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Sero-prevalence of Hepatitis C Virus Among Hospital Cleaners in Khartoum State

الإنتشار المصلي لفيروس إلتهاب الكبد (ج) لدى عمال النظافة في مستشفيات ولاية الخرطوم

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

BY

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الآية

قال تعالى:

قُل لَن يُصيبَنا إِلّا ما كَتَبَ اللّـهُ لَنا هُوَ مَولانا وَعَلَى اللّـهِ فَليَتَوَكَّلِ المُؤمِنونَ

صدق الله العظيم

سورة التوبة الآية 51

DEDECATION

To My Father, Mother, Brothers and Sisters

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ABSTRACT

Exposure to Hepatitis C Virus is considered as animportant occupational hazard for hospital cleanersand it can result in liver cirrhosis or hepatocellular carcinoma, which are responsible for hundreds of deaths each year.

This study was conducted on ninety hospitals cleaners working in hospitals in Khartoum State during the period from February to April 2017. The objective of this study was to determine the sero-prevalence of Hepatitis C Virus (HCV) infection among hospital cleaners in Khartoum State.

Five hospitals were included in this study, 44(48.9%) Specimens were collected from Omdurman Teaching Hospital, 26(28.9%) from Al-Zara Hospital , 10(11.1%) from Antalia Medical Center, 8(8.9%) from Al-Shap Teaching Hospital and 2(2.2%) from Bahari Teaching Hospital. Blood specimenes were collected from each participants and screened for anti-HCV using Enzyme Linked Immunosorbent Assay (ELISA). Some demographic data were collected by structured questionnaires.

The results showed that out of 90 samples investigated only one was positive 1(1.1%) for anti-HCV. When compared with the risk factors it was found that there was no statistical significant relation (P value >0.05) between the presence of anti-HCV and all risk factors were tested.

The study concluded that although there was a high percentage of illiteracy 52(57.8%) among study population, still they have good attitudes, and practices towards dealing with medical waste. Low prevalence in this study may be due to small sample size for this large scale screening are required to validate the result of the presented study.

المستخلص

التعرض لفيروس إلتهاب الكبد الوبائي (ج) يعتبر من اهم المخاطر المهنية لعمال نظافة المستشفيات ويمكن ان ينتج عنه تليف الكبد او سرطان الكبد، وهو مسؤل عن مئات الوفيات سنويا.
هذه الدراسة أجريت علي 90 عامل نظافة يعملون في مستشفيات بولاية الخرطوم، في الفترة من فبراير وحتي ابريل 2017.هدفت الدراسة الي تحديد مدي إنتشار الاصابة بفيروس إلتهاب الكبد الوبائي (ج) بين عمال النظافة بمستشفيات ولاية الخرطوم.

شملتالدراسة 5 مستشفيات، 44(48.9%) عينة جمعت من مستشفي امدرمان التعليمي،26 (28.9%) من مستشفي الذرة، 10 (11.1%) من مركز انطاليا الطبي، 8(8.9%) من مستشفي الشعب التعليمي و 2(2.2%) من مستشفي بحري التعليمي.جمعت عينات دم من كل المشاركين و فحصت للكشف عن المستضد السطحي لفيروس التهاب الكبد (ج) بواسطة تقنية الانزيم المناعي المرتبط (الاليزا). جمعت بعض البيانات النوعية عن طريق استبيانات.

أظهرت النتائج أن من اصل 90 عينة تم فحصها، فقط واحدة (1.1%) كانت إيجابية للمستضد السطحي لفيروس التهاب الكبد (ج). ولم تظهر الدراسة اي علاقة ذات دلالة إحصائية (P value > 0.05) بين إنتشار المستضد السطحي لفيروس إلتهاب الكبد (ج) وعوامل الخطر المستهدفة.

وقد خلصت الدراسة أنه رغم النسبة العالية للامية52 (57.8%) وسط مجتمع الدراسة، إلا أن لديهم موقف وسلوكيات جيدة تجاه التعامل معالنفايات الطبية. و أن معدل إنتشار عدوي التهاب الكبد (ج) في هذه الدراسة كان منخفضا ربما لحجم العينة البسيط لذلك يجب إجراء مسح بعدد اكبر من العينات للتحقق من نتائج هذه الدراسة.

TABLE OF CONTENTS

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

CHAPTER TWO

LITERUTURE REVIEW

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER FOUR

RESULTS

CHAPTER FIVE

DISCUSSION

LIST OF TABLES

LIST OF Figures

LIST OF ABBREVIATIONS

C.O:Cut Off

HAV:Hepatitis A Virus

HBV: Hepatitis B Virus

HCC: Hepatocellular carcinoma

HCV: Hepatitis C Virus

HCWs: Health Care Workers

HIV: Human Immunodeficiency Virus

HRP: Hourseradish peroxidase

IFN-α: Interferon-α

IRES: Internal Ribosomal Entry Site

MWHs: Medical Waste Handlers

NANBH: Non-A Non-B Hepatitis

NS: Non-Structural protein

TMB: Tetramethylbenzidine

UK: United Kingdom

WHO: World Health Organization

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1.1. Introduction

Viral hepatitis is a major public health problem affecting several hundred million people world wide. It causes considerable morbidity and mortality from both acute infection and chronic sequelae including chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC) (Hatim, 2008). Hepatitis C is recognized as an important global infectious disease affecting primarily the liver, caused by Hepatitis C Virus (HCV), with more than 170 million people are chronically infected (Baha *et al.*, 2013). Hepatitis C is generally asymptomatic with up to 80% of infected cases will progress to persistent infection. About 15-20% of chronic HCV infection progressed to cirrhosis and 1-4% are found to be an annual risk of developing hepatocellular carcinoma within 20-30 years (Baha *et al*., 2013; Alavian *et al*., 2013). HCV is a major cause of end stage liver disease in many parts of the world (Lauer *et al*., 2001).

Infection with HCV is an important occupational hazard for Health Care Workers (HCW) (Yazdans *et al*., 2005). An occupational hazard is any harmful condition associated with work place, which can result in illness or injury (Faremi *et al*., 2014). One of the occupational hazards among HCW is exposure to blood born disease (Lee *et al*., 2005).

Cleaners in hospital settings are particularly vulnerable to occupational hazard considering the fact that they are directly involved in the collection and disposal of medical waste, some of which are particularly infectious, toxic and could constitute a potential or actual threat to the well being of the worker (Azzawi, 2012).There are numerous consequences of occupational diseases among hospital workers, these include economic, physical and mental harm to worker and their families (Bdour *etal*., 2015; Manyele *et al*., 2008). According to world Health Organisation (WHO); injuries from contaminated needles and poor handling of medical waste is responsible for about 8 to 16 million new cases of Hepatitis B Virus (HBV), 2.3 to 4.7 million cases of Hepatitis C

Virus (HCV) and 80,000 to 160.000 cases of HIV annually (WHO 1999, Townsend and Cheeseman, 2005).

