CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1.1. INTRODUCTION

Acquisition of blood borne pathogens is a potential occupational health hazard for health care workers (HCWs) across the world (Sharma et al., 2010). Transmissions of over 20 different pathogens have been reported in HCWs because of occupational exposure (Yazdanpanah et al., 2006). The HCWs including clinicians, nurses, laboratory technicians, other hospital technicians, administration and cleaning staff are exposed to an increased risk of occupational infection with Hepatitis C virus (Tarantola et al., 2006).

Despite of continuous progressive preventive measures and employment of modern medical apparatus, HCWs performing exposures prone procedures run a risk for a vast array of blood born pathogen like HCV (Tansley et al., 2004). 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). Hepatitis C virus is predominantly a blood borne virus, with very low risk of sexual or vertical transmission (Shepard et al., 2005). Because of this mode of spread the key groups at risk are injecting drug users (IDUs), people who are transfused blood, recipients
of blood products and sometimes patients on hemodialysis. Common setting for transmission of HCV is also intra-hospital (nosocomial) transmission, when practices of hygiene and sterilization are not correctly followed in the clinic (Alter, 2011). A number of cultural or ritual practices have been proposed as a potential historical mode of spread for hepatitis C virus, including circumcision, genital mutilation, ritual scarification, traditional tattooing and acupuncture (Shepard et al., 2005). The estimated prevalence of HCV infection worldwide is 2.8% (Mohd et al., 2013). Studies of the molecular epidemiology and risk factors for hepatitis C virus (HCV) in health care workers (HCWs) of Peshawar, Khyber Pakhtunkhwa region found 4.13% of the HCWs were positive for HCV antibodies, while HCV RNA was detected in 2.79% of the individuals. Studies of hepatitis C virus in Sudan showed a low Seroprevalence of 2.2%–4.8% (Hatim, 2008).

1.2. RATIONALE

In Sudan the prevalence of HCV infection is 2.2%-4.8 % (Hatim, 2008). Hospitals personnel have an increased risk of being infected because of the exposure to inoculation with infected blood. Hepatitis C virus is one of serious hospital transmitting organism because of this reason the aim of my research is detection of Hepatitis C antibodies among HCW.
1.3. OBJECTIVES

1.3.1. GENERAL OBJECTIVE

To detect Hepatitis C virus seropositivity among health care workers in Khartoum State.

1.3.2. SPECIFIC OBJECTIVES

1- To detect seropositive of Hepatitis C virus antibodies.

2- To evaluate the seroprevalence of Hepatitis C virus among hospitals personnel in Khartoum State.

3- To detect relation between hepatitis C virus and different factor.
2.1. Background

Hepatitis C virus (HCV) is a globally prevalent pathogen and a leading cause of death and morbidity (Cooke et al., 2013).

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).

The hepatitis C virus particle consists of a core of genetic material (RNA), surrounded by an icosahedral protective shell of protein, and further encased in a lipid (fatty) envelope of cellular origin. Two viral envelope glycoproteins, E1 and E2 are embedded in the lipid envelope (Op De Beeck and Dubuisson, 2003).

Hepatitis C virus has a positive sense single-stranded RNA genome (Kato, 2000).

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

The most recent estimates of disease burden show an increase in seroprevalence over the last 15 years to 2.8%, equating to >185 million infections worldwide (Mohd et al., 2013).

Persistent HCV infection is associated with the development of liver cirrhosis, hepatocellular cancer, liver failure, and death (Lauer and Walker, 2001).
While the incidence rate of HCV infection is apparently decreasing in the developed world, deaths from liver disease secondary to HCV infection will continue to increase over the next 20 years. 130–150 million people globally have chronic hepatitis C infection. A significant number of those who are chronically infected will develop liver cirrhosis or liver cancer.

350,000 to 500,000 people die each year from hepatitis C-related liver disease (Razavi et al., 2013; WHO, 2014).

2.2. Geographical distribution

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).

2.3. Transmission

The hepatitis C virus is a blood borne virus. It is most commonly transmitted through: injecting drug users through the sharing of injection equipment, in health care settings due to the reuse or inadequate sterilization of medical equipment, especially syringes and needles, in some countries HCV is transmitted via the transfusion of unscreened blood and blood products, HCV can also be transmitted
sexually and can be passed from an infected mother to her baby however these modes are less common.

