCV virus and their distribution varies by region (WHO, 2014). In the United States, the annual number of newly acquired acute hepatitis C virus (HCV) infections has declined from an estimated 180,000 in the mid 1980s to an estimated 28,000 in 1995. Approximately 25% to 30% of these infections are clinically apparent cases that are sufficiently symptomatic to gain medical attention. Deaths from fulminant hepatitis C are rare. The prevalence of antibody to HCV (anti-HCV) in the general population of the United States is 1.8%, corresponding to an estimated 3.9 million Americans infected with HCV, and an estimated 8,000 to 10,000 deaths each year result from HCV–associated chronic liver disease. HCV infection affects persons of all ages, but most acute cases of hepatitis C and the highest prevalence of anti-HCV are found among young adults (Alter, 2011). Globally, there exist differences in the geographic distribution patterns of HCV genotypes and subtypes. In general, 1a, 1b, 2a, 2b, and 3a are found worldwide, whereas most of the others are restricted to certain geographic regions. However, such patterns are constantly evolving as a result of human migration and global travels (Simmonds *et al.,* 2005). Studies of hepatitis C virus in Sudan showed a low seroprevalence of 2.2%–4.8% (Hatim, 2008).

2.3 Classification

HCV is an RNA virus of the Hepacivirus genus of the Flaviviridae and is related to viruses of the animal Pestivirus genus (Haaheim *et al*., 2002).

HCV has been classified as the only member of the genus Hepacivirus and grouped together with the genera pestivirus, Flavivirus, and tentatively the GB-viruses, in the family flaviviridae. According to phylogenetic analyses, HCV is more closely related to the pestiviruses than to the flaviviruses. Based on genomic heterogeneity, six major genotypes, having more than 30% nucleotide sequence divergence, and more than 70 subtypes differing from each other by 10–30% at the nucleotide sequence level, have been defined. Subtypes are designated by lowercase letters following the number of the genotype (e.g., genotype 1 Subtype b¼1b). While genotype 1 and 2 viruses are prevalent almost worldwide, HCV genotypes 3–6 are to a large extent restricted to distinct geographical regions, including the Indian subcontinent and Southeast Asia (genotype 3), Africa and Middle East (genotype 4), South Africa (genotype 5), and Southeast Asia (genotype 6)(Brain and Marc, 2008).

A common system for nomenclature of HCV viral genome which has combined the various approaches has been proposed; the types classified as I, II, III, IV, and V (23) correspond to types la, lb, 2a, 2b, and 3a, respectively(Macdonald *et al*., 1996) . The hepatitis C virus (HCV) exhibits a high degree of genetic diversity and complexity and is classified into seven genotypes and 67 confirmed and 20 provisional subtypes (Smith *et al*., 2014).

2.4 Virological properties

2.4.1 Virion structure

The genome of HCV was sequenced and characterized long before virus-like particles were described. It was shown early on that HCV is inactivated by chloroform, indicating that particles are enveloped, and it was shown through filtration that the diameter is between 30 and 60 nm. Low levels of virus in plasma samples and problems of *in vitro* cultivation have made visualization of this virus difficult; however, virus like particles have been identified using electron microscopy shows spherical particles about 50 nm in diameter, (Major *et al*., 2001). By analogy to other flaviviruses, HCV particles are composed of at least the genomic RNA, the core protein, and the two envelope proteins E1 and E2 which are embedded into the lipid envelope. The core protein forms the internal viral capsid (presumably 30–35nm in diameter) that shelters the single stranded RNA genome (Brain and Marc, 2008).

2.4.2 Genome

The genome of HCV is single 9.5 Kb molecule of ssRNA of positive polarity, with gene order characteristic of family flaviviridae. A single long ORF encoding a polyprotein of about 3000 amino acid is flanked by untranslated 5´and 3´ seguences each containing short direct repeats and taking the form of hairpin. The 3´ is not poly adenylated. The structural proteins occupy the 5´quarter of ORF and the non structural proteins the reminder. The gene order, namely 5´C, E1, E2/ NS1, NS2, NS3, NS4, NS5-3´ closely resembles that of other members of flaviviridae. Structural protins C is highly basic and presumably represents the core(capsid) of virion E1and E2 are glycoproteins presumably both membrane proteins as in the genus pestivirus ,although it is possible E2 may be equivalent of the non structural proteins NS1 of genus flavivirus

which is otherwise missing from hepatitis C virus ,of the four other non structural proteins ,it can be deduced from characteristic motifs that as for other members flaviviridae , NS3 carries serine protease activity in its amino-terminal half and helicase activity in it is carboxy –terminal half ,where NS5 has RNA dependent RNA polymerase activity and NS4 ,NS2 may be comparable with the membrane binding proteins postulated to be required by other flaviviruses during membrane associated replication(White and Fenner ,1994). HCV genomes of different genotypes vary by 31–33% at the nucleotide level where as subtypes differ by 15–25 %(Li *et al*., 2014)