The level of awareness of occupational hazards among hospital cleaners varies depending on the locality of work (Manyele *et al*., 2008). Awareness is low in low income countries where majority of individuals employed as cleaners are of poor socio economic status, with little or no education and lacking in basic traning or orientation on how to handle hazardous waste (Manyele *et al*., 2008).

HCV infects an estimated 170 million persons world wide and thus represents a viral pandemic, one that is five times as wide spread as infection with the Human Immunodeficiency Virus type one (HIV-1). However, evidence has shown that the risk of transmission is higher with larger quantity of blood or other infectious body fluids, prolonged exposure to infectious materials, exposure to the blood of patients with advanced or severe disease, deep percutaneous injury and injuries from contaminated hollow bore instruments (Jed, 2006). In such situations hospital cleaners are constantly faced with high level risks of occupational hazards exposure during the course of their duties (Manyele *et al*., 2008).

1.2. Rationale

Medical wastes unlike other waste products constitute a serious health hazard to its handlers, and because infection with HCV is considered an important occupational hazard for cleaners in hospitals and it can ultimately result in liver cirrohosis, hepatic failure or hepatocellular carcinoma, which are responsible for hundreds of deaths each year. There are no approved methods for preventing HCV infection after exposure (no vaccine). However, the identification of factors determining an increased risk of HCV transmission would be important for post exposure counseling and management. Moreover, individuals living with undiagnosed HCV infection remain infectious to others and their families. Thus follow up based on HCV screening test to detect HCV infection as early as possible for an early course of therapy.

1.3. Objectives:

1.3.1. General objectives:

To investigate hepatitis C virus infection among hospital cleaners in hospitals in Khartoum state.

1.3.2. Specific objectives:

1.To determine the prevalence of HCV infection among cleaners in Khartoum hospitals using ELISA.

2. To identify the possible risk factors for the transmission of HCV to hospital cleaners .

3. To determine the relationship between the prevelance of HCV infection and certain factor such as past history of surgery, needle stick injury and blood transfusion.

ACHAPTER TWO

LITERATURE REVIEW

1.2. Background

Following the discovery of Hepatitis B Virus (HBV) in 1968 and the Hepatitis A Virus (HAV) in 1973, it became clear in 1974 and 1975 that most transfusion cases of hepatitis were due to neither virus from which the term Non-A, Non-B hepatitis (NANBH) was born (Tatsuo *et al*., 2016).

HCV was discovered in 1989 as a causative agent of NANBH in human when the genome of the virus was cloned and the agent was designated the HCV (James and Ellen, 2008). Despite the inability to grow the virus in culture or in a small animal model, the complete genome sequence of the virus was established using the methods of modern biotechnology and verified by injection of viral RNA produced from cDNA clones in to the liver of a chimpanzee (James and Ellen, 2008). Hepatitis C is a caused by HCV, a virus is a member of the Flaviviridae. The virus is transmitted by contaminated blood and blood products, and is thought to cause as much as 25% of acute viral hepatitis world wide (Edward *et al*., 2008). There is no current evidence of its being efficiently spread by arthropod vectors, but this possibility can not be ruled out (Edward *et al*., 2008). Chronic infection can last for many years with resulting accumulated liver damage and carcinoma (Edward *et al*., 2008).

Hepatitis C Virus it is the third genus in the family Flaviviridae, Hepacivirus (Arie *etal.*, 2009). HCV is a single-positive strand enveloped RNA virus which infects mainly the human hepatocytes (Tatsuo *et al*., 2016). HCV contains a positive stranded RNA genome of around 10,000 nucleotides which encodes a large polyprotein of over 3000 amino

acids which is cleaved co-and post-translationally into virion structural proteins (nucleocapsid (c) and envelope glycoproteins gpE1 and gpE2) and a plethora of non-structral proteins involved in viral replication and assemply (Lindenbach and Rice, 2013; Paul *et al.*, 2014).

Notable features of HCV replication include it is ability to induce a membranous web within the endoplasmic reticulum (Moradpour *et al.*, 2003) in which virus is replicated (Romero *et al.*, 2015), assembled on lipid droplets (Miyanari *et al.*, 2007) and secreted using the low density lipoprotein secretory pathway resulting in the production of apolipoprotein associated viral particles of very light density (Huang *et al.*, 2007).

HCV induces the recruitment of nuclear pore proteins into the membranous web possibly to facilitate a protective subcellulra environment from the cells innate immune response and to create a custom made virus factory (Neufeldt *et al.*, 2013).

2.2. Viral Structural Components

Cell culture derived HCV was reported to be very heterogeneous in size, with a diameter between 30 and > 100 nm(Lindenbach *et al.*, 2005, Wakita *et al.*, 2005 and Zhong *et al.*, 2005). The nucleocapsid consisting of mature core proteins and a viral genome is surrounded by an envelope composed of host lipids and viral envelope proteins (Tatsuo *et al.*, 2016).

HCV genome translation of viral depends on an internal ribosomal entery site (IRES) and yields a ~3,000 amino acid polyprotein. The polyprotein cleaved by host and viral proteases resulting in ten mature viral proteins. This includes three structural proteins (core protein, the nucleocapsid protein, and the envelope glycoproteins E1 and E2), a small integral transmembrane protein p7 and six non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B) (Tatsuo *et al.*, 2016). The core protein is the only HCV protein associated with nucleocapsid formation. The core protein consists of the first 191 amino acids in the HCV polyprotein. The C-terminal transmembrane segment serves the signal sequence for E1 and is removed by the action of host signal peptide peptidase. The mature protein is approximately 21KDa, consisting of the first 177 amino acids which are needed for infectious virion production (Kopp *et al.*, 2010).

Although originally classified as a non-structural (NS) protein, p7 is required for the production of infectious virions (Jones *et al*., 2007; Steinmann *et al*., 2007 and Wozniak *et al*., 2010).

2.3. HCV Genotypes

HCV is a single strand RNA virus that requires an RNA-dependant RNA polymerase to reproduce. The error rate of this polymerase is very high, and thus the viral genomes are highly heterogenous (Tatsuo *et al*., 2016). These viruses have been classified into a hierarchy of genotypes and subtypes, which have different biological and clinical properties. To determine the current status of HCV genotypes, complete genome sequences and their annotations were retrieved from public databases (Tatsuo *et al*., 2016). These viral sequences were arranged according to genotype / subtype and geographical distribution . HCV classified into seven major genotypes with serial subtypes (Tatsuo *et al*., 2016).

Genotyping is important to guide treatment because some viral genotypes respond better to therapy the others (Tatsuo *et al*., 2016).

•Genotype 1: Most common in USA and Europe (Important sub types: 1a and 1b).

•Genotype 2: Less common than genotype1 in USA and Europe (Important sub types: 2a and 2b).

•Genotype 3: Most common in India, the Far East and Australia.