Hepatitis C is not spread through breast milk, food or water or by casual contact such as hugging, kissing and sharing food or drinks with an infected person (WHO, 2014).

2.4. 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 (De Lucas et al., 2005; Karayiannis, 2005).
2.5. Signs and symptoms

Persons with newly acquired HCV infection usually are asymptomatic or have mild symptoms that are unlikely to prompt a visit to a health care professional. When symptoms occur, they can include: fever, fatigue, dark urine, clay-colored stool, abdominal pain, loss of appetite, nausea, vomiting, joints pain and jaundice (CDC 2008).

2.6. Diagnosis

Diagnosis of hepatitis is made by biochemical assessment of liver function.

Hepatitis C diagnosis depends on demonstration of anti-HCV detected by: EIA, ELISA and PCR

2.7. Previous Study

Approximately 2%–3% (130–170 million) of the world’s population has been infected with HCV. In many developed countries, including the United States, the prevalence of HCV infection is <2%. The prevalence is higher (>2%) in several countries in Latin America, Eastern Europe, and the former Soviet Union, and certain countries in Africa, the Middle East, and South Asia; the prevalence is reported to be highest (>10%) in Egypt (CDC, 2008).

In the UK, the majority of dental procedures are classified as exposure prone. In order to gauge the prevalence and determinants of infection among dental healthcare workers, a voluntary anonymous survey of HCV infection among
primary care dental workers employed in the West of Scotland was undertaken, in which occupational and personal risk data were collected in parallel with a blood specimen. The overall prevalence of HCV antibodies was 0.1% (1/880, 95% CI 0-0.6); this is no greater than the estimated prevalence of HCV infection in the local population. Personal risk data collected suggested that the single infection identified was acquired through a non-occupational route. These results suggest that HCV infection is not a major occupational risk for dental healthcare workers (Roy et al., 2003).

Another study conducted by Jochen to evaluate the prevalence of hepatitis C virus antibodies (anti-HCV) in patient groups and hospital personnel. The prevalence of anti-HCV in hemophiliacs, intravenous drug users, male homosexuals, and hemodialysis patients was 86, 63, 28, and 9 percent, respectively. Eight of 738 (1.1 percent) sera from health care workers were positive for anti-HCV (Jochen, 1991).

Another study conducted in Japan to determine the best way to protect workers, this study examined viral hepatitis infection in dental care workers in regions with a high prevalence of HCV infections. In total, 141 dental care workers (including dentists, dental hygienists and dental assistants) were enrolled. After a questionnaire to elicit demographic information was administered by an oral surgeon, antibody to HCV (anti-HCV) was measured. When necessary, HCV RNA in serum was measured. No one was positive anti-HCV (Nagao et al., 2008).
In Poland viral hepatitis is the most frequent occupational disease among medical workers. It has been estimated on 72% of all work-related infection diseases diagnosed in the year 2001, and was hepatitis C. The study was conducted to estimate incidence of viral hepatitis type C among medical workers in Silesian voivodeship in years 1996 to 2003. The source of their data was standardized documents of occupational diseases collected in sanitary-epidemiological centers in the voivodeship. The highest incidence was reported among auxiliary workers (48/10,000) and laboratory staff (46/10,000). The smallest incidence of hepatitis type C was achieved for nurses and midwives (18/10,000). Obtained results revealed a large county-to-county variation in HCV incidence and simultaneously increase of incidence (Braczkowska et al., 2005).

Healthcare workers especially nurses have an elevated risk of acquiring and transmitting parenteral Infections. Study was conducted to evaluate the prevalence of Hepatitis B virus (HBV) and Hepatitis C virus (HCV) markers with the final goal to encourage HBV vaccination of the non-immune Indian Nurse. A total of 442 samples were screened from July 2010 to June 2011. They were screened for the presence of hepatitis B surface antigen (HBs Ag), antibodies to Hepatitis B core antigen (anti-HBc) and Hepatitis B Surface antigen (anti-HBs) and anti-HCV antibodies by the third generation ELISA. The HBsAg, anti-HBc antibodies and anti-HBs antibodies prevalence were 1.13 %, 14% and 28% respectively. Anti-HCV antibody was not detected in any of the nurses screened. The presence of
anti-HBc increased with age from 5% in those 18-24 years old to 12% in those more than 50 years old (Anand and Mahesh, 2012).