2.4.3 Genome organization

Its genome consists of a relatively long (341-nt) 5′ noncoding region (5′NCR), a large open reading frame, which encodes 3010-3033 amino acid residues, and a short 3′ NCR followed by a homopolymer tail of A (Yamada *et al*., 1996) or U residues By analogy with flaviviruses, this polypeptide can be divided into a 5′ structural region consisting of the putative core and envelope proteins and a 3′ region corresponding to nonstructural (NS1 to NS5) proteins. HCV shows substantial NT sequence diversity throughout the viral genome and has been classified into several genotypes to date. The 5′ NCR is highly conserved among all genotypes and it shares significant sequence and secondary structure homology with those of pestiviruses, suggesting an important role for the 5′ NCR in both replication and polypeptide translational initiation (Yamada *et al.*, 1996). The polypyrimidine tract between NT 191 and 199 is an important site for translation initiation and is the binding site for the cellular protein p89 (Yamada *et al*., 1996). Contrary to the 5′ NCR, the sequence of the 3′-NCR consists of a short sequence (27–70 NT), which was largely diversified among different genotypes, and a homopolymer tail of U residues. Putative stem-loop structures have been identified in

the region both proximal to the 3´ end of the coding region and the 3´ NCR of the genome in all HCV groups in spite of considerable primary sequence differences (Yamada *et al*., 1996)

2.5 The viral proteins

The functional HCV proteins are generated from the polyprotein following co translational and post translational cleavage by cellular and viral proteases. . The structural proteins are characterized by hydrophobic domains at the C termini that are important for membrane association and subsequent cleavage by the signal peptidases, localized in the lumen of the endoplasmic reticulum. Cleavage at the junction between NS2 and NS3 occurs auto proteolytically by a protease encoded by NS2 and the N-terminal portion of NS3 , whereas the cleavages of the other downstream NS proteins are mediated by a distinct virus-encoded serine protease located in the N-terminal third of NS3 (Major *et al*., 2001). The first 191 amino acids of the HCV polyprotein represent the putative nucleocapsid or core protein with a molecular weight of 21 to 23 kDa, this region is highly conserved. The glycoproteins (gp) E1 (gp31) and E2 (gp70), representing the putative viral envelope proteins. The NS2 protein is cleaved from the polyprotein at its N-terminus by a cellular signal peptidase and at its C-terminus by an HCV protease encoded by most of the NS2 region and part of the NS3 domain. The NS3 encodes serine protease, NTPase, and RNA helicase activities. The serine protease activity has been particularly well characterized and probably plays an essential role in HCV processing. The NS4 region encodes two viral proteins designated NS4A and NS4B. The function of NS4B, a very hydrophobic protein, is unknown, although it is required for the phosphorylation of NS5A together with NS4A and NS3. NS4A has also been shown to act as a cofactor in NS3 protease activity. The NS5 region is processed into the proteins NS5A and NS5B (Major *et al*., 2001).

2.5.1 Structural proteins

HCV core is the viral nucleocapsid protein with numerous functionalities involving RNA binding, immune modulation, cell signaling, oncogenic potential and autophagy. HCV core protein also associates with the lipid droplets where HCV assembly also takes place. HCV E1/E2 is glycosylated envelope glycoproteins that surround the viral particles. HCV envelope is targeted by virus neutralizing antibody selection pressure with high degree of sequence variation that may render antibody responses ineffective and contributes to HCV persistence. The small ion channel protein p7 is downstream of the envelope region and is required for viral assembly and release (Kim and Michang, 2013).

2.5.2 Nonstructural proteins

NS2 is the viral auto protease that plays a key role in viral assembly, mediating the cleavage between NS2 and NS3. NS3 encodes the N-terminal HCV serine protease and C-terminal RNA helicase-NTPase. NS3 protease play a critical role in HCV processing by cleaving downstream of NS3 at 4 sites (between NS3/4A, NS4A/4B, NS4B/NS5A, NS5A/NS5B). It also cleaves the TLR3 adaptor protein TRIF and mitochondrial antiviral signaling protein MAVS, thereby blocking the cellular type I IFN induction pathway. NS3 is one of the key targets for HCV antiviral drug development. NS4A forms a stable complex with NS3 and is a cofactor for NS3 protease. The role of NS4B is not well understood, although it is known to induce the membranous web formation. NS5A is a dimeric zinc-binding metalloprotein which binds the viral RNA and various host factors in close proximity to HCV core
And lipid droplets. Inhibitors of HCV NS5A showed antiviral effect in patients and are in rapid clinical development. Finally, NS5B is the RNA-dependent RNA polymerase (RdRp) which is also being actively targeted

for antiviral drug development. Collectively, these proteins also contribute to various aspects of HCV life cycle, including viral attachment, entry and fusion, HCV RNA translation, posttranslational processing, HCV replication, virus assembly and release (Kim and Michang, 2013).

