CHAPTERONE

INTRODUCTION AND OBJECTIVES

1.1. Introduction

Hepatitis C virus (HCV) infection affects 3-4 million people every year and 170 million people are chronically infected with this virus (Tatsuo *et al.*, 2013).The virus causes acute and chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC) (Biscedlie, 2012). It was reported that HCC is one of the most common malignant tumors representing more than 5% of all cancers and ranks at fifth most common cancer in the world (Bruix*et al.*, 2001).

Epidemiological studies show that annual incidence rate of HCC is 3 to 7 cases per 100,000 in North and South America, Northern and Central Europe and Australia (Kao, 2003).

After the discovery of the HCV in 1989 and its linkage to non-A, non-B hepatitis, HCV was first thought to be an infection of minor importance, affecting selected drug users and blood product recipient populations in developed countries. More than 20 years later, it is now well established that HCV is of global importance which affecting all countries leading to a major global health problem that requires widespread active interventions for its prevention and control(Lavancy, 2011).

The two most important risk factors for HCC are chronic HBV and HCV infections an estimated 78% of HCC cases and 57% of liver cirrhosis cases are caused by chronic HBV and HCV infections (Perz*et al.,* 2006).

Both chronic HBV and HCV infections can lead to HCC, a type of liver cancer, and liver disease (But *et al.,* 2008*).*

Infection with HCV is considered one of the major causes of end-stage liver disease including cirrhosis and hepatocellular carcinoma. HCV infects more than 170 million people worldwide (Nelson *et al.*, 2011).

Studies on the epidemiology of HCV have suggested that the Nile delta region of Egypt has one of the highest prevalence rates of HCV infection in the world with seroprevalence rates approaching 20% in villagers over the age of 30 years (Nafeh *et al.,* 2000).

Chronic hepatitis C has been linked to the development HCC in many areas of the world. Of the more than 500 000 new cases of liver cancer occur each year, 22% (>100 000) are attributable to HCV infection. Prospective studies have shown that 80% of cases of acute hepatitis C progress to chronic infection;10–20% of these will develop complications of chronic liver disease, such as liver cirrhosis, within two to three decades of onset and 1–5% will develop liver cancer (Klein *et al*., 1991).

Hepatitis C virus can leads to HCC via oxidative stress, insulin resistance (IR), fibrosis, liver cirrhosis and HCV induced steatosis (Alter, 1997).

Oxidative stress is a key contributor in HCV-induced pathogenesis, previous reports showed that HCV Core protein regulates gene expression and alter cell signaling pathways (Penin *et al.*, 2004)

HCV Core protein also up regulates cyclooxygenase-2 (COX -2) expression in hepatocytes and causes oxidative stress leading to HCC (Nunez *et al.*, 2004).

1.2. Rationale

Hepatitis C Virus causes chronic liver disease. It is major cause of liver fibrosis and leads to development of hepatocellular carcinoma. World Health Organization (WHO) estimate that 3% of world population chronically infected with HCV most of these cases occur in Africa.

Chronic hepatitis C has been linked to the development of hepatocellular carcinoma (HCC) in many areas of the world, of the more than 500 000 new cases of liver cancer that occur each year, 22% (>100 000) are attributable to HCV infection (lavancy, 2011).

Hepatitis C virus (HCV) is a major cause of end stage liver disease in many parts of the world. One hundred and seventy million people are estimated to be infected worldwide.

In Sudan conducted a number of studies addressed the prevalence of HCV such as among Hepatitis B positive patients (Emad aldin*et al.*, 2013). And pre-surgery screened patients (Ikram*et al.*, 2015). But among HCC patients remains untouched.

Due to above facts the current study was designed to determine the frequency of HCV among hepatocellular carcinoma patients.

1.3. Objectives

1.3.1. General objective

To determine the frequency of HCV among hepatocellular carcinoma patients in Khartoum State during 2015.

1.3.2. Specific objectives

1. To detect HCV antibodies in blood samples collected from hepatocellular carcinoma patients.

2. To estimate the percentage of HCV among hepatocellular Carcinoma patients.

3. To determine difference in infection with hepatitis C virus among hepatocellular carcinoma patients according to the gender.