•Genotype 4: Most common in Africa and the Middle East. Is emerging in Europe among drug users and other high risk populations.

•Genotype 5: Most common in South Africa.

•Genotype 6: Most common in Hong Kong, Vietnam and Australia.

•Genotype 7: Unspecified location.

HCV genotypes differ from each other by 31-33%, and subtypes by 20-25%, at the nucleotide level (Simmonds *et al*., 2005).

2.4. Epidemionology

HCV is world wide in distribution, it has been estimated that ~3% of the world population, 170 million people, are infected by virus. The highest infection rate found was among Egyptian blood donors, where up to 19% were seropositive for HCV, which may have resulted in part from past treatment for bilharziasis using inadequately sterilized needles. In Africa and Asia, the virus has been endemic and the different genotype have different geographic distributions (James and Ellen, 2008). Almost 4 million Americans (1.8% of the population of the United States) have antibody to HCV, indicating previous infection with this virus. Higher rates have been found in southern Italy, Spain, center Europe, Japan and parts of the Middle East. Current data indicate that in some 40-50% of patients in industrialized countries the source of infection cannot be identified; 35% or more of patients have a history of intravenous drug misuse; house hold contact and sexual exposure appear not to be major factors in the epidemiology of this common infection, and occupational exposure in the health-care setting accounts for about 2% of cases. Transmission of HCV from mother to infant occurs in about 10% of viraemic mothers and the risk appears to be related to the level of viraemia (Arie *et al*., 2009).

2.5. Transmission

HCV can transmitted throught blood transfusions or blood products or tissue transplantation .Sexual intercourse and in rare cases household contact with infected patients under poor hygiene conditions. Hospital staff are endangered by injuries with needles. Also the virus can be transmitted vertically from mother to child during pregnancy or at birth. However , a HCV infection of the gestating mother is not considered an indication for caesarean section (Susanne *et al*., 2013). There are additional mechanisms of transmission that are not well understood. In some developing countries, circumcision or scarification practices may be important in the spread of the virus (James and Ellen, 2008).

2.6. Clinical Features

A generally slight liver inflammation appears after an average incubation period of 6-8 weeks. About 75% of infection are asymptomatic, sever clinical course are rare. Acute

infection with clinical symptoms have a favourable prognosis. Up to 80% of all infected individuals develop a chronic persistent hepatitis or reactivated chronic hepatitis. Chronic infection are characterized by elevated transaminase levels and can be temporarily normal: the more active the infection, the higher the values.10-20% of patients with chronic infection develop cirrhosis over the years, and 4% of them develop a primary liver cell carcinoma in the course of decades. Additional complications include periarteritis nodosa, membrane-proliferative glomerulonephritis and idiopathic sjogren syndrome (Susanne *et al*., 2013).

2.7. Pathogenesis

The virus directly enters the circulatory system, mainly through contaminated blood or blood products, and is transported by infected macrophages into the liver, where it infects hepatocytes. The result the liver inflammation accompanied by cell necrosis. Cellular damage seems to be induced by the immune response during hepatitis C (Susanne *et al*., 2013). Tubular structures have been observed in the cytoplasm of infected liver cells by electron microscopy. Antigen-antibody complexes are formed in the chronic infection form, and can be deposited in the glomeruli.They seem to be responsible for the membrane proliferative glomerulonephritis in such patients (Susanne *et al*., 2013).

The HCV exhibits a high mutation rate and changes in patients in the course of infection. The mutations arise during replication. They are attributed to the fact that the viral RNA-dependant RNA polymerase, unlike cellular DNA polymerase, dose not possess an exonucleolytic proofreading mechanism to control and enhance the accuracy of RNA synthesis. Specific mutations are important for the virulence of the different quasispecies (Susanne *et al*., 2013).

The association of HCV with host derived lipoproteins has a number of significant advantages for the virus and important implications for it is pathogenesis. HCV exploits the lipoprotein biosynthetic machinery to assemble and release nascent virions. An additional intriguing aspect of this interaction is that it might contribute to the hepatotropism of this virus, since the liver plays a key role in lipoprotein clearance. By

circulating in the blood stream disguised as lipoviral particles, HCV virions may have found a unique and efficient mode of viral entery into liver cells. Indeed, HCV attachment to hepatocytes and entry involves lipoproteins and their receptors (Zeisel *et al*., 2013).

It has not been conclusively resolved in which way HCV promotes carcinogenesis.there is evidence that specific sequences of the C protein interact with cellular Ras proteins and that this interaction induces transformation (Susanne *et al*., 2013). The time span between infection and the formation of a primary hepatocellular carcinoma is approximately 20-40years (Susanne *et al*., 2013).

2.8. symptoms and sings

Most people 80% with acute HCV infection have no symptoms. If symptoms occur, they may include loss of appetite, abdominal pain, fatigue, nausea, dark urine and jaundice. Of those who develop chronic HCV infection, the most common symptom is fatigue (CDC, 2016).

2.9. Treatment of Hepatitis C

2.9.1. Acute Hepatitis C

Early identification of acute hepatitis C is important, but may be difficult as the disease may be relatively silent in the acute phase; 75% of patients are not jaundiced and have nonspecific symptoms. Therapeutic trials of IFN-α have been undertaken. Recent studies have indicated that treatment benefits those patients who have been treated early, but it may be reasonable to allow one to three months to determine which patients might convalesce spontaneously (Jaeckel *et al*., 2001). In those don't appear to be convalescing two to four months after onset of the disease, antiviral treatment should be considered, as a high percentage of patients (>80%) may respond. The optimal form of treatment for acute hepatitis C is not yet determined but weekly pegylated IFN-α and ribavirin can be considered (Simmonds *et al*., 2005).

2.9.2. Chronic Hepatitis C

There is evidence that alcohol and hepatitis C may synergistically aggravate hepatic injury. The aim of therapy is to achieve an undetectable HCV RNA six months following therapy. HCV RNA should be measured in all patients to confirm viraemia. If the test is positive, serum aminotransferases, bilirubin, alkaline phosphatase and prothrombin time should be measured (Arie *et al*., 2009). A liver biopsy is helpful in grading the degree of inflammation and staging the degree of fibrosis. Earlier guidelines recommended antiviral therapy for those patients with chronic hepatitis C (Arie *et al*., 2009).

.The current treatment for chronic HCV infection is injection of IFN-α conjugated to polyethylene glycol, which increases its stability, together with the inhibitor ribavirin. This treatment results in curing the infection in about half the cases but the cure rate depends upon the genotype of the virus (James and Ellen, 2008).

The drug Sofosbuvir now represents one major corner-stone in effective therapy (Alqahtani *et al*., 2015). Various combinations of Sofosbuvir, non-nucleoside polymerase inhibitors, Daclatasvir along with second and third generation protease inhibitors, become the only curable chronic viral infection, with nearly all patients now curable after short regimen treatments and with little resistance (Nyalakonda and Utay, 2015).