2.6 Replication

Viral entry into the host cell involves a complex series of interactions Including attachment, entry and fusion. The initial viral attachment to its receptor/co-receptors may involve HVR1 in HCV E2 (Rosa *et al*., 1996) with facilitation by heparan sulfate proteoglycans expressed on hepatocyte surface. (Barth *et al*., 2003) While LDL receptors (LDLR) can bind HCV and promote its cellular entry(Angello *et al*., 1999) HCV-LDLR interaction may be non-productive and can potentially lead to viral particle degradation (Albecka *et al*., 2012). Following attachment to the entry factors, HCV is internalized into the target cells via a pH-dependent and clathrin mediated endocytosis (Blanchard *et al*., 2006). Multiple cellular receptors and entry factors for HCV have been identified, including the scavenger receptor class B type I (SRB1) and CD81 as well as tight junction proteins, claudin-1 (CLDN1) and occludin (OCLN), Additional recently identified entry factors include the receptor tyrosine kinases (RTK) epidermal growth factor receptor (EGFR), ephrin receptor A2 (EphA2) and Niemann-Pick C1-like 1 cholesterol absorption receptor (NPC1L1) (Kim and Michang, 2013). The first two HCV entry factors (SRB1 and CD81) were identified as binding partners of HCV E2 (Scarselli *et al*., 2002).

Following target cell entry through receptor-mediated endocytosis, HCV particle undergoes pH-dependent membrane fusion within an acidic endosomal compartment to release its RNA genome into the cytoplasm. HCV polyprotein is translated in rough ER with the positive strand HCV

RNA as the template, with the translation initiated in a cap-independent manner via the IRESin the 5'NTR. HCV translation yields a single polyprotein precursor of approximately 3000 amino acid in length that is further processed by cellular (e.g. signal peptidases) and viral proteases (NS2, NS3/4A) to generate 10 individual viral proteins, including core and envelope glycoproteins, E1 and E2, p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B as mentioned above(Kim and Michang K.2013). In the course of polyprotein processing, the HCV proteins are seen to be associated with a "membranous web" which includes double-membrane vesicles containing HCV nonstructural proteins, HCV RNA, ER membranes and lipid droplets. The membranous web in HCV-expressing cells appears to be induced by HCV NS4B possibly in combination with NS5A. Viral RNA replication is believed to occur in these webs with the positive strand RNA genome as a template for the NS5B RdRp to generate the negative strand replicative intermediate, to produce further positive sense genomes. Nascent positive strand RNA genomes can be further translated to produce new viral proteins, or serve as templates for further RNA replication, or be assembled to infectious virions. Various cellular factors are involved in HCV replication, including cyclophilin A and phosphatidylinositol 4 kinase IIIα (PI4KIIIα). Cyclophilin A can modulate RNA-binding capacity of NS5B polymerase and interact with NS5A. As such, cyclophilin inhibitors have antiviral effect against HCV with clinical development ongoing.76-80 As for PI4KIIIα; it is a lipid kinase that is recruited to the membranous web by NS5A, required for HCV replication and provide integrity to the membranous viral replication complex (Kim and Michang, 2013).