CHAPTER TWO

LITERATURE REVIEW

2.1. Hepatitis

Hepatitis is a general term meaning inflammation of the liver and can be caused by several mechanisms, including infectious agents. Viral hepatitis can be caused by a variety of different viruses such as hepatitis A, B, C, D and E. Since the development of jaundice is a characteristic feature of liver disease and not just viral hepatitis, a correct diagnosis can only be made by testing patients sera for the presence of specific anti-viral antibodies (Houghton, 1996).

The first demonstration that most cases of transfusion-associated hepatitis were caused by neither hepatitis A virus (HAV) nor hepatitis B virus (HBV) the only two known human hepatitis viruses at the time, came in 1975. This new form of disease was called non-A non-B hepatitis and the presumed etiologic agent, non-A non-B hepatitis virus (Purcell, 1994).

2.2. Hepatites C virus (HCV)

In 1989 the virus responsible for most transfusion-associated non-A non-B hepatitis was identified and cloned, and named hepatitis C virus (HCV) (Purcell, 1994).

HCV proteins are reported to interact with host-cell factors that are involved in cell cycle regulation, transcriptional regulation, cell proliferation and apoptosis Severe inflammation and advanced liver fibrosis in the liver background are also associated with the incidence of HCV-related HCC (Tatsuo *et al*., 2013).

HCV is a positive-sense single-stranded RNA virus, the HCV genome is approximately 9,600 nt. in length and consists of a 5' non translated region (5'NTR), a single open reading frame that encodes a poly protein precursor of about 3,000 amino acids, and a 3' NTR.

Both structural (core, E1, E2, and p7) and non structural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) are cleaved from the single open reading frame by both viral and host proteases. The HCV genome also has an internal ribosomal entry site (IRES) that can promote 5'-end-independent initiation of RNA translation (Tatsuo *et al*., 2013).

HCV is an RNA virus, and it is thought to be unable to integrate its genome in to The host genome, in contrast to hepatitis B virus (HBV) or human immunodeficiency virus (HIV). However, HCV proteins and the interaction between them and host proteins mainly contribute to the viral oncogenic processes (Kanda *et al.*, 2012).

2.2.1. Structure of HCV

HCV particle is small 55-65 nm in size, consist of core genetic material (RNA) surrounded by classical icosahedral scaffold protective shell of protein, and further encased in lipid envelope of cellular origin (Beek and Dubussion, 2003).

2.2.2. Classification of HCV

HCV is the only known member of the hepatitis C virus genus in the family flaviridae. There are six major genotypes of HCV, which are indicate numerically (1-6) with several subtypes within each genotypes to represent letters subtypes are further broken down quasi species based on their genetic diversity (Beek and Dubussion, 2003).

2.2.3. HCV genotypes

HCV is classified into eleven major genotypes (designated 1-11), many subtypes (designated a, b, c, etc.), and about 100 different strains (numbered 1,2,3, etc.) based on the genomic sequence heterogeneity (Simmonds, 1999).

The variability is distributed throughout the genome. However, the non-coding regions at either end of the genome (5'-UTR and 3'-UTR; UTR-un translated region) are more conserved and suitable for virus detection by PCR (Simmonds, 1999).

The genes coding for the envelope E1 and E2 glycoproteins are the most variable. Amino acid changes may alter the antigenic properties of the proteins, thus allowing the virus to escape neutralizing antibodies (Simmonds, 1999).

The distribution of HCV genotypes vary according to the geographical region. Genotypes 1-3 are widely distributed throughout the world. Subtype 1a is prevalent in North and South America, Europe, and Australia. Subtype 1b is common in North America and Europe, and is also found in parts of Asia. Genotype 2 is present in most developed countries, but is less common than genotype 1(Anita *et al.*, 2011).

2.2.4 Replication of Hepatitis C Virus

Involve several steps, the viruses need a certain environment to be able to replicate, first move to such area. HCV has high rate of replication with approximately one trillion particles produced each day in an infected individual. Due to lack of proof reading by HCV RNA polymerase as HCV also has exceptionally high mutation rate, a factor that may help it elude the host´s immune response (Linderbash*et al.*, 2005).