2.10. Immune response and Diagnosis

IgM antibodies against the NS4 and C proteins can be found in acute infections. IgG antibodies against the C protein can be detected a few days to a few weeks after the onset of symptoms; IgG antibodies against non-structural proteins (NS3, NS4, NS5) are detectable later (Susanne *et al*., 2013). Immunoglobulins against envelope proteins E1 and E2 are detected at an early stage in only about 10% of acute infections. Cytotoxic T lymphocytes can be detected in the blood of patients after stimulation by peptides that are derived from viral proteins (Susanne *et al*., 2013).

Increased transaminase levels may be elucidative for the diagnosis of HCV infection, although this does not allow any further assignment of the pathogen (Susanne *et al*., 2013).

The main approach for diagnosis of HCV infections is:

2.10.1. ELISA

Which is used for screening. If the test findings are positive, it can be inferred that a fresh, chronic or past HCV has occurred. Used to detect specific antibodies (Susanne *et al*., 2013).

2.10.2. Quantitative RT-PCR

For detection of viral RNA genomes is the most important method immediately provides the level of the viral load. In general, serum or plasma is used as the source material; liver biopsies are used only in exceptional cases. In addition, the genotype is usually determined by PCR and hybridization tests (Susanne *et al*., 2013).

2.11. HCV Vaccine

A vaccine candidiate is currently undergoing phase 2 clinical efficacy trails in the USA and is based on the i/m delivery of non-structural genes via a pair of replication defective viral vectors (Swadling *et al*., 2014). If successful, a high risk groups such as healthcare workers, cleaners in hospitals, paramedics would benefit from vaccination (Tatsuo *et al*., 2016).

2.12. Previous Studies

Health care waste can cause serious harm if not managed properly, for example in 2000 WHO estimated world wide that injections with contaminated syringes caused two million HCV infections (40% of all new infections) (WHO 2000).

Occupational risk related to hepatitis virus exposure is a major concern for medical waste handlers (MWHs), in developing nations where a guideline for waste handling is not strictly followed, this is study was conducted in Southern Ethiopia, to assess the rate of and risk factors for exposure to HBV and HCV among MWHs from December 2014 to January 2015. A total of 152 MWHs and 82 non-MWHs were studied and was found the prevalence of HCV (0.7%) in MWHS and (1.2%) in NMWHs (Anteneh *et al*., 2015).

Another study conducted among healthcare waste handlers in Belo Horizonte, Brazil that was found the risk of HCV infection was higher among healthcare waste workers compared with domestic waste workers, probably because of needlestick accidents owing to deficient sharps management systems (Mol *et al*., 2016).

Another study conducted among MWH to detect prevalence of HBV, HCV and HIV in Tripoli, Libya from January to December 2004, blood sample were collected from 300 MWHs and 300 sample from NMWHs a that reported a higher rate of anti-HCV 8 (2.7%) among MWHs and (0.0%) among NMWHs (Franka *et al*., 2009).

Another study conducted among MWHs in Gondar, northwest Ethiopia was found HCV 1% (Anagaw *et al*., 2012).

Study was conducted by (Eltaj *et al*., 2015) to measure the prevalence of HCV infection and to identify risk factors associated with HCV infection among healthcare workers at dialysis centers in Khartoum State, Sudan was found 1.8% (2/109).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

3.1.1. Type of study

This is a descriptive cross-sectional study conducted to determine the prevalence of Hepatitis C virus infection among hospital cleaners in Khartoum State Hospitals.

3.1.2. Study area and duration

The study was conducted in Omdurman Teaching Hospital, Al-Zara Teaching Hospital, Al-Shaab Teaching Hospital and Antalia Medical Center, from February to April 2017. The practical part of this study was carried out in the Research Laboratory, College of Medical Laboratory Science, Sudan University of Science and Technology (SUST).

3.1.3. Study population

The study population inthis study were cleaners working in hospitals.

3.2. Size of sample

A total of ninety (n=90) hospital cleaners were enrolled in this study.

3.3. Ethical consideration

This study was approved by the College Ethics Committee, College of Medical Laboratory Science, SUST. After explaining the study and it is goal. An informed consent was obtained from each participants before collecting the demographic and clinical data.

3.4. Data collection methods

3.4.1. Questionnaire

A structured questionnaires were used for collection of both qualitative and quantitative data. Which include demographic information such as age, sex, marital status, education level, work experience, previous needle stick injury, surgery, blood transfusion and hemodialysis and Protective equipment.

3.4.2. Laboratory procedure

The laboratory procedure such as blood collection, preparation of specimens and analysis of specimens was done to obtain data.

3.4.2.1. Blood collection

Three-ml blood Specimens were obtained by venipuncture. The skin was cleaned by 70% (v/v) ethanol, sterile syringe (5ml) was used to collect 3 ml of blood, then the blood was dispensed in a sterile EDTA container.

3.4.2.2. Preparation of specimens

Specimens were centrifuged at 3000 rpm for 5 min and plasma were separated immediately. Plasma were stored at -20 °C until the serological analysis.

3.4.2.3. Analysis of specimens

The specimens were analyzed for the presence of HCV antibody by a commercially available ELISA kit (4^{th} Generation) (fortress diagnostics, UK). The assays were performed following the instructions of the manufacturer. Positive and negative controls were included in each assy. According to the information included in the kits, the immunoassay used has specificity 99.94%.

3.4.2.4. Principle of the assay

This kit employs solid phase, indirect ELISA method for detection of antibodies to HCV in two-step incubation procedure. Polystyrene microwell strips are pre-coated with recombinant, highly immunoreactive antigens corresponding to the core and the non-structural regions of HCV (4^{th} generation HCV ELISA).

During the first incubation step, anti-HCV specific antibodies, if present, will be bound to the solid phase pre-coated HCV antigens.

The wells are washed to remove unbound serum proteins, and rabbit anti-human IgG antibodies (anti-IgG) conjugated to hourseradish 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 colour product. The blue colour turns yellow after stopping the reaction with sulphuric acid. The amount of colour intensity can be measured and is proportional to the amount of antibody captured in the wells, and to the sample respectively.

Wells containing samples negative for anti-HCV remain colourless.

3.4.2.5. Procedure

All reagents and samples were allowed to reach room temperature for 15minutes. Washing buffer was prepared 1:20 from stock wash buffer with distilled water.

100µl of sample diluents was added into each will except the blank. 20 µl from positive control, negative control and specimen was added into their respective wells and mixed by pipette gently. The plate was covered with the plate cover and incubated for 30minutes at 37°C. After the end of the incubation, plate cover was removed and each well was washed 5 times with the diluted wash buffer. Each time allowed the microwells to soak for 30 second. After the final wash, the plate was turned onto clean towel, and taped it to remove any remainders. 100µl HRP-conjugate was added to each well except the blank. Then the plate was covered and incubated for 30 minutes at 37°C.the plate was washed 5times as above.