Another study in which prevalence of hepatitis C virus infection was investigated in health care workers in Pistoia General Hospital (central Italy). Serum samples collected from 511 health care employees engaged in direct clinical task and 222 clerical and nurse school attendees have been tested by ELISA and confirmed by RIBA. Total seroprevalence was 3.8%: 4.7% in the first group; 1.8% in the second group (Catalani et al., 2004).

Study conducted in Pakistan screened 4202 subjects, of these, 681 individuals were reactive either with hepatitis B or C. One hundred and thirty three (3.17%) were hepatitis B reactive and 548 (13.0%) were diagnosed with hepatitis C. After adjusting for age, security personnel, prisoners and IV drug users were 5, 3 and 6 times more likely to be hepatitis B reactive respectively as compared to the health care workers. IDUs were 46 times more likely to be hepatitis C positive compared with health care workers (Abdul Rauf et al., 2012).

Another study in Damascus that found the prevalence of hepatitis C virus antibodies among health care workers was 3%. The positivity of anti-hepatitis C was 0% in the laboratory group, dentistry group, and surgery group. Whereas, it was 6% in the hemodialysis group, and 10% in the other medical workers group (Othman and Monem, 2001).
Previous study among health care worker of Khyber pakhtunkhwa collect blood samples of 824 HCWs, aged between 20-59 years were analyzed for anti-HCV antibodies, HCV RNA and HCV genotypes by Immunochromatographic tests and PCR. All relevant information was obtained from the HCWs with the help of a questionnaire. The study revealed that 4.13% of the HCWs were positive for HCV antibodies, while HCV RNA was detected in 2.79% of the individuals (Sanaullah et al., 2011).
CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

3.1.1. Type of study

This is a descriptive cross sectional study

3.1.2. Study area

Blood samples for this study were collected from personnel of different hospitals in Khartoum State. The experimental work was carried out in the research laboratory, College of Medical Laboratory Science, Sudan University of Science and Technology.

3.1.3. Study duration

The study was conducted in period between February and May - 2015

3.1.4. Study population

Health care workers including (physicians, nurses and laboratory technicians).

3.1.5. Sample size

Ninety HCW were enrolled in this study.
3.2. Sampling technique

This study was based on non probability convenience sampling technique

3.3. Ethical consideration

Approval to conduct this study was obtained from College Ethical Committee, college of Medical Laboratory Science, Sudan University of Science and Technology.

3.4. Specimen

Five ml whole blood was withdrawn using sterile single use syringe. The blood was put in sterile small tube until used.

3.5. Laboratory methods

3.5.1. Separation of sera

The blood was centrifuged at 3000rpm for 5 min at room temperature.

The sera was collected into epindorff tube and stored at -20°C until serological analysis.

3.5.2. Serological analysis

All samples were analyzed for specific anti HCV-antibodies using Enzyme Linked Immunosorbant Assay (ELISA) (Fortress Diagnostic Limited, Antrim, BT 41 1QS United Kingdom.)
3.6. Principle

This kit is employs solid phase indirect ELISA method for detection of antibodies to HCV in two steps incubation procedure. Polystyrene microwell strips are pre-coated with recombinant, highly immunoreactive antigens corresponding to the core and non-structural region 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 antigen.

The wells are washed to remove unbound serum proteins, and rabbit anti-human IgG antibody (antiIgG) conjugated to horseradish peroxidase (HRP-conjugate) is added. During the second incubation step, these HRP-conjugated antibodies will be bound to any antigen-antibody (IgG) complexes previously formed and the unbound HRP-conjugate is then removed by washing. Chromogen solution containing Tetramethylbenzidine (TMB) and urea peroxide are added to the well and in presence of the antigen-antibody-antiIgG (HRP) immunocomplex, the color less Chromogen are hydrolyzed by bound HRP conjugate to a blue-color product. The blue color turns to yellow after the stopping the reaction with sulphuric acid. The amount of color 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 colorless.
3.7. Assay Procedure

All reagents and sera were maintained to room's temperature (18-30°C) for at least 15-30 minutes.