Once inside the hepatocytes, HCV mainly replicates within them. However there is controversial evidence for replication in lymphocyte or monocytes by mechanism of host tropism. Circulating HCV particles bind to receptors on the surface of hepatocytes and subsequently enter the cells. Two putative HCV receptors or CD8- 1 and human scavenger receptor class B1 (SB-B1) (Linderbash*et al.*, 2005).

However, these receptors are found throughout the body. The identification of hepatocyte – specific factors that determine observed HCV liver tropism is currently under investigation (Linderbash*et al.*, 2005).

Once inside the hepatocytes, HCV initiates the lytic cycle accomplish it is own replication. The poly protein is then proteolytically processed by viral and cellular protease to produce three structural (virion associated) and no seven structural protein (Linderbash*et al.*, 2005).

Alternatively, a frame shift may occur in core region to produce an alternate reaching from protein (ARFP) HCV include two proteases. The NS protein then recruits the viral genome into RNA replication complex, which is associated with rearranged cytoplasmic membranes (Linderbash*et al.*, 2005).

RNA replication takes place via the viral RNA dependent RNA polymerase NS5B, which produce strand RNA then serves as a template. Production of new positive strand viral genomes. Ascent genomes can then be translated further replicated, or packaged within new virus particles, new virus particles are through to bud into secretary pathway and release at the cell surface (Linderbash*et al.*, 2005).

2.2.5. Stability

HCV is inactivated by exposure to lipid solvents or detergents, heating at 60°C for 10 h or 100°C for 2 min in aqueous solution, formaldehyde (1:2000) at 37°C for 72 h, ß-propriolactone and UV irradiation. HCV is relatively unstable to storage at room temperature and repeated freezing and thawing (Purcell, 1994).

2.2.6. Pathogenesis

HCV is a non-cytopathic virus that enters the liver cell and undergoes replication simultaneously causing cell necrosis by several mechanisms including immune-mediated cytolysis in addition to various other phenomena such as hepatic steatosis, oxidative stress and insulin resistance. The proteins peptides encoded by different sub-genomic regions of the HCV genome and their quasispecies influence the above mechanism, and thus, have a significant role in HCV pathogenesis and disease causation (Mohammad *et al*., 2013).

HCV proteins are reported to interact with host-cell factors that are involved in cell cycle regulation, transcriptional regulation, cell proliferation and apoptosis (Banerjee *et al.,* 2010).

2.2.7. Diagnosis

During HCV infection, every attempt is made to diagnose and differentiate acute from chronic hepatitis C infection. Acute HCV infection is typically mild. It is often not diagnosed, and the infection may be recognized only when it becomes chronic. The diagnostic tests used, including the presence of anti-HCV antibodies in serum, cannot differentiate between acute and chronic HCV infection because anti-HCV IgM, used as marker of acute

infection, is variable in acute infectious disease and is also detected at high rates in patients with chronic HCV infection (Mohammad *et al.*, 2013).

The diagnostic procedures for hepatitis C virus infection used in laboratories are based on the detection of anti-HCV antibodies against recombinant HCV proteins using enzyme immunoassay (EIA).

Non-structural and recombinant antigens are used in these assays. Four different generations of anti-HCV test kits have been developed all these anti-HCV assays had the disadvantages of giving high false positive results and a lack of sensitivity to detect antibodies during the window period. In addition, these antibody-based assays could not distinguish between acute, past and chronic infections. This was followed by the development of supplementary tests involving the recombinant immunoblot assay (RIBA) which was commercialized RIBA had the disadvantages of difficulty in performance and a high percentage of indeterminate results. Therefore these are no longer used in diagnostic laboratories.