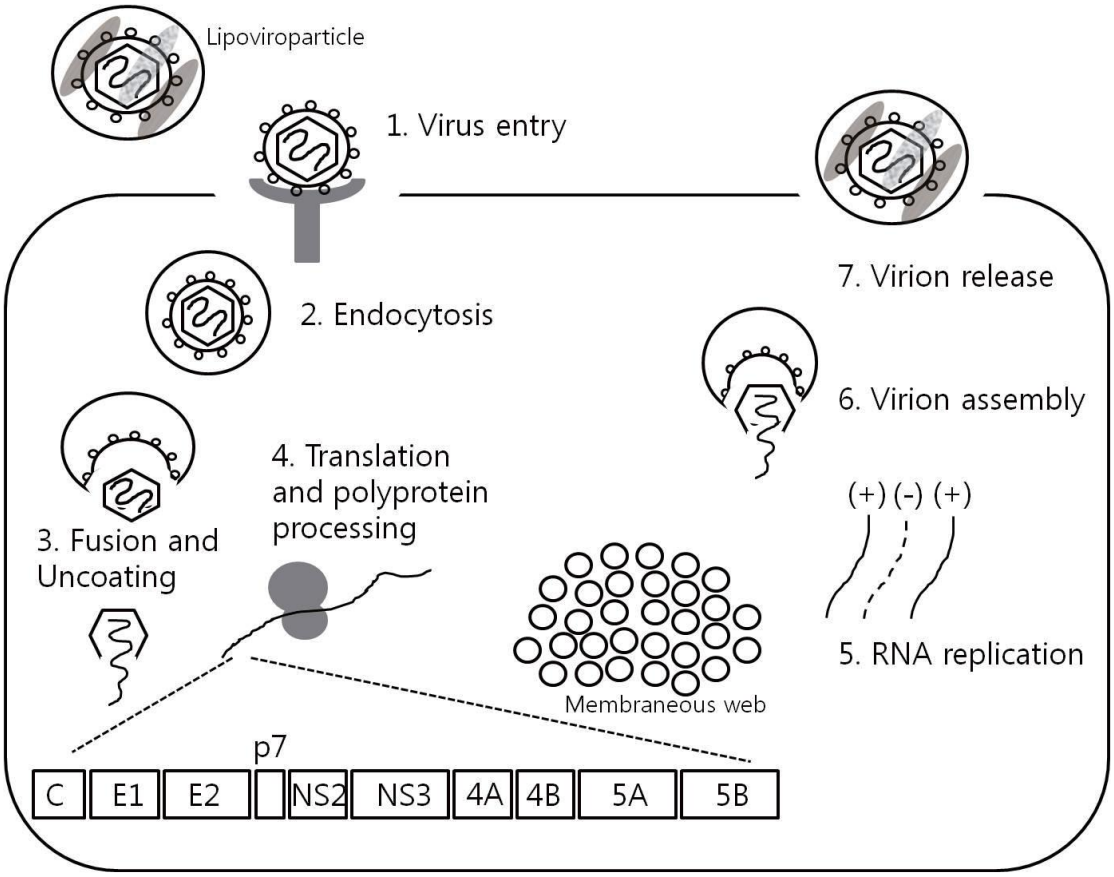

Figure1. Schematic representation of the HCV life cycle (Ploss and Dubuission, 2012)

2.7. Transmission

HCV is mainly transmitted by parenteral exposure to blood and blood products. The development of effective screening tests for blood and blood products and the implementation of viral disinfection procedures have almost excluded this route of transmission in countries where these measures are in place. Thus, the major remaining risk factor for acquiring HCV infection in developed countries is the use of contaminated needles in injection drug use. In some countries, HCV infection has been spread primarily by the use of inadequately sterilized medical instruments. In contrast to HBV infection, sexual transmission and maternal–infant spread of HCV is much less frequent (Brain and Marc, 2008)

2.7.1 Risk of transmission to health care workers

Health care workers may be at greater risk of hepatitis C than the general UK population but the prevalence of anti-HCV in such staff is lower than among health care workers in the US, and western Europe. In the UK, the overall prevalence of infection was estimated to be 0.23% among all health care workers and 0.28% in those at risk of occupational contact with blood and body fluids (Ramsay *et al*., 1999). In the US, a review of published studies in health care workers who received a needle stick injury from an anti-HCV positive source estimated the risk of transmission to be 1.8% (range 0%-7%). In a recent meta-analysis, the risk of transmission was shown to be greater if the source patient was known to be positive for HCV RNA; no transmission occurred from HCV RNA negative sources (Dore et *al*., 1997).

2.7.2 Risk of transmission from health care workers

Only two episodes of transmission from an HCV infected surgeon to patients have been described to date (Esteban et *al.*, 1996). In the UK, transmission from an HCV infected surgeon was implicated in a single case of acute hepatitis C detected after cardiothoracic surgery (CDSC, 1995). In the look back investigation that followed, 277 patients were tested but no other infected individuals were identified. This suggests that the risk of transmission from health care worker to patient is much lower than the risk of transmission from surgeons positive for hepatitis B e antigen (Duckworth *et al*., 1999). Based on this evidence, health care workers with HCV infection in the UK are not restricted from performing exposure prone procedures unless they have been shown to transmit hepatitis C to a patient (CDSC, 1995). Nevertheless, health care workers with HCV infection should be seen in occupational health departments to be advised on scrupulous adherence to the optimal precautions for control

of blood borne virus infections in order to reduce the potential risk of transmission during exposure prone procedures (Ramsay, 1999). In addition, infected health care workers should be advised about the local arrangements for the reporting, assessment, and management of any incidents in which patients appear to have been exposed to a health care workers blood. Patients who sustain a significant exposure to blood should be managed in the same way as exposed health care staff (Ramsay, 1999).

2.8 Tissue Tropism and Host Range

Hepatocytes are considered to be the natural target cells for HCV. Viral RNA was also detected in peripheral blood mononuclear cells (PBMCs) and bone marrow cells but it is unclear whether productive infection occurs in these cells. In cell culture, HCV RNA replication was demonstrated in non-liver cells like human T- and B-cell lines or embryonic kidney cells. Furthermore, certain mouse cell lines can support replication of HCV replicons demonstrating that the viral replication machinery is also functional in a murine host cell environment (Brain and Marc, 2008)

2.9 Clinical features

2.9.1 Acute hepatitis

The incubation period for hepatitis C averages about 7 weeks, with a range of 2 to 26 weeks, after exposure as measured by prospective studies of transfusion-associated hepatitis and needle sticks. The clinical picture of acute hepatitis C illustrated in resembles other forms of acute viral hepatitis. However, symptoms of malaise, nausea, and right upper quadrant pain followed by dark urine and jaundice appear in only about one third of patients. Biochemical evidence of hepatitis, such as elevated levels of serum ALT, is observed in more than 80% of cases, with levels

typically 10 times normal or higher. ALT elevations usually coincide with symptoms, and both tend to resolve in 2 to 12 weeks. Hepatitis C RNA can be detected in the serum early after exposure, usually within the first 3 weeks. In acute resolved cases, the RNA disappears as then disease resolves. HCV is rarely associated with fulminant hepatitis, but it has been reported. There are no recognized sequelae in patients whom resolve hepatitis C infections. However, patients in whom HCV resolves do not in general appear to be protected from re-infection. Multiple bouts of hepatitis C have been reported in patients repeatedly treated with blood or plasma products. Liver biopsy is not recommended for the diagnosis of acute viral hepatitis, but in cases in which it has been performed, the histologic changes in addition to lymphocytic infiltrates include distinctive eosinophilic clumping of hepatocyte cytoplasm, acidophilic bodies, microvesicular steatosis, and activation of the sinusoidal cells (Major *et al.*, 2001).