50µl of chromogen A and 50µl of chromogen B was dispensed into each well including the blank and mixed, then incubated at 37°C for 15minutes.

Finally 50µl stop solution was added into each well by using multichannel pipette and mixed by tapping the plate gently.

3.4.2.6. Calculation of the results

The plate reader was calibrated with blank well and the absorbance was read at 450nm. The results were calculated by relating each sample optical density (OD) value to the cut off value of plate. Calculation of cut off (C.O) value:

C.O = *Nc + 0.12

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

Interpretation of the result:

Positive more than cut off value.

Negative less than cut off value.

CHAPTER FOUR

RESULTS

Ninety hospital cleaners at different Khartoum hospitals participated in this study between February to April 2017. 44(48.9%) from Omdurman Teaching Hospital, 26(28.9%) form Al-Zara Hospital, 10(11.1%) from Antalia medical Center, 8(8.9%) from Al-Shaap Teaching Hospital and 2(2.2%) from Bahari Teaching Hospital (Table1).

The participants consisted from 58(64.4%) females and 32(35.6%) males (Table2). The age range was between 18 and 70 years, The majority of study 23(25.6%) population were at age between 26-35 years (Figure 1).

The nationality of study population 65(72.2%) of the participants were Sudanese, while 25(27.8%) were Ethiopians (Table 4).

Regarding the education level the majority of study population are illiterate 52(57.8%), basic were 29(32.2%) and high school were 9(10%) (Figure 2).

The study indicated that almost all of study population 74(82.2%) have training program of medical waste collection and 16(17.8%) have not trained (Table 3)

Regarding the clinical data, 9(10%) had a history of surgical operation, 7(7.8%) had a history of blood transfusion, 1(1.1%) had a history of family with hepatitis, 23(25.6%) had a history of needle stick injury and no history of haemodialysis (Table 5).

HCV antibodies were detected in one subject of study population, the prevalence rate of HCV in the study group was 1.1% (Table 6).

There was no statistical significant relationship (P value >0.05), between the presence of HCV and all variables tested including gender (Table 7), previous

needle stick injury (Table 8), history of surgery (Table 9), history of blood transfusion (Table 10) and family history with hepatitis (Table 11).

Table 1. Distribution of cleaners according to hospital

Hospitals	Frequency	%
Omdurman Teaching Hospital	44	48.9%
Al-Zara Hospital	26	28.9%
Al-Shap Hospital	8	8.9%
Antalia medical Center	10	11.1%
Bahari Teaching Hospital	2	2.2%
Total	90	100.0

Table 2. Frequency and percentage of participants according to the gender

Gender	Frequency	%
Female	58	64.4
Male	32	35.6
Total	90	100.0

Table 3. Frequency of study population according to training of medicalwaste collection

Training	Frequency	%
Yes	74	82.2
No	16	17.8
Total	90	100.0

Table 4. Frequency and percentage of participants according to nationality

Nationality	Frequency	%
Sudanese	65	72.2
Ethiopians	25	27.8
Total	90	100.0

Table 5. Clinical data of study population

Clinical data	Yes	No	Total
History of surgical operation	9(10.0%)	81(90.0%)	90
History of blood transfusion	7(7.8%)	83(92.2%)	90
Family history of hepatitis	1(1.1%)	89(98.9%)	90
History of needle stick injury	23(25.6%)	67(74.4%)	90

Table 6. Frequency and percentage of seropositive of HCV among hospitalcleaners.

Result	Frequency	%
Positive	1	1.1
Negative	89	98.9
Total	90	100.0

Table 7. Relation between gender and seropositive HCV

Gender		Result		Total
		Positive	Negative	
Female	Count	1	57	58
	%of Total	1.1%	63.3%	64.4%
Male	Count	0	32	32
	%of Total	.0%	35.6%	35.6%
Total	Count	1	89	90
	%of Total	1.1%	98.9%	100.0%

PV= 0.455 insignificant value

Table 8. Relation between needle stick injury and seropositive HCV

Needle Stick injury		Result		Total
		Positive	Negative	
Yes	Count	0	23	23
	% of Total	.0%	25.6%	25.6%
No	Count	1	66	67
	% of Total	1.1%	73.3%	74.4%
Total	Count	1	89	90
	% of Total	1.1%	98.9%	100.0%

PV= 0.556 insignificant value

Table 9. Relation between the history of surgery and seropositive HCV.

History of surgery		Result		Total
		Positive	Negative	
Yes	Count	0	9	9
	% of Total	.0%	10.0%	10.0%
No	Count	1	80	81
	% of Total	1.1%	88.9%	90.0%
Total	Count	1	89	90
	% of Total	1.1%	98.9%	100.0%

PV= 0.737 insignificant value

Table 10. Relation between the history of blood transfusion and seropositive HCV.

History of blood transfusion		Result		Total
		Positive	Negative	
Yes	Count	0	7	7
	% of Total	.0%	7.8%	7.8%
NO	Count	1	82	83
	% of Total	1.1%	91.1%	92.2%
Total	Count	1	89	90
	% of Total	1.1%	98.9%	100.0%

PV= 0.770 insignificant value

Table 11. Relation between the family history with the hepatitis and seropositive HCV.

Family history with hepatitis		Result		Total
		Positive	Negative	
Yes	Count	0	1	1
	% of Total	.0%	1.1%	1.1%
No	Count	1	88	89
	% of Total	1.1%	97.8%	98.9%
Total	Count	1	89	90
	% of Total	1.1%	98.9%	100.0%