Recently, fourth generation anti-HCV assays incorporating additional non structural proteins are being used as screening tests The diagnosis and differentiation of acute from chronic HCV infection poses another problem. Therefore, discrimination between acute and chronic infection in the same patient is sometimes very difficult. HCV RNA in the serum or liver appears to be the earliest detectable marker of acute HCV infection, preceding the appearance of anti-HCV by several weeks (Mohammed *et al.*, 2013)

2.2.8. Treatment

The rationales for treatment of chronic hepatitis are to reduce inflammation to prevent progression to fibrosis, cirrhosis, and HCC through the

eradication of the virus in chronically infected patients, and to decrease infectivity and control the spread of the disease (WHO, 1999).

Interferon has been shown to normalize liver tests, improve hepatic inflammation and reduce viral replication in chronic hepatitis C and is considered the standard therapy for chronic hepatitis C. Currently, it is recommended for patients with compensated chronic hepatitis C (anti-HCV positivity, HCV RNA detection, abnormal ALT levels over at least 6 months, fibrosis shown by liver biopsy). Combination therapy results in better treatment responses than mono therapy the highest response rates have been achieved with pegylated interferon in combination with ribavirin. Interferon has been shown to normalize liver tests, improve hepatic inflammation and reduce viral replication in chronic hepatitis C and is considered the standard therapy for chronic hepatitis C (WHO, 1999).

2.3. Hepatocellular carcinoma

Hepatocellular carcinoma (HCC), also called malignant hepatoma is the most common type of liver cancer. Most cases of HCC are secondary to either a viral hepatitis infection (hepatitis B or C) or cirrhosis (alcoholism being the most common cause of liver cirrhosis) (Kumar *et al.*, 2003).

Treatment options of HCC and prognosis are dependent on many factors but especially on tumour size and staging. Tumour grade is also important. High-grade tumours will have a poor prognosis, while low-grade tumors may go unnoticed for many years, as is the case in many other organs. (Kumar *et al.*, 2003).

HCC is a relatively uncommon cancer in the United States. In countries where hepatitis is not common, most cancers of the liver are not primary HCC but metastasis (cancers spread from elsewhere in the body such as the colon). Severe inflammation and advanced liver fibrosis in the liver back ground are also associated with the incidence of HCV-related HCC (Tarao *et al.*, 1999).

2.3.1. Clinical feature

The classic clinical features of HCC include right upper quadrant pain and weight loss. Other clinical scenarios that suggest this diagnosis include worsening liver function in a patient known to have cirrhosis, acute abdominal catastrophe from rupture of a liver tumor with intra-abdominal bleeding, and some rare extra hepatic manifestations. More and more commonly though, patients are being diagnosed with HCC at an asymptomatic stage while they are being evaluated for liver transplantation or as part of routine screening in those with cirrhosis. Symptoms at initial presentation in a series of 461 Italian patients with HCC showed approximately 23% were asymptomatic, 32% had abdominal pain, 9% malaise, 8% fever, 8% ascites, 8% jaundice, 6% anorexia, 4% weight loss, 4% hemorrhage, and 2% encephalopathy (Alfx and Adrian 2002).

2.3.2. Diagnosis

Tumors are multifocal in the liver in 75% of cases at diagnosis. Diagnosis is usually made by history, physical examination , imaging (ultrasound, MRI or CT scan showing a liver mass consistent with HCC) and optionally elevated serum AFP (>400 ng/ml), because AFP is elevated in only50%-75% of cases. A suspicious lesion on the sonogram generally requires additional imaging studies to confirm the stage of the tumour and sensitivity

for detection of small nodules may be low. The addition of arterial phase imaging to conventional CT scanning increases the number of tumour nodules detected, but in nodular cirrhotic livers the sensitivity to detect HCC is low. The overall sensitivity of MRI is similar to that of triphasic CT scan but in patients with nodular cirrhotic livers MRI has better sensitivity and specificity. Confirmation of diagnosis is made by fine needle aspiration or biopsy. Elevation of AFP >400 ng/ml can be used instead of fine needle cytology for diagnosis of HCC in patients with liver cirrhosis and a focal hyper vascular liver lesion (>2 cm) in at least one imaging technique. Patients with potentially resectable liver mass and AFP >400 ng/ml should undergo surgery without preoperative fine needle aspiration cytology or biopsy. Any deterioration in liver function in a patient with known liver cirrhosis of any aetiology should raise a suspicion of HCC (Julic and Sotiropoulos, 2010).