2.9.2 Chronic hepatitis

The single most important feature of HCV infection is the propensity of the virus to cause persistent infection. About 85% of patients infected remain so for more than 6 months, most for the remainder of their lives. These chronically infected patients are the source of almost all new infections and themselves are at increased risk for the development of significant chronic liver disease, cirrhosis, and HCC. Many chronically infected patients do not have symptoms and would not be detected unless tested by their physicians or if they donate blood. Others may develop severe chronic liver disease over only a few years or, more commonly, over several decades Patients with chronic HCV infection may experience increased fatigue as their only symptom. Others may have overt symptomatic liver disease with anorexia, nausea, right upper quadrant pain, dark urine, and pruritus. ALT levels frequently fluctuate

over time and may be normal or significantly elevated in the same patient measured at different times; whereas others have persistently normal or persistently elevated ALT levels (Major *et al*., 2001).

2.10 Mechanism of Pathogenesis and interferon resistance

Once the virus enters the hepatocytes through receptor mediated endocytosis and starts replication, it initiate damaging of hepatocytes, the major component of which is through the host's own immune response (Nelson, 2001). Interferon is the most potent natural weapon of the host against intra-cellular viral infection. HCV, however, owing to intricate actions of its genomic proteins is equipped with ability to evade the natural interferon-mediated clearance. HCV core protein has been reported to decrease the robustness of the host's immune response by decreasing transcription of interferon induced antiviral genes, HCV NS3/4A protease also has been concerned in inhibiting the interferon amplification loop which otherwise results in suppression of HCV replication. Inhibition of HCV protease can reverse the effects of HCV infection that make protease inhibitors one of the most noteworthy potential therapeutic agents for HCV (DeLucas *et al.,* 2005; Karayiannis, 2005).

2.11 Risk factor of HCV infection

Any behavior, occupation, or medical condition that results in frequent percutaneous exposure to blood presents the most important risk for acquiring hepatitis C shows the changing importance of several selected risk factors associated with acute HCV infection from 1983 through 1996. In studies performed before specific screening tests for HCV infections were available, there was a significant association of acute NANBH and a history of transfusion within the 6 months preceding the transfusion. The other major risk factors include sexual exposure,

occupation, and household contact. At least 10% of acute HCV cases have no epidemiologic factors identified. In a study of the risk factors associated with chronic hepatitis among blood donors who tested positive for anti-HCV, a history of drug use, including cocaine snorting, was the most common risk factor, followed by sexual promiscuity, transfusion, imprisonment, tattooing, needle stick, and acupuncture. Only sexual promiscuity would not be considered a parenteral exposure (Major *et al.*, 2001).

2.12 Complications

The major long-term risks of chronic HCV infection are cirrhosis with hepatic failure and hepatocellular carcinoma. Cirrhosis develops in 20% of cases and has a poor prognosis. About 25% of cirrhotic patients will develop liver failure. About 1 in every 20–30 infections will die from complications of the liver disease caused by the infection. Hepatocellular carcinoma occurs most frequently in cirrhotic patients who have longstanding liver disease. HCV is now the most common risk factor for hepatocellular carcinoma in many areas of the world, including Japan.

Extra hepatic manifestations of infection include mixed essential cryoglobulinaemia and glomerulonephritis. Although cryoglobulinaemia is common (450% of patients with cirrhosis) it is not often symptomatic or progressive (Haaheim *et al.*, 2002).

2.13 Immunity

2.13.1 Innate Immunity

Both in cell culture and in the majority of patients treated with IFN-a, a rapid and efficient block of HCV replication occurs. This result is somewhat surprising given the high rate of persistence of HCV infections (50–80%) and the finding that, in infected liver, type 1 IFN-induced genes are activated. In several studies it was concluded that HCV