PV=0.915 insignificant value

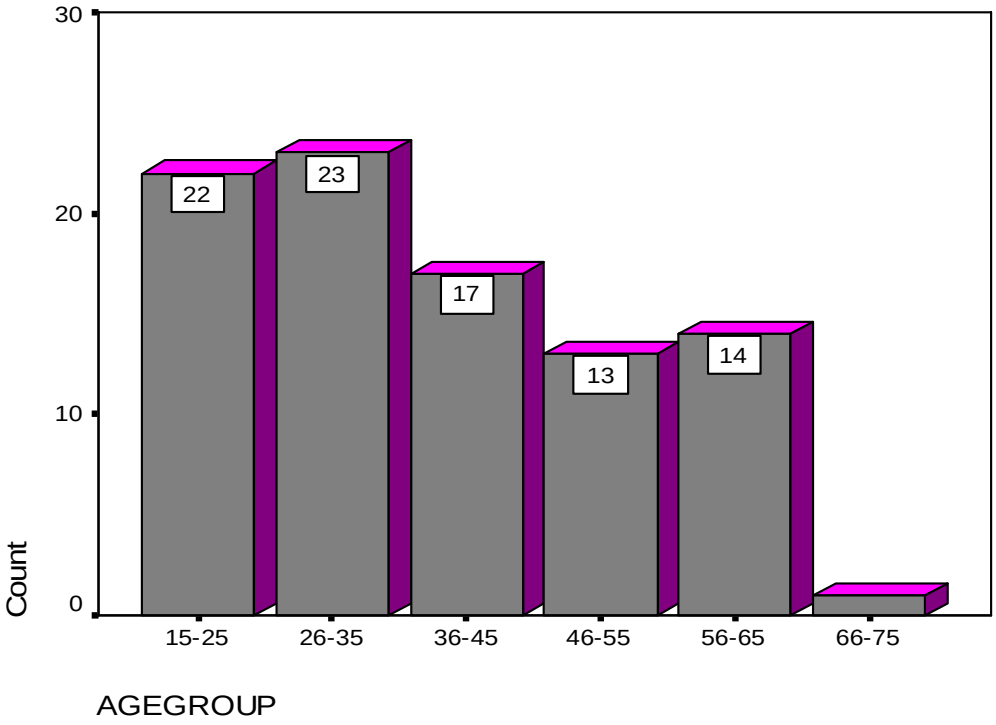

Figure 1. Distribution of study population according to their age group

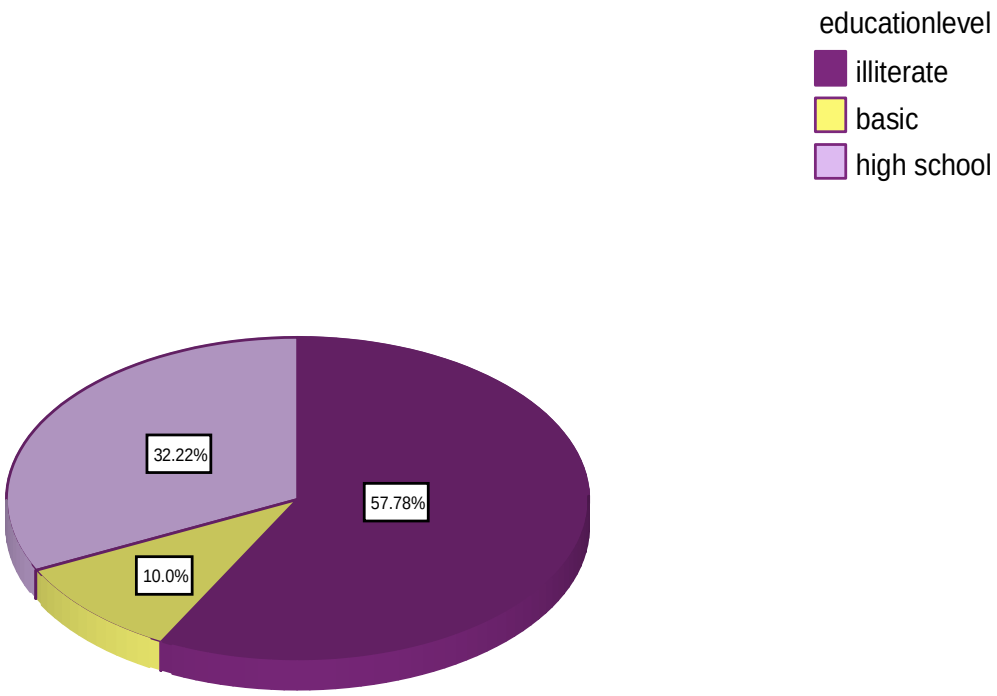

Figure 2. Distribution of study population according to education level

CHAPTER FIVE

DISCUSSION

Although HCV infection is considered as a health problem in high risk groups such as hospitals cleaners, it is prevalence in this groups is still negotiable and there are yet no published data in Sudan investigating the presence of HCV among hospital cleaners.

The result of this study showed that one (1.1%) of the included cleaners were infected by HCV using 4th generation ELISA method. This result is in line with study conducted among medical waste handlers (MWHs) in Gondar town, Northwest Ethiopia (Anagaw *et al*., 2012) who reported (1.0%). Also similar to that obtained by Jochen (1991) that found seroprevalence of HCV in HCWs is (1.1%). And higher compared with the prevalence of HCV among MWHs in Southern Ethiopia was reported (0.7%) (Anteneh *et al*., 2015), also higher compared with the prevalence of HCV among health care workers (HCWs) in Sudan (Omdurman) which was found zero (0,00%) (Nail *et al*., 2008), also higher than result obtained by Mohamoud (2015) among HCWs in Sudan which was reported the prevalence of HCV is zero (0.00%). Also the prevalence in this study was lower than that found in studies among MWHs in Tripoli,libya was found (2.7%) (Frank *et al*., 2009), and in Brazil (3.3%) (Mol *etal*., 2016), and lower than that found among HCWs at dialysis centers in Khartoum stat which was found (1.8%) (Eltagi and kamil, 2016), and also lower than that found among HCWs in Saudi Arabia (9.7%) (El-Hazmi and Al-Majid, 2008). Also lower compared with the prevalence of HCV among blood donors at the national laboratory for public health that found (3.0%) (Sahar, 2015). And lower than that found among Sudanese population (2.2%-4.8%) (Hatim, 2008). The highest prevalence of HCV infection in Sudan was noted in patients with end stage renal disease on regular heamodialysis with a seroprevalence of (23.7%) (El-Amin *et al*., 2007).

The important of training on the hazards associated with handling of medical waste and its disposal cannot be over emphasized, (82.2%) of the cleaners had received some forms of training on occupational hazards and safety, and they were equally more informed than those not trained.

5.2. CONCLUSION

The study revealed that although there was a high percentage of illiteracy among study population, still they have good attitudes, and practices towards dealing with medical waste. Low prevalence of HCV in this study may be due to small sample size for this large scale screening is recommended.

5.3. RECOMONDATIONS

1. All cleaners in hospitals should be trained on risks associated with their job and how to mitigate them.

2. Maintain use personal protective equipment to protect the cleaners from direct exposure to hazard.

3. Maintain use engineering controls, such as autodisposable syringes, needle-free devices, and sheathed needles.

4. Proper management of medical waste by the health authorities, may reduce the risk of acquiring infectious agent by hospital cleaners.

5. Further research it is important to define the prevalence of HCV among hospital cleaners and to evaluate their associated risk factors so as to adopt effective preventive strategies.