2.4. HCV and hepatocellular carcinoma (HCC)

An important late complication of hepatitis C is primary HCC, usually occurring in patients with cirrhosis (Walker, 1999*).*

The mechanisms by which HCV may lead to HCC are still unsolved. Unlike HBV, HCV does not integrate into the host genome, and HCV does not seem to encode a transforming protein. HCV itself may therefore not be directly oncogenic. The feature that links HCV with cancer may rather be the repeated cycles of hepatocyte destruction and regeneration over many years. These repetitive-destruction- regenerative cycles may cause neoplastic changes, which then progress to carcinoma (WHO, 1997).

Specific diagnosis requires liver biopsy, the clinical usefulness of liver biopsy is to establish the diagnosis, to identify or exclude other lesions, to

obtain a grading of necro-inflammatory activity, to stage the progression of the disease (clinical follow-up), and to assess the effects of treatment (Dienes *et al.,* 1999).

2.5. Previous studies

Study in Pakistan showed that 35.70% patients had HCC of HCV origin, 31.63% with HBV origin, 21.42% as the etiology of HCC(Junaid *et al.*, 2009).

Another study carried in Nigeria, reveled that out of 108 patients consisting of 81 males and 27 females were studies, giving a male to female ratio 4:1 their ages ranged from 22 to 75 years with mean of 48 years. The highest incidence of hepatocellular carcinoma was found among those aged 40-48 years. A total of 19 patients (17.6%) were positive for HCV antibodies. There was no significant difference in the frequencies of these antibodies between the male and female patients (Mustapha *et al*., 2006).

A study conducted in Milan, Italy, a total of (n=163) pantients with cirrhosis attending the out patient clinic at Sanpaolo Hospital, were enrolled in a prospective study at the early recognition of HCC. The study concluded one hundred four patients (64%) were infected with genotype 1b, 52 (32%) were infected with genotype 2a/c, 1 was infected with genotype 1a, and 2 were infected with genotype 3a. In 4 patients, the HCV genotype could notbe assigned (Savino*et al*., 2007).

A study conducted in New Delihi, India, which enrolled 300 chronic liver disease patients screened for the presence of anti-HCV antibodies, 145 were positive. These HCV antibody positive patients were tested for the presence of HCV RNA and 71 patients were found to be HCV RNA positive. All

HCV RNA positive samples were subjected to genotype determination. The analysis revealed the presence of genotypes 1, 2 and 3 using RFLP and type specific PCR followed by direct sequencing. The genotype 3 was observed in 45 (63.38%) patients. Of these, 31 showed infection with subtype 3a (68.8%), 12 had subtype 3b (26.6%) and 2 patients showed unique subtype 3i and 3f (4.44%). Genotype 1 was seen in 22 (30.98%) patients. Of these 22 cases, 8 had subtype 1a, 10 had subtype 1b and only 5 patients had subtype 1c infection. Genotype 2a was seen in four patients only (Anita *et al*., 2011).

Studywasconducted in Lahore city in Pakistan, show that out of 4246 blood samples, 1914 were collected from male and 2332 from female subjects. Moreover, all the individual samples were categorized into six age groups. The PCR results revealed that 210 (4.9%) individuals had active HCV infection. Gender-wise prevalence of active HCV infection was estimated to be 5.27% in male (101 positive out of 1914 samples) and 4.67% in female subjects (109 positive out of 2332 samples), respectively. Although the probability trends were slightly higher among males of all age groups than females (Muhammad *et al*., 2013).

Study carried in Yemem, to investigate the prevalence of HCV infection among general population in central region of Yemen. The study population comprised 2379 apparently healthy subjects who were screened for HCV antibodies status using ELISA quantitative technique, seroprevalence was calculated and stratified by age, sex, educational level, and monthly income. The study showed that out of 2379 subjects, 31 (1.3%) were HCV antibodies positive. Higher prevalence of HCV antibodies was found among females 24 (1.01%), than males, 7 (0.29%) (Rajesh and Sadiq., 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

3.1.1. Type of study

This is a cross-sectional study.

3.1.2. Study area

This study was carried out in Khartoum State, The State lies between longitudes 31.5 to 34 °E and latitudes 15 to 16 °N.

3.1.3. Study duration

This study was conducted during the period from January to April 2015.

3.1.4. Study population

Hepatocellular carcinoma patients confirmed by CT scan and ultrasound both males and females with different ages were enrolled in this study.

3.2. sample size

Seventy blood samples (n=70) was collected from different hospitals in Khartoum State.

3.3. Sampling technique

This study was based on non probability convenience sampling technique.

3.4. Ethical consideration

This study was approved by the Collage Ethical Committee board, Sudan University of Science and Technology and an informed consent was obtained from each patients before collecting the demographic and clinical data.

3.5. Laboratory methods

3.5.1. Blood Samples

A volume of 5 ml of whole blood was obtain from each patients by venipuncture and collected into a dry tube, allowed it to stand in the pinch until clotted.

3.5.2. Samples processing

Blood samples were then centrifuged at 3000 g for 5 min. Serum was gently collected into eppendorf tube and stored at −20°C until the serological analysis.

3.5.3. Samples analysis

We analyzed the serum samples of the participants for anti-HCV IgG antibodies by a commercially available enzyme -linked immune sorbent assay “HCV-IgG ELISA” kit (fortress diagnostics limited, unit 2C Antrim technology park, Antrim, BT4I IQS (United Kingdom), The assays were performed following the instructions of the manufacturer. Positive and

negative controls were included in each assay. According to the information included in the kit's insert.

3.6. Detection of anti HCV IgG by indirect ELISA

3.6. 1. principle

The ELISA test kit provides a qualitative detection of antibodies to Hepatitis C virus in human serum or plasma, The test 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, immune reactive antigens corresponding to the core and non structural regions of HCV. During the first incubation step, anti-HCV specific antibodies, if present will be bound to the solid phase pre-coated HCV antigens.

The wells are washed to remove unbound serum proteins, and rabbit anti-human IGg antibodies (anti-IGg) conjugated to horseradish peroxidase (HRP-conjugate) 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 removed by washing.

Chromogen solutions containing tetramethylbenzidine (TMB) and urea peroxide are added to the wells and in presence of the antigen-antibodoy-anti-IGg (HRP) immunocomplex. The colorless chromogens are hydrolyzed

by the bound HRP conjugate to a blue color product. The blue color turns yellow after stopping the reaction with sulphuric acid. The amount of color intensity can be measured and is proportional to the amount of antibody captured in the wills and to the sample respectively. Wells containing samples negative for anti-HCV remain colorless.

3.6.2. Procedure

100ul specimen diluents was added to each well except the blank, 10ul of positive control and negative control and specimen added to their respective well. The plate covered with plate cover and incubated for 30 minutes at 37°C, after the end of incubation each well washed 5 times with diluted wash buffer, then 100ul of HRP-conjugate added to each well except the blank, the plate is covered by plate cover and incubated for 30 minutes at 37°C,each well washed 5 times with diluted wash buffer, 50ul of chromogen A and chromorgen B solution is added to each well including blank, the plate incubated at 37°C for 15 minutes, 50ul of stop solution is added to each well, intensive yellow color developed in positive control and anti-HCV positive sample wells, the absorbance was readied within 5minutes after stopping the reaction.

3.6.3. Interpretation of result and Quality control

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

Calculation of cut-off value(C.O.) =* Nc + 0.12.

*Nc = the mean absorbance value for three negative controls

Quality control range: the OD value of the blank well, which contains only choromogens and stop solution, is less than 0.080 at 450nm.

The OD value of the positive control must be equal to or greater than 0.080 At 450/630nm or at 450 after blanking.

The OD value of the negative control must be less than 0.100 at 450/630 or At 450 after blanking.

Interpretations of the result: (S= the individual absorbance (OD) of each specimen).

Negative results (S/C.O <1), positive results (S/C.O.>1).