proteins, such as core, interfere with the various steps of the IFN-a/b-induced signaling and that some HCV proteins appear to block individual IFN-a/b-induced effectors. One prominent example is NS5A, assumed to Block activity of the double-strand RNA-activated protein kinase PKR by binding to PKR via a particular NS5A region. This region overlaps with the so-called interferon sensitivity- determining region in NS5A, assumed to correlate with outcome of antiviral therapy. However, this original Assumption is still contradictory and it is still unclear whether HCV indeed interferes with one or several type 1 IFN-induced effectors molecule(s). It also remains to be clarified by which mechanism interferons block HCV RNA replication. Much less controversial are the mechanisms by which HCV interferes with the induction of innate antiviral defense. Several studies have shown that the NS3 protease proteolytically cleaves two signal-transducing molecules: TRIF, linking the activation of Toll-like receptor 3 to kinase complexes responsible for the phosphorylation of interferon response factor-3 (IRF-3) and CARDIF (also called MAVS, ips-1, VISA) relaying the activation of retinoic-acid-inducible gene 1 (RIG-1) also to IRF-3 phosphorylation. As a result, IRF-3-dependent genes are not expressed including IFN-b and IRF-7 and cells Remain sensitive to virus infection. Although linking the block of IFN-b expression to persistence is attractive, it is unclear if and to what extent that is the case. On one hand blocking IRF-3 activation would not affect the antiviral program induced by type 1 IFN (e.g., produced by activated dendritic cells or administered during therapy), whereas on the other hand this block may affect the secretion of cytokines required for the development of a vigorous adaptive immune response and its attenuation may facilitate persistence(Brain and Marc, 2008).

2.13.2 Adaptive Immunity

The role of HCV-specific antibodies in controlling viral infection is not clear. They appear to be dispensable for viral clearance and do not protect from reinfection, neither in experimentally infected chimpanzees nor in Humans after multiple exposures to HCV. However, there is evidence that the presence of HCV-specific antibodies at least partially attenuates infection. For instance, antibodies neutralizing HCV virions of different genotypes have been detected in sera of chronic hepatitis C patients but the frequency of these antibodies appears to be low. Control of acute HCV infection is primarily achieved by a rigorous and multi specific T-cell response. Thus, successful antiviral response generally encompasses Multiple major histocompatibility complex (MHC) class-I and class-II restricted T-cell epitopes and a profound expansion of CD8 and CD4Tcells. In contrast, persistent infections are characterized by oligoclonal T-cell responses and a low frequency of HCV-specific T cells. The underlying reasons for the weak response in the majority of patients are not clear but several possibilities have been suggested: (1) an impaired antigen presentation that might be due to interference of HCV With dendritic cell function; (2) CD4 T-cell failure due to deletion or anergy; (3) mutational escape in important T- (and B-) cell epitopes; and (4) functional impairment of HCV-specific CD8 T cells. How T-cell Impairment is brought about is unclear but one attractive possibility is that the defect induced in innate immunity results in a defect in CD4T-cell help. In fact, HCV-induced loss of T-cell help appears to be the key event of immune evasion (Brain and Marc, 2008).

2.14 Laboratory diagnosis

The common tests used for diagnosis of HCV infection were designed primarily for screening of blood donors. These assays are based on detection of serum antibody to various HCV antigens because these antibodies are nearly universally present in patients who are chronically infected with HCV. Acute HCV infections are relatively rare among blood donors, but the antibody tests often fail to detect these patients in the window period between the time of infection and the time of appearance of antibody detectable by the assay. Therefore, the antibody assays also have limited utility for diagnosis of patients with acute HCV infections. Tests for HCV RNA genome detection based on the PCR or other highly sensitive RNA detection systems have been used for the diagnosis of acute hepatitis (Major *et al.,* 2001).

2.14.1Serologic Assay

Routine screening tests for detecting HCV infections are based on serological assays measuring HCV-specific antibodies (most often by enzyme-linked immunosorbant assay (ELISA)) and nucleic acid-based tests to determine viral RNA. Current ELISA assays have specificity of >99% and they are positive in 99% or more of immunocompetent Patients in whom viral RNA is detectable (Brain and Marc, 2008). Supplemental tests have been developed to help exclude false-positive ELISA results. The most commonly used supplemental assay is the recombinant immunoblot assay RIBA (Major *et al.*, 2001).

2.14.2 Molecular assay

Sensitive tests for HCV RNA based on RT-PCR or other nucleic acid amplification techniques can be used during the window period (Major *et al.,* 2001). Qualitative RNA detection assays have been implemented in many blood banks in European Union countries and in the US. The risk to

acquire transfusion-associated hepatitis C in such countries has been reduced to <1/million blood donations. Determination of HCV genotypes, which is an important parameter for current antiviral therapy, is based on analyzing viral RNA either by hybridization or direct sequence analysis or by using genotype-specific primers for PCR (Brain and Marc, 2008).

2.14.3 Cell culture

Development of efficient cell culture systems for HCV propagation was difficult and was not successful until more than 10 years after the discovery of the virus. The first breakthrough was the development of sub genomic replicons composed of the NTRs, a selectable marker (neo), and a heterologous IRES directing translation of the HCV replicase (NS3–NS5B). When transfected into a human hepatocarcinoma cell line (Huh-7) and subjected to selection (e.g., with G418 in case of replicons containing the neo gene), stable cell lines were established that carry autonomously replicating HCV replicons. These viral RNAs replicate to very high levels and are maintained persistently when the cells are passaged under conditions of continuous selective pressure (e.g., G418). Owing to its high efficiency, this replicon system was of enormous value for studying HCV replication and HCV–host interaction, and for the development of antivirals targeting any of the viral replicase components (e.g., NS5B RdRp or the NS3 protease) (Brain and Marc, 2008).