REFERENCES

1. **Alavian S.M., Mahboobi N. and Karayiannis P. (2013).** Oral conditions associated with hepatitis C virus infection. *Saudi J Gastroenterol*. **19**: 245-251.
2. **Alqahtani S.A., Afdhal N., Zeuzem S., Gordon S.C., Mangia A. *et al*. (2015).** Safety and tolerability of ledipasvir/sofosbuvir with and without ribavirin in patients with chronic hepatitis C virus genotype 1 infection: analysis of phase 3 ION trail. *Hepatology*. **62**(1): 25-30.
3. **Anagaw B., Shiferaw Y., Belyhun Y., Erku W., Biadgelegn F. *et al*. (2012).** Seroprevalence of hepatitis B and C virus among medical waste handlers at Gondar town health institutions, Northwest Ethiopia. *BMC Res Notes*. **5**: 55.
4. **Anteneh A., Mesfin W., Endale T. and Techalew S. (2015).** The exposure rate to hepatitis B and hepatitis C viruses among medical waste handlers in three government hospitals, southern Ethiopia. *Epide Health*. **38**: 1-8.
5. **Arie J.Z., Jangu E.B., Barry D.S., Paul D.G. and Philip M. (eds.) (2009).***Principles and Practice of Clinical Virology*. 6th Ed. Wiley-Blackwell. John Wiley and Sons Ltd. Pp: 309, 312-313.
6. **Azzawi S.A. (2012).** Hospital waste and cleaning workers in Baquba Teatching Hospital. *Diyala J Med*. **2**(1): 79-83.

7. **Baha W., Foullous A., Dersi N., They-they T.P., Elalaoui K. *et al*. (2013).** Prevalence and risk factors of hepatitis B and C virus infections among the general population and blood donors in Morocco. *BMC Public Health*. **13**:50.
8. **Bdour A.N., Tarawneh Z., Al-Monani T. and El-Mashaleh M. (2015).** Analysis of hospital staff exposure risks and awareness about poor medical waste management- A case study of the Tabuk regional healthcare system- Saudi Arabia. *J Comm Dis*. **47**(2). 1-13.
9. **Centers for Disease Control and Prevention. (2016).** Hepatitis C FAQs for the public. Division of viral Hepatitis.
10. **Edward K. Wagner M. Hewlett D. Bloom. And David C. (eds.). (2008).** *Basic Virology*, 3rd Ed. USA. Black well. Pp: 57.
11. **El-Amin H.H., Osman E.M., Mekki M.O. *et al*. (2007).** Hepatitis C virus infection in hemodialysis patients in Sudan: two centers report. *Saudi J Kidney Dis Transpl*.**18**:101-6.
12. **El-Hazim M.M. and Al-Majid F.M. (2008).** Needlestick and sharps injuries among health care workers: A 5-year surveillance in a teaching center in Saudi Arabia. *Bio Res*. **19**(2): 133-140.
13. **Eltagi A.M. and Kamil M.A. (2016).** Hepatitis C virus infection among health care workers at dialysis centers in Khartoum state, Sudan. *IJSR*. **5**(10): 1258-1261.
14. **Faremi F.A., Ogunfowokan A.A., Olatubi M.I., Ogunlade B. and Ajayi O.A. (2014).** Knowledge of occupational hazards among cleaning workers. A case study of cleaners of a Nigerian University. *Int J Health Sci Res*. **4**(8):198-204.

15. **Franka E., El-Zoka A.H., Hussein A.H., Elbakosh M.M., Arafa A.K. and Ghenghesh K.S.(2009).** Hepatitis B virus and hepatitis C virus in medical waste handlers in Tripoli, Lipya. *J Hosp Infect*. **72**: 258-261.
16. **Hatim M.Y. (2008).** Epidemiology of viral hepatitis in Sudan. *CliniExper Gastroenterology*. **1**: 9-13.
17. **Huang H., Sun F., Owen D.M., Li w., Chen Y., Gale M. *et al*.(2007).** Hepatitis C virus production by human hepatocytes dependent on assembly and secretion of very low density lipoproteins. PNAS USA. **104**: 5848-5853.
18. **Jaeckel E., Cornberg M., Wedemeyerm H. *et al*. (2001).** Treatment of acute hepatitis C with interferon alfa-2b. *The new Engl J Med*. **345**: 1452-1457.
19. **James H. and Ellen G. (2008).***Viruses and Human Disease*. 2nd Ed. London: Elsevier. Pp: 120-122.
20. **Jed S.L. (2006).** A quick guide to postexposure prophylaxis in the healthcare setting. Mountain plains AIDS education and training center.
21. **Jochen A. (1991).** Prevalence of Hepatitis C virus antibodies in hospital personnel. *Zentralblatt fur Bakteriologie*. **274**(4): 543-547.
22. **Jones C.T., Murray C.L., Eastman D.K., Tassello J. and Rice C.M. (2007).** Hepatitis C virus p7 and NS2 proteins are essential for production of infectious virus. *J Virol*. **81**(16): 8374-8383.
23. **Kopp M., Murray C.L., Jones C.T. and Rice C.M. (2010).** Genetic analysis of the carboxy terminal region of the hepatitis C virus core protein. *J Virol*. **84**(4): 1666-1673.

24. **Laure G.M. and Walker B.D. (2001).** Hepatitis C virus infection. *N Engl J Med*. **345**: 41-52.

25. **Lindenbach B.D., Evans M.J., Syder A.J., Wolk B. *et al*. (2005).** Complete replication of hepatitis C virus in cell culture. *Science.* **309**(5734): 623-626.

26. **Lindenbach B.D. and Rice C.M. (2013).** The ins and outs of hepatitis C virus entry and assembly. *Nat Rev Microbiol.***11**(10): 688-700.

27. **Mahmoud A.M. (2015).** Serodetection of Hepatitis C virus among health care workers in Khartoum state. M.Sc thesis, Sudan University.

28. **Manyele S.V., Ngonyani H.M. and Eliakimu E. (2008).** The status of occupational safety among health service providers in hospitals in Tanzania. *Tanza J Healt Res*. **10**(3): 159-165.

29. **Miyanari Y., Atsuzawa K., Usuda N., Watashi K. *et al*. (2007).** The lipid droplet is an important organelle for hepatitis C virus production. *Nat Cell Biol*. **9**(9): 1089-1097.

30. **Mol M.P., Goncalves J.P., Silva E.A., Scarponi C.F., Greco D.B., Cairncross S. andHeller L. (2016).** Seroprevalence of hepatitis B and C among domestic and healthcare waste handlers in Belo Horizonte, *Brazil.Waste Manag Res*. **34**(9): 875-83.

31. **Moradpour D., Gosert R., Egger D., Penin F., Blum, H.E. and Bienz k. (2003).** Membrane association of hepatitis C virus nonstructural proteins and identification of the membrane alteration that harbors the viral replication complex. *Antiviral Res*. **60**(2): 103-109.

32. **Nail A., Eltiganni S. and Imam A. (2008).** Seroprevalence of Hepatitis B and C among health care workers in Omdurman Sudan. *Sudan JMS*. **3**: 201-206.

33. **Neufeldt C.J., Joyce M.A., Levin A., Steenbergen R.H. *et al.* (2013).** Hepatitis C virus induced cytoplasmic organelles use the nuclear transport machinery to establish an environment conducive to virus replication. *Plos Pathog*. **9**(10): **e** 1003744. 1371/*journal*.

34. **Nyalakonda H. and Utay N.S. (2015).** A new era of therapy for hepatitis C virus infection. *Curr Opin Infect Dis*. **28**(5): 471-478.