CHAPTER FOUR

RESULTS

This study was conducted during the period from January to April 2015. A total of seventy(n=70) blood samples were obtained from patients hospitalized with hepatocelluler carcinoma in four hospitals and center in Khartoum state. The samples were collected from Ibrahim Malik Teaching Hospital 36 (51%), Ibn Sina Specialized Hospital 21(31%), Soba University Hospital 8 (12%) and Antalya Diagnostic Centre 5(7%) (Table 1). The seventy samples were collected from 37(53 %) males and 33(47%) females that showed in table (2). All blood samples were examined for the presence of Anti-HCV antibodies using ELISA Kit. The results showed that out of 70 blood samples investigated,14(20%) were positive for Anti-HCV antibodies. The rest 56 (80%) negative (Table 3). Of the positive blood samples, 7(50%) were obtained from males 7(50%) from females. On the other hand, of the 37 males 7(19%) were positive and 30(81%) were negative and from 33 females 7(21%) were positive while 26(79%) were negative.

Table 1. Distribution of patients according to hospitals

Hospitals\ center	**Samples**	
	No	**%**
Ibrahim Malik Teaching hospital	36	51
Ibn Sina Hospital	21	30
Soba University Hospital	8	12
Antalya Diagnostic Center	5	7
Total	70	100%

Table 2. Distributions of patients according to gender

Gender	**Samples**	
	No	**%**
Males	37	53
Females	33	47
Total	70	100

Table 3. Frequency of HCV among hepatocellular carcinoma patients

Results	Samples	
	No.	**%**
Positive	14	20
Negative	56	80
Total	70	100

Table 4. Frequency of HCV among HCC according to gender

Result	**Males**	**Females**
Positive	7(19%)	7(21%)
Negative	30(81%)	26(79%)
Total	37(100%)	33(100%)

CHAPTER FIVE

DISCUSSION

5.1. Discussion

Hepatitis C Virus (HCV) infection is major public health problem worldwide it is amajor cause of end stage liver disease including cirrhosis and Hepatocellular carcinoma (HCC) in many parts of the world (Nelson *etal*., 2011).

This present study aimed at detection of HCV among HCC patients in Khartoum State. Out of 70 blood samples investigated only 14(20%) samples were positive for HCV antibodies. The result is slightly less than that reported by Mustapha *et al*., (2006) in Nigeria who reported that out of 108 patients, 19(17.6%) were positive for HCV antibodies. But is less than that reported by Junaid *etal*., (2009) in Pakistan who reported the percentage of HCV infection as 35.7%.

The little differences between the result of the present study and the study of Junaid and his colleagues, attributed to some factor like the samples size and different geographical area in which each study was conducted. This corresponded to some studies which says the geographic distribution of the

prevalence of HCC and HCV genotypes may provide in direct evidence that certain genotypes of the virus are more commonly associated with more severe disease progression including HCC.

This reinforces that there is a direct relationship between HCV infection

Incidence of HCC.

On the other hand, the present study showed that the prevalence of HCV among females 21%, is slightly higher than males 19%. This in consistence to study conducted in Yemen and concluded the higher prevalence of HCV antibodies was found among females 1.01% than males, 0.29%. This may be due to fact that the present study investigated HCC while the yememi study investigated the distribution of HCV in general. But different from study that carried in Lahore City in Pakistan which reveled that the prevalence of HCV is slightly higher among males 5.27% than females 4.57%. This attributable to the difference in the number of samples and distribution of HCV between different Geographical area in which the study was conducted.

Conclusion5.2.

The frequency of HCV among hepatocellular carcinoma patients is not so high.

There is association between the prevalence of HCC and infection with HCV.

Infection of HCV among males and females is all most equal.

5.3. Recommendations

1. Patients with chronic hepatitis C virus are highly recommended to screen for HCC on regular basis.

2. Education programs to target at risk populations and increase awareness in the general populations about hepatitis C.

3.Additional studies are requiredfor screening of HCV among HCC patients to validate the results of the present study.

4. Due to sensitivity, specificity and easy to perform the use of ELISA for detection of HCV-antibodies is highly recommended.

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