2.15 Treatment

Interferon (alpha or beta) is the only treatment known with activity against HCV. Interferon treatment reduces the chronicity rate in acute infection. Unfortunately most patients are asymptomatic during this phase and therefore do not come to medical attention. The current standard of care for chronic hepatitis C infection is pegylated interferon in combination with oral ribavirin. A 12 month course of this drug

combination is effective in permanently eradicating infection in about 55% of cases. Viral genotypes 2 and 3 respond better and 80% clear infection with just 6 months of treatment (Haaheim *et al.*, 2002).

2.16 Prevention

Prevention of hepatitis C at present is based on prevention of exposure to Contaminated blood by screening of blood and plasma donors, identification of carriers by testing high-risk individuals, and public health measures designed to prevent spread. No effective vaccine has been developed to prevent HCV infection. In developed countries, screening of blood donors has virtually eliminated transmission of HCV by transfusion (Major *et al.*, 2001). HCV infection can only be prevented by avoiding contact with the virus. There is no vaccine and the heterogeneity of the virus makes it difficult to develop a conventional vaccine in the near future. Pre- and post exposure prophylaxis with immunoglobulin is ineffective (Haaheim *et al.*, 2002).

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design

This was a descriptive cross sectional study.

3.2 Study area

The study was carried out in Ad Douiem locality in White Nile State.

3.3 Study duration

The study was conducted between January and May 2017.

3.4 Study population

Healthcare workers including (laboratory specialists, nurses, laboratory assistants, pharmacists and laboratory attendants).

3.5 Ethical consideration

Ethical approval to conduct this study in the region was obtained from the health services director in Ad Douiem locality and verbal consent was obtained from participants before collection of the blood samples.

3.6 Sample size

Ninety two blood samples (n=92) were collected in sterile plain containers from healthcare workers.

3.7 sample collection:

Five ml of venous blood were collected into plain containers under aseptic technique from each health care worker under study. The

specimens were centrifuged at 5000 rpm for five minute, the serum separated into plain vacutainers then stored at -20Cº until used.

3.8ELISA principle

This kit employs solid phase, indirect ELISA method for detection of antibodies to HCV in two-step incubation procedure. Polystyrene micro well strips are pre-coated with recombinant, highly immunoreactive antigens corresponding to the core and the non-structural regions of HCV (fourth generation HCV ELISA).during the first incubation step, anti HCV specific antibodies ,if present ,will be bound to the solid phase pre-coated HCV antigens. The wells are washed to remove unbound serum proteins and rabbit anti-human IgG antibodies (anti-IgG) conjugate 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 solutions containing tetramethylbenzidine (TMB) and urea peroxide are added to the wells and in the presence of the antigen –antibody-anti IgG (HRP) immunocomplex the colorless chromogens are hydrolyzed by the bound HRP conjugate to a blue color product. The blue color intensity can be measured and is proportional to amount of antibody captured in the wells, and to the sample respectively. Wells containing samples negative for anti-HCV remain colorless.

3.9 ELISA Procedure

All reagents and sera were maintained to room's temperature (18-30ºC) for at least 15-30minutes. The wash buffer concentrate was checked for the presence of salt crystals. If crystals have formed in the solution, re-solubilized by warming at 37°C until crystals dissolve. The stock wash

buffer was diluted 1 to 20 with distilled or deionized water and only cleans vessels were used to dilute the wash buffer. The strips needed were set in strip-holder and numbered the wells including three negative controls (e.g. B1, C1, D1), tow positive controls (e.g. E1, F1), and one Blank (A1, neither samples nor HRP-conjugates should be added into the Blank well). Hundred micro liters specimen diluents were added into each well except the Blank. Ten micro liters of positive control, negative control and specimens were added into their respective wells. The plate was covered with plate cover and incubated for 30 min at 37_oC. It recommended being used thermostat-controlled water tank to assure the Temperature stability and humidity during the incubation. After the end of incubation, the plate cover was removed and discarded. Each well was washed 5times with diluted wash buffer. Each time the micro wells were allowed to soak for 30-60 seconds. After the final washing cycle the strips plate were turned onto plotting paper or clean towel and taped to remove any remainders. Hundred micro liters HRP conjugate was added to each well except the blank, and then the plate was covered with plate cover and incubated for 30 minute at 37 °C. At the end of incubation the plate cover was removed and discarded and wash each well 5times with diluted wash buffer as in step 10.

Fifty micro liter for Chromogen A and B were dispensed into each well including the Blank and mixed by tapping the plate gently and incubated at 37° C for 15 min avoiding the light. The enzymatic reaction between the Chromogen A/B solutions produced blue color in positive control and anti-HCV positive wells. Fifty micro liter stop solution was added into each well and mixed. Intensive yellow color developed in positive control and anti-HCV positive wells. The plate reader was calibrated with the Blank well and read the absorbance at 450 nm. If a dual filer instrument

was used, set the reference wavelength at 630 nm, and the cut-off value was calculated and evaluated for the results.

Note: The absorbance was read with in 5 min after stopping the reaction.

Calculation of cut-off value: C.O = NC+0.12

NC = the mean absorbance value of three negative controls.

Interpretation of the result:

Negative result: less than cut-off value.

Positive result: more than cut-off value.

Borderline result: less than or equal cut-off value × 2.

Quality control

Reagent standard were checked for storage, stability and preparation before Starting work.

CHAPTER FOUR

RESULTS

CHAPTER FOUR

RESULTS

4.1 Results

Ninety two (n=92) HCWs were recruited from Ad Douiem locality in this study.

Table (4.1).Distributions of participants according to the gender (n=92)

Gender	Frequency	%
Male	47	51.1
Female	45	48.9
Total	92	100

Table (4.2).Distributions of participants according to the age groups (n=92)

Age groups	Frequency	%
20-40	78	84.8
41-60	14	15.2
Total	92	100

Table (4.3).Distributions of participants according to the social status (n=92)

Social status	Frequency	%
Married	47	51.1
Single	45	48.9
Total	92	100

Based on the occupation of the recruits the most frequent were nurses 33(35.9%) followed by laboratory specialists 22(23.9%), laboratory assistants 18 (19.6%), cleaning staff 16(17.4%) and pharmacists 3(3.3%).

Table (4. 4). Distributions of participants according to occupation (n=92)

Occupation	Frequency	%
Laboratory specialists	22	23.9
Laboratory assistants	18	19.6
Cleaning staffs	16	17.4
Pharmacists	3	3.3
Nurses	33	35.9
Total	92	100

Twenty four (24) of the recruits had accidental injury during their work (26.1%). (12) Of recruits had Surgery during life (13%). (2) Of recruits had blood transfusions during life (2.2%). All participants not receive renal dialysis. Nurses had the highest rate of accidental injury during work (8) (8.6%) among all the participants. In all HCWs the results of anti-HCV antibodies were Negative. The prevalence of HCV in heath care workers was zero.

Table (4.5) Distributions of participants according to their exposure to injuries (n=92)

Occupation	Frequency of Injury	
	Yes	No
Laboratory specialists	5	17
Laboratory assistants	6	12
Cleaning staffs	5	11
Pharmacists	0	3
Nurses	8	25
Total	24	68

CHAPTER FIVE

DISCUSSION

CHAPTER FIVE

DISCUSSION, CONCLUSOINS AND RECOMMENDATIONS

5.1 DISCUSSION

HCWs are at a high risk of contracting blood borne infections; however, according to the various range of endemicity of HCV infection, the prevalence of HCV among HCWs are different in countries with low endemicity (Taher *et al*., 2016). This study was conducted to determine the seroprevalence of HCV in HCWs at Ad Douiem hospital and health centers in Ad Douiem locality. All of the participants (92) were found seronegative for HCV antibodies. This result is similar to those reported in Omdurman, Sudan among healthcare workers that found the prevalence of HCV was zero (Nail *et al.*, 2008). The results are similar to those reported in Tehran, Iran among HCWs that found the prevalence of HCV was zero (Taher *et al*., 2016) as well as in India among HCWs where the prevalence of HCV was zero (Doddaiah *et al.*, 2013; Anand and Mahesh, 2012) and in Japan among dental care workers including dentists, dental hygienists and dental assistants where the prevalence of HCV was zero (Nagao *et al*., 2008). These results were less than those reported in Khartoum state, Sudan among healthcare workers at Dialysis Centers that found the prevalence of HCV was (1.8%) (Abdalla and Shappan, 2013). The results are less than those reported in Brazil among healthcare workers that found the prevalence of HCV was (4.8%) (Parana et *al*., 2007) and those reported in UK among dental workers where the prevalence of HCV was (0.1 %) (Roy *et al.*, 2003). The lower rate of exposure in this study may be explained by the large group of HCWs in this study had never reported their needle stick injuries, history of blood

transfusions or surgery also HCV has low infectivity rate and the Seroprevalence of HCV in this study may affected by low endemicity of HCV in Sudan.

5.2 Conclusions

Hepatitis C virus infection was uncommon in the samples collected from healthcare workers in Ad Douiem Locality; all participants were free from HCV.

5.3 Recommendations

Further studies with a large number of samples and other techniques are required to confirm these results.

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APPENDICES

APPENDICES

Questionnaire on Study of Detection of Hepatitis C Virus among health care workers Ad Douiem locality, White Nile State –Sudan.

Data collection Sheet

General data

1. Name……..
2. Gender

Male ( ) female ( )

3. Age……

4. Housing……

5. Social status

Married ( ) single ( )

6. Type of occupation……...

7. Period of work in hospital…….

8. Have you taken a sharp instrument?

Yes ( ) No ( )

If the answer is yes, what is the procedure used in the hospital to treat the injured person.....

9. Blood transfusion

Yes ( ) No ( )

10.Surgical operation

Yes ( ) No ( )

11.Renal dialysis

Yes ( ) No ( ).

- **ELISA micro titer plate show the results**