35. **Paul D., Madan V. and Bartenschlager R. (2014).** Hepatitis C virus RNA replication and assembly: living on the fat of the land. *Cell Host Microbe*. **16**(5): 569-579.

36. **Romero B.I., Berger C., Kallis S., Kolovou A., Paul D., Lohmann V. and Bartenschlager R. (2015)**. NS5A domain 1 and polyprotein cleavage kinetics are critical for induction of doublemembrane vesicles associated with hepatitis C virus replication. *Mol Biol*. **6**(4). Pii: **e** 00759-15.

37. **Sahar A.I. (2015).** Serodetection of Hepatitis B and C virus among blood donors at the national laboratory for public health, M.Sc thesis, Sudan University.

38. **Simmonds P., Bukh J., Combet C. *et al.* (2005).** Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. *Hepatology*. **42**. 962-973.

39. **Steinmann E., Penin F., Kallis S., Patel A.H., Bartenschlarger R. and Pietschmann T.(2007).** Hepatitis C virus p7 protein is crucial for assembly and release of infectious virions. *PLoS Pathog***. 3**(7): **e** 103.

40. **Susanne M., Dietrich F., Uwe T. and Hermann S. (eds.). (2013).** *Molecular Virology*. london: Spektrum Akademischer Verlag, Heildelberg. Pp: 278-281.

41. **Swadling L., Capone S., Antrobus R.D., Brown A., Richardson R. *et al*. (2014)**. A human vaccine strategy based on chimpanzee adenoviral and MVA vectors that primes, boots, and sustains functional HCV- specific T cell memory. *Sci Transl Med*. **6**(261): 261 ra 153.

42. **Tatsuo M., Stanley M., Christopher M. and Takaji W. (eds.). (2016).***Hepatitis CVirus 1, Cellular and Molecular Virology*. Japan. Springer. Pp: 3-82.

43. **Townsend W.K. and Cheeseman C.R. (2005).** Guidelines for the evaluation and assessment of the sustainable use of resources and of wastes management at health care facilities. *J Waste Manag Res*. **23**: 398-408.

44. **Wakita T., Pietschmann T., Kato T., Miyamoto M. *et al*. (2005).** Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. *Nat Med*. **11**(7): 791-796.

45. **World Health Organisation. (1999).** Unsafe injection practices and transmission of blood borne pathogens. *Bull World Health Org*. **77**: 787-819.

46. **Wozniak A.L., Griffin S., Rowlands D., Harris M., Yi M., Lemon S.M. and WeinmanS.A. (2010).** Interacellular proton conductance of the hepatitis C virus

p7 protein and its contribution to infectious virus production. *PLoS Pathog*. **6**(9): **e** 1001087.

47. **Yazdan Y., Caril G., Migueres B., lot F., Campins M.*et al*. (2005).** Risk Factors for Hepatitis C Virus Transmission to Health Care Workers after Occupational Exposure: A European case control study. *Clini Infec Dis*. **41**: 1423-30.
48. **Zeisel M.B., Felmlee D.J. and Baumert T.F. (2013).** Hepatitis C virus entry. Curr Top Microbiol Immunol. **369**: 87-112.
49. **Zhong J., Gastaminza P., Cheng G., Kapadia S. *et al*. (2005).** Robust hepatitis C virus infection in vitro. *Proc Natl Acad Sci USA*. **102**(26): 9294-9299.

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Questionnaire

Prevalence of hepatitis hepatitis C (HCV) among hospital cleaners

- Serial no: ……
- Age: …… sex: male ☐ female ☐
- Residence: ……
- Marital status:

Single: ☐ rried: ☐ ers: ☐

- Duration of employment:……
- Educational level:
- Basic ☐ high school ☐ university ☐ others ☐
- General health status:

Recent or previous disease:……

History of drugs: ……

Haemodialysis: Yes ☐ No ☐

History of surgery: Yes ☐ No ☐

- Family history of hepatitis:

Yes ☐ NO ☐

- Job training or educationally and advisory lectures :

Yes ☐ No ☐

If yes; how many time per year: ……

- Type of protective equipment regularly used:

Gloves ☐ ks ☐ es ☐ s ☐

Others ☐ mention:……..

…….

…….

- History of blood transfusion:

Yes ☐ No ☐

- Vaccination:

Yes ☐ No ☐

- Accidental needle stick injury:

Yes ☐ No ☐

If yes; how many times:…….

What precaution you took: ……

- Common accidents at work: ……

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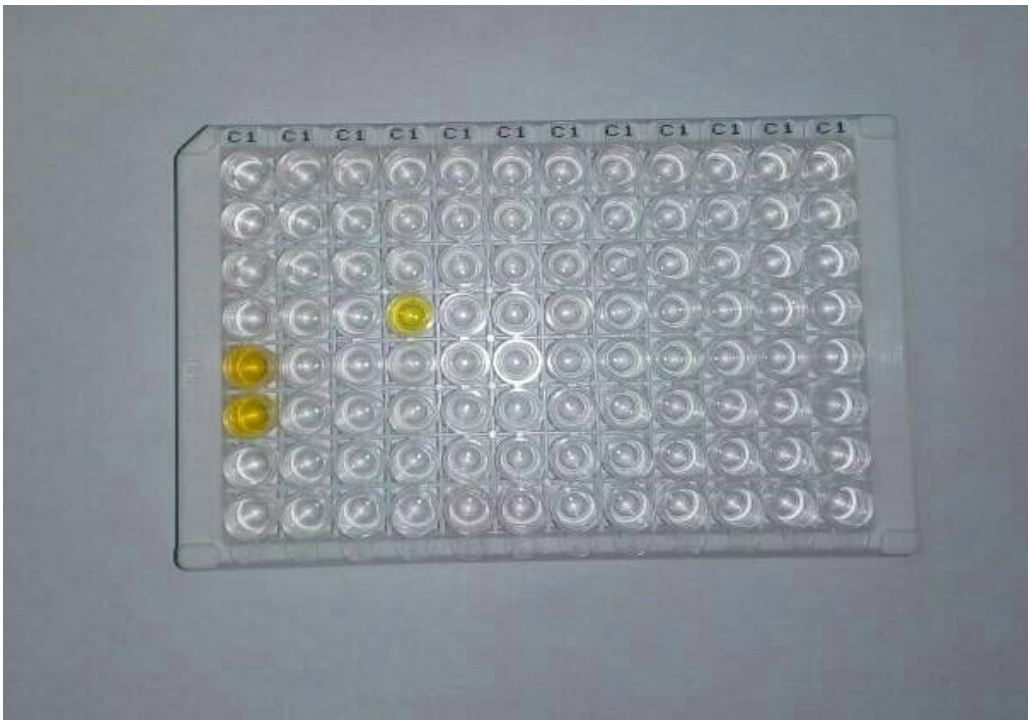

Color Plate of ELISA Kit