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), two 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°C. 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 5 times 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, 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 5 times 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 solution 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 filter 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 \( \times 2 \).

**Quality control**

Reagent standard were checked for storage, stability and preparation before starting work.
A total of 90 of HCWs were participated in this study. The workers appointed in different hospitals in Khartoum State. These were Omdurman Teaching Hospital, AL-Naow Teaching Hospital, AL-Dosogi specialized Hospital, Khartoum Teaching Hospital, Dar-ELEelage Hospital and Omdurman Military Hospital (Table 1). The workers including Males 59(65.6%); and females were 31(34.4%) (Table2).

Considering the occupation of the study subjects, the most frequent were laboratory technologists 55(61.1%), followed by nurses 20 (22.2%) and 15(16.7%) physicians (Table 3).

Study on the detection of HCV among participants is revealed that all blood samples were negative for anti-HCV antibodies.

The prevalence of HCV in health care workers is zero.
Table 1. Frequency of participants according to hospitals (n=90)

<table>
<thead>
<tr>
<th>Hospital</th>
<th>Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Omdurman Teaching Hospital</td>
<td>30</td>
</tr>
<tr>
<td>AL-Naow Teaching Hospital</td>
<td>20</td>
</tr>
<tr>
<td>AL-Dosogi Specialized Hospital</td>
<td>14</td>
</tr>
<tr>
<td>Khartoum Teaching Hospital</td>
<td>12</td>
</tr>
<tr>
<td>Omdurman Military Hospital</td>
<td>5</td>
</tr>
<tr>
<td>Dar-ELelage Hospital</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
</tr>
</tbody>
</table>
Table 2. Frequency and percentage of participants according to the gender (n=90)

<table>
<thead>
<tr>
<th>Gender</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>59</td>
<td>65.6</td>
</tr>
<tr>
<td>Female</td>
<td>31</td>
<td>34.4</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 3. Frequency and percentage of participants according to occupation (n=90)

<table>
<thead>
<tr>
<th>Occupation</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physician</td>
<td>15</td>
<td>16.7</td>
</tr>
<tr>
<td>Nursing</td>
<td>20</td>
<td>22.2</td>
</tr>
<tr>
<td>laboratory technologist</td>
<td>55</td>
<td>61.1</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>100.0</td>
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</table>
CHAPTER FIVE

DISCUSSION

This study was conducted to determine the seroprevalence of HCV in health care workers. Out of 90 blood samples all of them were seronegative for HCV antibodies. This result less than that obtained by Jochen (1991) that found seroprevalence of HCV in health care worker is 1.1%. Also less than result obtained by Roy et al., (2003) in UK throw dental worker who reported that the prevalence of HCV is 0.1 %. But similar to that obtained by Nagao et al., (2008) in Japan throw dental care workers including dentists, dental hygienists and dental assistants that found the prevalence of HCV is zero typically to this study. Also this result similar to that obtained by Anand and Mahesh (2012) in Indian throw nurse that found the prevalence of HCV is zero. Also this result less than result obtained by Othman and Monem (2001) in Damascus throws HCW that found the prevalence of HCV is 3% (the positivity of anti-hepatitis C was 0% in the laboratory group, dentistry group), and surgery group. Whereas, it was 6% in the hemodialysis group and 10% in the other medical workers group), in general the prevalence is high more than present study but the prevalence of laboratory workers is similar. This difference may be due to the high endimicity of Damascus with HCV or large sample size that was used.
5.2. CONCLUSION

The health care workers in this study are free from HCV; HCV is very rare among health care workers.

5.3. RECOMMENDATIONS

1- More care must be taken by care workers when received HCV patients.

2- Further studies with large number of samples and more advanced technique are required to validate the results of the present study.
REFERENCES


6. CDC. (2008). URL: Division of Viral Hepatitis and National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention


