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Sero- detection of IgG antibodies of Hepatitis C Virus in El-obeid City

الكشف المصلي للأجسام المضادة لفيروس التهاب الكبد الوبائي (ج) في مدينة ألابيض

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BY

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الآيـــــة

قال تعالى

(قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَاۖ إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ (32))

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DEDECATION

To my family

To my friends

KNOWLEDGEMENT

I am grateful to Allah without his support I would never have been able to complete anything.

I would like to express my deep thanks to my supervisor Prof. Humodi Ahmed Seed for this continuous help, kindness and construction criticism and comment to all stage of this research, and I am so grateful to Dr. Nasr Mohamed Nasr for their support and technical help.

Thank to my father who toiled me and labored to educate me, and I am very thankful to my gorgeous friend Samah Youssef and Zeinab Abdalglil for their help and support me.

ABSTRACT

Hepatitis C is an infectious disease caused by Hepatitis C Virus (HCV). It is a major public health problem, and infection with this virus may lead to serious consequences such as liver cirrhosis and liver carcinoma. The objective of this study to detect IgG antibodies of Hepatitis C Virus in Elobeid City. The study was conducted during the period from March to October 2017. Ninety (90) subjects were enrolled in this study from both males and females, the age of the subjects range from (18-65) years old, participants were interviewed for risk factors using predesigned form. 5ML of blood were collected randomly from each subject. The blood was centrifuged at 2000 rpm for 5 minutes to obtain serum. The sera were tested by using fourth generation ELISA machine. Data was entered and analyzed with SPSS version 16 statistical software and was summarized and presented in tables. Reveal after risk factors including: (hemodialysis, history of jaundice, tattooing, surgery, multiple sex partners, intravenous drug and blood transfusion) the majority of subjects examined were males 67(73.4%) while females 23 (25.6%).

All samples were tested give Negative result. According to the assay used this study indicates HCV is not prevalent in Elobeid City.

Further studies with large sample size are needed to determine the rate of spreading of HCV among general population in Elobeid City and plans for prevent the spread of infection.

المستخلص

إلتهاب الكبد الفيروسي هو عباره عن مرض معدي يسببه فيروس التهاب الكبد(ج). يعتبر فيروس إلتهاب الكبد الوبائي (ج) واحد من المعضلات الصحيه عالميا والاكثر خطوره وإنتشار وألاصابه به تودي إلي تليف الكبد وسرطان الكبد.

هدفت هذه الدراسه إلي تحديد مدى إنتشارالاجسام المضاده لإلتهاب الكبد الوبائي (ج)في مدينة ألابيض. كان عدد الاشخاص المشاركين بالدراسه 90 شخص من الرجال والنساء وكانت اعمارهم تتراوح بين 18-65 عاما. تم أخذ البيانات من كل فرد من المشاركين علي حسب تعرضه لإحد العوامل التي تودي إلي إلتهاب الكبد الوبائي، وقد اجريت هذه الدراسة في الفترة من مارس إلي أبريل لعام 2017.

تم اخذ 5 مل من الدم لكل فرد من المشاركين وطردها في جهاز الطرد المركزي(2000 دوره) لمدة خمسه دقائق للحصول علي البلازما(سيرم) ثم تم إختبارها لمضاد إلتهاب الكبد الوبائي (ج) عن طريق مقاييس الممتز المناعي المرتبط بي الإنزيم(إليزا).

تم إدخال البيانات وتحليلها عن طريق برنامج الحزم الاحصائيه للعلوم الاجتماعيه(16)، ثم تم إختصارها وعرضها في جداول . كشفت هذه الدراسه تبعا لتعرض اي فرد من المشاركين لإحد العوامل التي تودي إلي إلتهاب الكبد (الاستصفاء الدموي، اليرقان، الجراحه، نقل الدم، وعوامل أخري) ان غالبية المشاركين الذين تم إختبارهم كانو من الذكور 67 (73.4%) والإناث 23(23.6%).

كل العينات التي تم إختبارها اعطت نتيجه سالبه مما يعني انه لا يوجد إنتشار لفيروس التهاب الكبد الوبائي (ج) في ولاية شمال كردفان.

يجب عمل دراسات اخري وإستخدام مجموعه كبيره من العينات لتحديد معدل إنتشار التهاب الكبد الوبائي (ج) في مدينة الابيض وعمل خطط لمنع إنتشار المرض.

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LIST OF ABBREVIATIONS

AHC:	Acute Hepatitis C infection
ALT:	Alanine aminotransferase
CD4:	Helper T cells
CD8:	Cytotoxic T cells
CHC:	Chronic Hepatitis C infection
EIA:	Enzyme immune assay
ELISA:	Enzyme Linked Immuno Sorbent Assay
FDA:	Food and Drug Administration
HCV:	Hepatocellular Carcinoma
HCC:	Hepatitis C Virus
HRP:	Horse Radish Peroxidase
IRES:	Intravenous Drug Use
IDU:	Internal ribosome entry site
NAT:	Nucleic Acid Amplification Test
OCLN:	Occluding
POCT:	Point of care test
PCR:	Polymerase chain reaction
RVR:	Rapid Virological Response
RNA:	Ribo Nucleic Acid
RIBA:	Recombinan immunoblot assay
RT-PCR:	Reverse transcription Polymerase chain reaction
SRBI:	Scavenger Receptor Class B Member 1
SVR:	Sustained Virologic Response
SPSS:	Statistical Packages Of Social Science Soft Program
TMA:	Thrombotic Micro Angiopathy
TMB:	Tetra methylbenzidine

CHAPTER ONE

AND OBJECTIVES INTRODUCTION

CHAPTER ONE

INTRODUCTION AND OBJECTIVE

1.1. Introduction

Hepatitis viruses are the main cause of liver cancer. Among those, hepatitis C virus is the one that causes hepatocellular carcinoma. It can be ended with development of cirrhosis and hepatocellular carcinoma (HCC) (Gunn *et al*., 2001). Hepatitis C virus (HCV) is RNA virus known to infect humans and chimpanzees, causing similar disease in these two species. HCV is most often transmitted parenterally but is also transmitted vertically and sexually (CDC, 1998). World health organization estimated that approximately 170 million people are infected with HCV, about 130 million are carriers, three to four million persons are newly infected in each year and more than 350,000 people estimated to die from hepatitis C related liver diseases in each year worldwide (WHO, 2006). Data from the adult population suggest that approximately 10–20% of patients with HCV can go on to develop cirrhosis and hepato cellular carcinoma and the probability of becoming a chronic carrier is inversely related to age at the time of infection (Castello *et al*., 2010). Transmission is very important some of the risk factors for contracting HCV include sharing of injecting equipment, tattooing or piercing with unsterile equipment or procedures working in environments where there is contact with bodily fluids receiving blood transfusions, receiving dialysis, surgery, sharing of equipment with a person having HCV, practicing unsafe sex or having contact with blood without adequate protection (Jain *et al*., 2007). Hepatitis C virus infection is a major public health concern particularly in African countries, which have the highest prevalence rates of HCV in the world (1–26%) (Madhava *et al*., 2002). In

Africa over 28 million people are chronically infected with HCV and it is difficult to speculate about current and future trends (Onyekwere and Hameed, 2015). Sudan is the largest country in the Nile valley .The Sudanese community is characterized by great social and demographic diversity reflected in the epidemiology of microbial diseases in the country. Studies on HCV in Sudan are few and lack specific national goals. The few studies on HCV infection in Sudan demonstrated a sero prevalence ranging from 2.2% in the Gezira state, in which schistosomiasis is endemic, to 4.8% in patients with schistosomal periportal fibroses (Mudawi, 2008). The prevalence of HCV infection among asymptomatic male Sudanese blood donors was 4.4%. Other studies reported prevalence rates of 3% and 1.5% in southern and northern regions of Sudan, respectively. Furthermore, HCV transmission was evident in healthcare settings, and occupational risk is expected to be high. In presurgery screened patients in Khartoum, central Sudan, prevalence of HCV was 2 % (Osman *et al*., 2012), Unprotected sexual activity (20%) was the most apparent predisposing risk factor for HCV seroreactors, followed by razor sharing (13.3%), parenteral drug injection (10%), tattooing, and surgical procedures. The highest prevalence of HCV infection in Sudan was noted in patients with end stage renal disease who were on regular hemodialysis (seroprevalence of 66.7%). Major risk factors for infection were longer duration of dialysis. Dialysis in multiple centers, and an age over 30 years. Genotype 4 was the most frequently isolated genotype among HCV positive patients in Sudan (Mudawi *et al.*, 2007). The HCV status in Sudan is not well documented, particularly for high risk groups and in healthcare settings. Further studies are urgently needed, including but not limited to population-based studies that are representative of entire communities, and a national cooperative registry system should be established in Elobeid City.

1.2. Rationale

The prevalence of HCV is most common problem in world, because many persons with chronic HCV infection are asymptomatic. Currently, there are no vaccine and post exposure prophylaxis to prevent HCV infection and standard treatment which are highly expensive, associated with side effect and only partially effective, for this reasons population based serological studies are needed to estimate the prevalence of the infection, development and evaluate prevention effort in Elobeid City.

1.3. Objectives

1.3.1. General objective

To investigate Hepatitis C Virus in Elobeid City.

1.3.2. Specific objectives

1. To detect IgG antibodieos of HCV.

2. To determine incidence of Hepatitis C Virus in Elobaid City.

3. To determine the association between the presence of Hepatitis C Virus and certain factor such as sex, age, hemodialysis, history of jaundice and blood transfusion.

CHAPTER TWO

LITERATURE REVIEW

CHAPTER TWO

LITERATURE REVIEW

2.1 Hepatitis C Virus

2.1.1 History of the virus

The hepatitis C virus (HCV) is discovered in 1989 and is quickly established as the major cause of non-A, non-B hepatitis (Choo *et al*., 1989).

2.1.2 Taxonomy

The hepatitis C Virus belongs to the genus Hepacivirus, a member of the family Flaviviridae. Hepatitis C Virus (HCV) is a small (55–65 nm in size) (Ray *et al*., 2009).

2.1.3 Genotypes of Virus

Based on genetic differences between HCV isolates, the hepatitis C virus species is classified into six genotypes (1–6) with several subtypes within each genotype (Simmonds *et al*., 1993; Nakano *et al*., 2011), Subtypes are further broken down into quasi species based on their genetic diversity. Genotypes differ by 30–35% of the nucleotide sites over the complete genome the difference in genomic composition of subtypes of a genotype is usually 20–25%. Subtypes 1a and 1b are found worldwide and cause 60% of all cases (Ohno *et al*., 2007).

2.1.4 Structure of the Virus

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 (Beeck *et al.*, 2003).

2.1.5 Genome Structure and organization

Genome organization of Hepatitis C Virus has a positive sense single stranded RNA genome. The genome consists of a single open reading frame that is 9600 nucleotide bases long (Kato, 2000). This single open reading frame is translated to produce a single protein product, which is then further processed to produce smaller active proteins. The viral proteome only consists of 2 proteins. At the 5' and 3' ends of the RNA are the UTR that are not translated into proteins but are important to translation and replication of the viral RNA. The 5' UTR has a ribosome binding site (Jubin, 2001) that starts the translation of a very long protein containing about 3,000 amino acids. The core domain of the hepatitis C Virus (HCV) IRES contains a four way helical junction that is integrated within a predicted pseudoknot (Berry *et al.*, 2011). The conformation of this core domain constrains the open reading frame's orientation for positioning on the 40S ribosomal subunit. The large pre-protein is later cut by cellular and viral proteases into the 10 smaller proteins that allow viral replication within the host cell, or assemble into the mature viral particles. Structural proteins made by the hepatitis C virus include Core protein, E1 and E2; nonstructural proteins include NS2, NS3, NS4A, NS4B, NS5A, and NS5B (Dubuisson, 2007).

2.1.6 Replication of virus

Hepatitis C Virus nonstructural proteins and viral RNA have been detected in livers of infected patients or experimentally inoculated chimpanzees, confirming that the liver is a site of HCV replication. HCV can also replicate

in peripheral blood mononuclear cells. The HCV replication rate is high, with high mutation rate, leads to great heterogeneity in its presentations, which are known as quasispecies. Following initial binding of the hepatitis C Virus particle to scavenger receptor class B member 1 (SRB1) and CD81, the particle engages in further interactions with the tight junction proteins claudin 1 (CLDN1) and occludin (OCLN) and finally enters cells by receptor mediated endocytosis then The viral RNA genome is released into the cytoplasm and translated at the rough ER, giving rise to a poly protein that is cleaved into mature proteins. Viral proteins, in conjunction with host cell factors, induce the formation of a membranous compartment composed of single, double, and multi membrane vesicles as well as lipid droplets. RNA replication occurs at an unspecified site within the membranous web and proceeds via a negative sense copy ((–)RNA) that serves as a template for the production of excess amounts of positive sense progeny RNAs ((+)RNA) , then assembly of HCV particles probably initiates in close proximity to the ER and lipid droplets, where core protein and viral RNA accumulate. The viral envelope is acquired by budding through the ER membrane in a process that is linked to lipoprotein synthesis. HCV particles are thought to be released via the constitutive secretary pathway. (Blight and Gowans, 1995).

2.1.7 Pathogenesis

Hepatitis C Virus is a non cytopathic Virus (Irshad and Dhar, 2006). After entering a susceptible host, HCV invades, infects and replicates within the blood stream, repeating the process in various tissues, as well as in peripheral B and T lymphocytes, as it proceeds to the liver by tropism, passing through various tissues such as those of the pancreas, thyroid, adrenal glands, spleen and bone marrow (Koziel *et al.,* 1992), Since HCV can also directly infect the lymphatic tissue, its stimulation can lead to the

development of B-cell lymphomas. It is known that the liver is the principal site of HCV replication, and various studies have shown that this virus infects approximately 10% of hepatic cells (Dustin and Rice, 2007). Infection with HCV at extra hepatic sites can promote the appearance of HCV variants (Maggi *et al.,* 1997). Thereby decreasing the chance that the immune system will recognize the virus. 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 quasi species influence the above mechanism, and thus, have a significant role in HCV pathogenesis and disease causation script. The progression of fibrosis in chronic hepatitis C has been associated with the diversity of HCV quasi species (Wang *et al.,* 2007). The production of new viruses is counter balanced by the destruction of infected cells through tissue apoptosis or degradation in peripheral blood, since the half life of the virus in peripheral blood is approximately 2.7 hours (Pawlotsky, 2004*)*. Experimental studies have shown that NS3 and NS5 proteins induce apoptosis in infected hepatocytes (Herzer *et al.,* 2007). In individuals infected with HCV, the persistence of the virus can be attributed the large inoculums and the high rate of viral replication, which allow the virus to evade the host immune response (Cerny and Chisari, 1999). There is controversy over whether the sequence of nucleotides is directly associated with more intense hepatic lesions. There is some evidence of direct cytopathic lesion caused by HCV, including HCV induced histological lesions with scant inflammatory infiltrate (Miller and Purcell, 1990) fulminant hepatitis C after chemotherapy in liver transplants (Naoumov, 1999) and HCV related acute cholestatic syndrome after renal transplantation (Delladetsima *et al.,* 2001). Studies suggest that specific

genotypes, such as genotype 1, can be more cytopathic (Dusheiko *et al.*, 1994) or can induce more rapid progression of the disease than do other genotypes .Genotype 1 has been shown to be the genotype most strongly associated with chronic HCV infection (Amoroso *et al.*, 1998). The risk of cirrhosis and hepatocarcinoma has been shown to be greater in individuals presenting genotype 1b than in those presenting genotypes 2 and 3 (Bellentani *et al.*, 1999). Other studies evidenced that HCV genotype and viral load do not influence the progression of the disease it is known that steatosis is a cofactor that influences the progression of fibrosis in chronic hepatitis C (Cholet *et al.*, 2004). Various studies have directly associated steatosis with HCV genotype 3.Therefore; genotype 3 is considered cytopathic (Rubbia *et al*., 2004).

2. 1.7.1 Humoral Immune Response

Hepatitis C Virus can establish persistent infection despite an active humoral and cellular immune response that is generally targeted against all viral proteins. The virus may escape from the humoral immune response if the kinetics of infection and viral replication do not allow complete neutralization of the virus by HCV specific antibodies after primary infection. Although virus specific antibodies may interfere with viral entry into host cells and opsonize the virus for elimination by macrophages, they cannot eliminate HCV from infected cells. In addition, HCV has a high mutation rate, especially in the hyper variable region of the envelope proteins that can be recognized by neutralizing antibodies (antibodies that can bind and eliminate virus) (Kurosaki *et al.,* 1993). Several studies have demonstrated that the humoral immune response can select HCV variants with sequence changes that allow escape from antibody recognition (Kato *et al.,* 1993). However, recent studies in chimpanzees have suggested that HCV can cause persistent infection in the absence of mutations in the hyper

variable region Thus, progression to persistent HCV infection is most likely a multi factorial process that depends on multiple aspects of virus host interaction (Bassett *et al*., 1999).

2.1.7.2 Cellular Immune Response

The cellular immune response probably plays an important role in the outcome of HCV infection because of its ability to recognize and eliminate virus from infected cells. The antigen specific immune response is mediated by CD4-positive helper T cells and CD8-positive cytotoxic T cells. Because chronic rather than acute infection is diagnosed in most patients, immunologic studies have been performed on patients with persistent infection who could not clear HCV. Only a few studies have analyzed the cellular immune response during the acute phase of infection. These studies suggest that the strength and quality of both helper T-cell (Diepolder *et al.*, 1997) and cytotoxic T-cell responses differ between patients who recover and those who develop chronic infection. More important, the viral sequences that are recognized most frequently and vigorously by HCV specific T cells vary little among all the HCV genotypes. Furthermore, several of these frequently recognized viral peptides bind with high affinity to many different classes II MHC molecules, suggesting that they can be efficiently presented and recognized by patients with different MHC haplo type's.Thus, these viral sequences could be explored for development of preventive or therapeutic vaccines against HCV. The cellular response against HCV could be interfered with in several ways. First, HCV elicits only a weak T-cell response in patients who develop chronic infection (Cooper *et al.,* 1999). Certainly, general immune tolerance or immune suppression is not the cause of persistent HCV infection, because most chronically infected patients display normal immune responses against other viral agents (Rehermann *et al*., 1996). The emergence of viral mutants or

quasi-species with sequence variations in T-cell epitopes may contribute to the apparent ineffectiveness of cell-mediated immune response (Weiner *et al*., 1995). There is also increasing evidence that several HCV proteins, such as core, E2 and NS5A, interfere with the immune response. Furthermore, infected hepatocytes, which lack co-stimulatory molecules, may be relatively inefficient in priming the immune system, and the liver has been proposed as the major site where activated T cells are destroyed. Finally, the cellular immune response is a double edged sword. An immune response that is ineffective in clearing HCV infection may be more harmful to the liver, causing chronic inflammation, hepatocellular injury and over several decades, liver fibrosis and cirrhosis. Progression to persistent infection and the immunologic mechanisms of liver injury are the consequence of complicated interactions between the virus and host (Nester *et al*., 2009).

2.2 Symptoms of hepatitis C

Many people with hepatitis C don't have any symptoms and are unaware they have the infection. They may develop symptoms later on as their liver becomes increasingly damaged (Cheesebraough, 2006).

2.2.1 Early symptoms

Only around one in every three or four people will have any symptoms during the first six months of a hepatitis C infection. This stage is known as acute hepatitis C (Wilkins *et al*., 2010). If symptoms do develop, they usually occur a few weeks after infection. Symptoms may include :a high temperature of 38C (100.4F) or above, tiredness, loss of appetite, tummy (abdominal) pains, feeling and being sick, around one in every five people who experiences symptoms will also have yellowing of the eyes and skin. This is known as jaundice. In around one in every four people infected with

hepatitis C, the immune system will kill the virus within a few months and the person will have no further symptoms, unless they become infected again. In the remaining cases, the virus persists inside the body for many years, this is known as chronic hepatitis (Nelson *et al.*, 2011).

2.2.2 Later symptoms

The symptoms of long term (chronic) hepatitis C can vary widely. In some people, symptoms may be barely noticeable. In others, they can have a significant impact on their quality of life. The symptoms can also go away for long periods of time and then return. Some of the most common problems experienced by people with chronic hepatitis C include: feeling tired all the time, joint and muscle aches and pain feeling sick, problems with short term memory, concentration and completing complex mental tasks such as mental arithmetic, many people describe this as "brain fog", mood swings, depression or anxiety, indigestion or bloating , itchy skin and abdominal pain, If left untreated, the infection can eventually cause the liver to become scarred (cirrhosis). Signs of cirrhosis can include jaundice, vomiting blood, dark stools, and a buildup of fluid in the legs or abdomen (Rosen, 2011).

2.3 DIAGNOSIS

The purpose of diagnosis of viral infection is to allow the infected persons to be identified and treated. Thus, diagnosis of viral infection is important to prevent disease progression and viral spread. Majority of primary HCV infected patients are asymptomatic, thus, symptoms could not be used as specific indicators for HCV infection. HCV viremia could still exist despite a normal serum alanine aminotransferase (ALT) level. Therefore, virological methods rather than ALT levels are used to diagnose HCV infection

(Chevaliez and Pawlotsky, 2009) In general; the virological methods for examining viral infections include indirect and direct tests. The indirect tests are to detect antibody induced by viral infection, including IgM for recent infection and IgG for recent or past infection. The direct tests include virus isolation, detection of viral antigens and viral nucleic acids. At present, it is difficult to isolate and culture HCV using clinical specimens. Furthermore, anti-HCV IgM could be detected not only in 50%-93% of patients with acute hepatitis C but also in 50%-70% of CHC patients (Sagnelli *et al.*, 2003). Therefore, anti-HCV IgM cannot be used as a reliable marker for the acute HCV infection, and IgM assays have not been used in clinical practice (Farci *et al.*, 1992). At present, diagnostic assays for anti-HCV total antibody, viral core antigen, and viral genomic RNA are used in clinical practice, this assays are: POCT: Point of care test; EIA: Enzyme immunoassay; RIBA: Recombinant immunoblot (Tillmann, 2014).

2.3.1 Detection of antibody production

In general, serological tests for detecting anti-HCV antibodies include tests for screening and confirmation. Screening tests are used first to screen the antibody positive specimens while confirmatory tests are then used to verify the positive screening specimens (Wilkins, 2010).

2.3.1.1 Screening test: EIA

At present, the third generation test of EIA for the anti-HCV antibody detection is commonly used in the diagnostic laboratory (Alborino *et al.*, 2011). Conserved antigens from the HCV core, NS3, NS4 and NS5 regions are used in these tests to detect anti HCV antibodies. The sensitivity of third generation EIAs was estimated at 98.9% and the specificity was found at 100% in patients with chronic liver disease (Colin *et al.*, 2001). EIAs are

easy to use and inexpensive. Furthermore, this assay could be fully automated and adapted to large volume testing. Therefore, EIAs to detect anti-HCV antibody are generally recommended for screening the HCV infections. However, this assay should not be used in infants younger than 18 months due to the possibility of reactivity with maternal antibody. However, the time between HCV infection and the appearance of detectable antibodies (serological window period) is generally more than 40 days using the third generation EIAs (Barrera *et al.,* 1995). The fourth generation EIA could detect the anti-HCV antibody significantly earlier than the other assays. The antigens utilized in the fourth generation anti-HCV assay are derived from the core (two different epitope clusters), NS3, NS4A, NS4B, as well as the NS5A regions. NS3 and NS4 antigens are derived from genotypes 1a, 1b, 2 and 3 (Alborino *et al.,* 2011).

2 .3.1.2 Screening test: The rapid, point-of-care test

Point-of-care tests are used directly at the site of patient care, outside of the diagnostic laboratory (Chevaliez, 2013). Several point-of-care tests (POCTs) have been developed to detect anti-HCV antibodies with a relatively high sensitivity and specificity (Scalioni *et al.,* 2014). It is use in patients over 15 years old, for screening persons who are considered at risk for HCV infection. This test detects anti-HCV antibodies in different specimens, *e.g.*, finger stick and veni puncture whole blood, serum, plasma, or oral fluid. Recombinant proteins or synthetic peptides of core, NS3 and NS4 antigens are immobilized on a nitrocellulose membrane to perform an indirect lateral flow immunoassay, and the results are directly visualized using colloidal gold labeled protein A, which generates a reddish-purple line within 20 to 40 min in the presence of anti-HCV antibodies in the specimens. These rapid tests are cheap, simple to perform and fast (Lee *et al.,* 2011).

2.3.1.3 Confirmatory tests: Recombinant immunoblot assays Recombinant immunoblot assays (RIBA) can be used to confirm the presence of anti-HCV antibodies for individuals who have showed positive reactivity by EIAs (Saludes *et al.*, 2014). This assay is highly specific, as the presence of antibodies against each of the several HCV proteins is assessed as individual bands on a membrane strip. This assay includes recombinant proteins and synthetic peptides from E2 hyper variable region, NS3 helicase, and NS4A, NS4B and NS5A regions. However, these indirect virological tests to detect anti-HCV antibody cannot distinguish current from past infection (Yuki *et al*., 1995). Active HCV infection must be confirmed by the direct diagnostic methods.

2.3.2 Detection of viral RNA

Based on the items used for amplification, nucleic acid amplification tests (NAT) are divided into target amplification, signal amplification and probe amplification methods (Persing and Landry, 1989). Target amplification methods [*e.g*., reverse transcription-polymerase chain reaction (RT-PCR) and transcription-mediated amplification (TMA) and signal amplification methods for example branched DNA (bDNA) was commonly used to detect the presence of HCV RNA. The presence of HCV RNA in the serum is a reliable marker of viremia. Universal standardization for HCV RNA titer is important. The World Health Organization (WHO) has established an international standard for HCV RNA quantification units *i.e.*, an HCV RNA international unit (IU), which is currently used in all of the commercial HCV RNA quantitative assays no matter what the techniques used (Pawlotsky, 2002).

2.3.2.1 Qualitative HCV RNA detection: Qualitative detection assays are based on the principle of target amplification using either RT-PCR or TMA.

HCV RNA is extracted and converted into complementary DNA (cDNA) using reverse transcriptase. The cDNA is subsequently processed via cyclic enzymatic reactions leading to the generation of a large number of double stranded DNAs in PCR-based assays or single stranded RNAs in TMA. Detection of these amplified products is achieved by hybridizing the produced amplicons onto specific probes. In general, the highly conserved 5'UTR region is the target of choice for HCV genomic RNA detection across different genotypes (Mack *et al*., 2012; Saludes *et al.*, 2014).

2.3.2.2 Quantitative HCV RNA detection: HCV RNA can be quantified by means of target amplification techniques (real-time RT-PCR or TMA) or signal amplification techniques (bDNA assay). Real-time RT-PCR is the method of choice for the quantification of HCV RNA levels in clinical practice. This assay is highly sensitive with wide dynamic range of quantification and can prevent carry over contamination. Not all HCV genotypes are detected equally by NAT assays, most likely because of nucleotide a mismatch which has occurred before (Chevaliez *et al*., 2013). HCV RNA in the serum is probably the earliest detectable marker of acute HCV infection, preceding the appearance of anti-HCV antibody by several weeks. CHC infection is defined as the presence of HCV RNA more than 6 month. HCV RNA levels remain relatively stable over time in CHC patients. Therefore, after a positive reaction screened by the anti-HCV antibody test, NATs to detect HCV RNA is often used as the confirmatory tool to diagnose CHC infection (Butcher *et al.*, 2014). Detection of HCV RNA is also used to determine the viral load both prior to and during antiviral treatments. On the other hand, the HCV RNA level has no prognostic value. The level of HCV genomic RNA, reflection of HCV replication, does not correlate with the severity of liver disease, not with the risk of liver disease progression to cirrhosis or HCC (Pawlotsky, 2010).

2.3.3 Detection of viral core antigen:

Compared to other diagnostic methods like EIA, the advantages of NATs are having higher specificity and sensitivity (Tillmann, 2014). However, the disadvantages of these assays are time-consuming and require sophisticated technical equipment, trained technicians, dedicated laboratory space and expensive reagents. In patients with HCV infection, it has been demonstrated that the HCV core antigen level strongly correlates with the HCV RNA level for various genotypes. Thus, due to cheap and easy-to-perform, the HCV core antigen quantification assay can be used as an alternative method to NATs to detect HCV RNA. In general, about 90% of HCV RNA positive samples are positive with a viral load above 10000 IU/mL, well in the sensitivity range of the HCV core antigen assay. Therefore, HCV antigen detection might be the next step following a positive antibody screening test. Several combination assays for detection of both anti-HCV antibodies and HCV core antigen have been developed (Laperche *et al*., 2005). At present, EIA to detect HCV core antigen is too insensitive to replace the NATs to detect HCV RNA in the blood bank setting, and in the treatment monitoring according to the current clinical practice guidelines. However, it could be used as a supplemental test in resource-limited settings (Chevaliez *et al*, 2014).

2.3.4 Interpretations of diagnostic results

The presence of HCV RNA in the absence of anti-HCV antibodies is strongly indicative of acute hepatitis C (AHC), which can be confirmed by sero conversion (*i.e*., the appearance of anti-HCV antibodies) a few days or weeks later. However, there are still other possibilities for the presence of HCV RNA in the absence of anti-HCV antibodies for example CHC infection in the immune depressed patients, hemodialysis patients or

agammaglobulinemic subjects. Possible diagnostic results for hepatitis C Virus infection. Individuals are in high risk, *e.g*., persons who have been exposed to HCV; persons with elevated alanine aminotransferase; persons who are immunecompromised ,The presence of both anti-HCV and HCV RNA does not allow one to distinguish AHC from an acute exacerbation of CHC. The anti-HCV IgG avidity index within the first 8 d following the onset of clinical symptoms may be useful in identifying actual AHC (Coppola *et al.,* 2007). If the antibody test is positive and the HCV RNA test is negative, this result indicates a resolution of HCV infection or AHC during a period of low-level viremia. If the HCV RNA assay is negative and remains negative for more than 6 month, then the individuals are recovered from a past HCV infection. CHC is defined as the persistence of HCV RNA for more than 6 month. In patients with clinical signs of chronic liver disease, CHC is certain when both anti-HCV antibodies and HCV RNA are present (Chevaliez and Pawlotsky, 2009).

2.3.5 Genotyping

Different HCV genotypes would result in different responses to antiviral treatments. Thus, genotyping is important to predict the likelihood of response and determine the optimal duration of therapy (Mack *et al.,* 2012).

2.3.5.1 Serological method

The HCV genotype can be determined by detection of antibodies against HCV genotype-specific epitopes using a competitive EIA. The currently available assay (Murex HCV serotyping 1-6 HC02, Abbott Laboratories, North Chicago, Illinois) could identify the six HCV genotypes (1-6) but not subtypes, and provide interpretable results in approximately 90% of chronically infected immunocompetent patients (Montenegro *et al*., 2013).

2.3.5.2 Molecular techniques

The reference method for HCV genotyping is genome sequencing of the core/E1 or the NS5B regions and subsequent phylogenetic analysis. This in house method is restricted to reference centers. The Linear Array HCV Genotyping Test (Roche Molecular Systems) targets the 5'UTR, this assay is based on conventional PCR amplification followed by reverse hybridization onto membrane strips containing specific probes. The obtained band pattern can be either visually interpreted or read by a scanner. Assays targeting other regions in addition to the 5′UTR have been recently developed to better discriminate between subtypes 1a and 1b. The Versant HCV genotype 2.0 assays are also based on reverse hybridization and targets the 5'UTR and core regions. On the other hand, the Abbott Real Time HCV Genotype II (Abbott Molecular) targets the 5'UTR and NS5B regions. This assay is based on a single step real time RT-PCR with labeled genotype-/subtype-specific probes that minimize contamination with amplified products. (Shinol *et al.*, 2012).

2.3.6 Sub typing

Hepatitis C Virus sub typing is important for epidemiological studies, especially in the case of outbreaks, but it is not considered to be clinically relevant for the treatment of interferon-α and ribavirin. Sub typing may be clinically relevant in the era of DAAs. For example, the phase 3 studies of telaprevir, boceprevir, faldaprevir and simeprevir showed lower sustained virologic response (SVR) rates for HCV-subtype 1a than those for subtype 1b. In addition, BILB 1941, a non-nucleoside inhibitor of HCV NS5B, has been shown to have better antiviral efficacy in patients with subtype 1b than in those with subtype 1a. Therefore, methods to determine the HCV subtypes should be important in the era of DAAs. The second generation

line probe assay, a reverse hybridization assay that uses probes targeting both the 5'UTR and core coding region, correctly identified HCV subtypes 1a and 1b in more than 99% of cases. Thus, this assay could be used to differentiate HCV subtypes 1a and 1b in clinical trials and practice (Chevaliez *et al.*, 2009).

2.4 Epidemology

2.4.1 Natural reservoir: Human is only natural reservoir of HCV, and chimpanzee remains the only proven model for experimental HCV infection (Shors and Teri, 2011).

2.4.2 Transmission

The primary route of transmission in the developed world is intravenous drug use (IDU), while in the developing world the main methods are blood transfusions and unsafe medical procedures (Maheshwari and Thuluvath, 2010). The cause of transmission remains unknown in 20% of cases; many of these are believed to be accounted for by IDU (Pondé, 2011).

A. Drug use

Intravenous drug use (IDU) is a major risk factor for hepatitis C in many parts of the world (Xia *et al*., 2008) of 77 countries reviewed, 25 (including the United States) were found to have prevalences of hepatitis C in the intravenous drug user population of between 60% and 80%. Twelve countries had rates greater than 80%. It is believed that ten million intravenous drug users are infected with hepatitis C; China (1.6 million), the United States (1.5 million), and Russia (1.3 million) have the highest absolute totals. Occurrence of hepatitis C among prison inmates in the United States is 10 to 20 times that of the occurrence observed in the general

population; this has been attributed to high-risk behavior in prisons such as IDU and tattooing with non sterile equipment Shared intranasal drug use may also be a risk factor (Moyer, 2013).

B. Healthcare exposure

Blood transfusion, transfusion of blood products, or organ transplants without HCV screening carry significant risks of infection. Those who have experienced a needle stick injury from someone who was HCV positive have about a 1.8% chance of subsequently contracting the disease themselves (Wilkin *et al.*, 2010). The risk is greater if the needle in question is hollow and the puncture wound is deep. There is a risk from mucosal exposures to blood, but this risk is low, and there is no risk if blood exposure occurs on intact skin. Hospital equipment has also been documented as a method of transmission of hepatitis C, including reuse of needles and syringes; multiple use medication vials; infusion bags; and improperly sterilized surgical equipment, among others (Alter, 2007).

C. Sexual intercourse

Whether hepatitis C can be transmitted through sexual activity is controversial. While there is an association between high-risk sexual activity and hepatitis C, and multiple sexual partners are a risk factor for hepatitis C, there is no conclusive evidence that hepatitis C can be transmitted by sexual activity, since people who report transmission with sex as their only risk factor may actually have used drugs but denied it. The majority of evidence supports there being no risk for heterosexual couples with only one sexual partner. Sexual practices that involve higher levels of trauma to the anogenital mucosa, or that occur when there is a concurrent sexually

transmitted infection, including HIV or genital ulceration, do present a risk (Tohme and Holmberg, 2010)**.**

D. Body modification

Tattooing is associated with two to three fold increased risk of hepatitis C. This can be due to either improperly sterilized equipment or contamination of the dyes being used. Tattoos or piercings performed either before the mid-1980s, "underground," or non professionally are of particular concern, since sterile techniques in such settings may be lacking. The risk also appears to be greater for larger tattoos. It is estimated that nearly half of prison inmates share unsterilized tattooing equipment. It is rare for tattoos in a licensed facility to be directly associated with HCV infection (Jafari *et al.,* 2010).

E. Shared personal items

Personal care items such as razors, tooth brushes, and manicuring or pedicuring equipment can be contaminated with blood. Sharing such items can potentially lead to exposure to HCV. Appropriate caution should be taken regarding any medical condition that results in bleeding, such as cuts and sores. HCV is not spread through casual contact, such as hugging, kissing, or sharing eating or cooking utensils. Neither is it transmitted through food or water (Wong and Lee, 2006).

F. Mother to child transmission

Mother-to-child transmission of hepatitis C occurs in less than 10% of pregnancies. There are no measures that alter this risk. It is not clear when transmission occurs during pregnancy, but it may occur both during gestation and at delivery. A long labor is associated with a greater risk of transmission. There is no evidence that breast feeding spreads HCV;

however, to be cautious, an infected mother is advised to avoid breast feeding if her nipples are cracked and bleeding, or if her viral loads are high (Lam *et al.*, 2010).

2.4.3. Treatment

HCV induces chronic infection in 50–80% of infected persons. Approximately 40–80% of these clear with treatment (Torresi *et al.*, 2011). In rare cases, infection can clear without treatment. Those with chronic hepatitis C are advised to avoid alcohol and medications toxic to the liver, and to be vaccinated for hepatitis A and hepatitis B. Ultrasound surveillance for hepatocellular carcinoma is recommended in those with accompanying cirrhosis. (Wilkins *et al.*, 2010).

2.4.3.1 Medications

Treatment with antiviral medication is recommended in all people with proven chronic hepatitis C who are not at high risk of dying from other causes. People with the highest complication risk should be treated first, with the risk of complications based on the degree of liver scarring. The initial recommended treatment depends on the type of hepatitis C virus with which a person is infected (AASLD and IDSA, 2015).

.**A**. HCV genotype 1a: 12 weeks of ledipasvir and sofosbuvir OR 12 to 24 weeks of paritaprevir, ombitasvir, dasabuvir, and ribavirin.

B. HCV genotype 1b: 12 weeks of ledipasvir and sofosbuvir OR 12 weeks of paritaprevir, ombitasvir, and dasabuvir.

C. HCV genotype 2: 12 to 16 weeks of sofosbuvir and ribavirin.

D. HCV genotype 3: 12 weeks of sofosbuvir, ribavirin, and pegylated interferon.

E. HCV genotype 4: 12 weeks of ledipasvir and sofosbuvir OR paritaprevir, ritonavir, ombitasvir, and ribavirin, OR 24 weeks of sofosbuvir and ribavirin

F. HCV genotype 5 or 6: sofosbuvir and ledipasvir (AASLD and IDSA, 2015).

Sofosbuvir with ribavirin and interferon appears to be around 90% effective in those with genotype 1, 4, 5, or 6 diseases. Sofosbuvir with just ribavirin appears to be 70 to 95% effective in type 2 and 3 disease but has a higher rate of adverse effects. Treatments that contain ledipasvir and sofosbuvir for genotype 1 has success rates of around 93 to 99% but is very expensive in genotype 6 infection, pegylated interferon and ribavirin is effective in 60 to 90% of cases. There is some tentative data for simeprevir use in type 6 disease as well (Bunchorntavakul *et al.,* 2013). Prior to 2011, treatments consisted of a combination of pegylated interferon alpha and ribavirin for a period of 24 or 48 weeks, depending on HCV genotype. This produces cure rates of between 70 and 80% for genotype 2 and 3, respectively, and 45 to 70% for genotypes 1 and 4. Adverse effects with these treatments were common, with half of people getting flu like symptoms and a third experiencing emotional problems. Treatment during the first six months is more effective than once hepatitis C has become chronic (Ozaras and Tahan, 2009).

2.4.3.2 Surgery

Cirrhosis due to hepatitis C is a common reason for liver transplantation though the virus usually (80–90% of cases) recurs afterwards. Infection of the graft leads to 10–30% of people developing cirrhosis within five years.

Treatment with pegylated interferon and ribavirin post transplant decreases the risk of recurrence to 70% (Coilly *et al*., 2013).

2.4.3.3 Alternative medicine

Several alternative therapies are claimed by their proponents to be helpful for hepatitis C including milk thistle, ginseng, and colloidal silver. However, no alternative therapy has been shown to improve outcomes in hepatitis C, and no evidence exists that alternative therapies have any effect on the virus at all (Liu *et al.,* 2003).

2.4.3.4 Prognosis

The responses to treatment is measured by sustained viral response (SVR), defined as the absence of detectable RNA of the hepatitis C virus in blood serum for at least 24 weeks after discontinuing the treatment, and rapid virological response (RVR) defined as undetectable levels achieved within four weeks of treatment. Successful treatment decreases the future risk of hepatocellular carcinoma by 75%. Prior to 2012 sustained response occurs in about 40–50% in people with HCV genotype 1 given 48 weeks of treatment. A sustained response is seen in 70–80% of people with HCV genotypes 2 and 3 with 24 weeks of treatment. A sustained response occurs about 65% in those with genotype 4 after 48 weeks of treatment. The evidence for treatment in genotype 6 disease is sparse and what evidence there is supports 48 weeks of treatment at the same doses used for genotype 1 disease (Fung *et al.,* 2008).

2.4.4 Hepatitis C vaccine

A hepatitis C vaccine, a vaccine capable of protecting against hepatitis C, is not available. Although vaccines exist for hepatitis A and hepatitis B,

Development of a hepatitis C vaccine has presented challenges (Randal, 1999). No vaccine is currently available, but several vaccines are currently under development. Most vaccines work through inducing an antibody response that targets the outer surfaces of viruses. The Hepatitis C virus is highly variable among strains and rapidly mutating, making an effective vaccine very difficult (Strickland *et al.,* 2008).

2.4.5 Prevention

Currently there is no vaccine against the hepatitis C virus. So, to avoid the spread of the disease, people should: Cover cuts and scratches with appropriate dressings, hygienically dispose of blood-stained items such as bandages and sanitary napkins, a void sharing personal items that may be contaminated with blood (such as toothbrushes and razors),avoid sharing drug injecting equipment, avoid tattooing, acupuncture or body piercing where the equipment is not known to be adequately sterilized, Practice safe sex, People with the hepatitis C virus should advise their dentist or any other health professional that they are carriers of the virus and the screening of blood donors is important at a national level, as is adhering to universal precautions within healthcare facilities (Ray *et al*., 2009).

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design

This is a cross- sectional study.

3.1.1 Study area

This study was conducted in Elobeid City.

3.1.2 Study population

The study was carried out on Sudanese who lived in Elobeid City.

3.1.3 Study duration

The study was conducted during the period from March to October 2017.

3.1.4 Inclusion criteria

Any person who lived in Elobeid or patient came to Elobeid hospital for investigation such as (liver disease and hemodialysis) if they were male or female and the age range from 18-65 years old, and were exposed to any risk factors which transmit the Hepatitis C Virus, this factors include (hemodialysis, history of jaundice, tattooing, surgery, multiple sex partners, intravenous drug and blood transfusion).

3.1.5 Exclusion criteria

Any person who did not live in Elobeid, or patient came to the Elobeid hospital for other investigation except liver disease and hemodialysis, male or female below 18 years old and over 65 years old, and not exposed to any risk factor which transmit the Hepatitis C Virus.

3.1.6 Sample size

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

3.1.7 Data collection

Data were collected from each subject using interviewed form, interview Include: age, sex and possible risk factors (hemodialysis, history of jaundice, tattooing, surgery, multiple sex partners, intravenous drug and blood transfusion) associated with HCV.

3.1.8 Ethical consideration

Approval to conduct this study was obtained from the College of Graduate Studies, Sudan University of Science and Technology. Permission to take blood specimens was obtained from Elobeid Teaching Hospital administration, written consent was taking from each person before filling the form or taken blood.

3.2 Collection of specimens

Under strict sterile condition, 5 ml of whole venous blood were obtained from the people .The specimens were collected in sterile plain container, left to clot and serum was separated by centrifugation at 2000rpm for 5 minute and then stored at -20c until tested.

3.3 Laboratory diagnosis

Fourth generation ELIZA was used to detect HCV Ab according to the manufacture's guidelines.

3.3.1 ELISA for detection of HCV Abs

The reagent were supplied by Fortress Diagnostics (fourth generation) for determination of specific antibodies to HCV in human plasma or serum.

3.3.2 Principle

The kit employs solid phase, indirect ELISA method for detection of antibodies to HCV in two step incubation procedure Polystyrene micro well strips are pre coated with recombinant, highly immune reactive antigens corresponding to the core and the non structural regions of HCV (Fourth generation HCV ELISA).

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

The wells are washed to remove unbound serum proteins, and rabbit antihuman 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 conjugated is then removed by washing.

Chromogen solutions contained Tetra methylbenzidine (TMB) and urea peroxide are added to the wells and in presence of the antigen antibody anti IgG (HRP) immunocomplex; the colorless Chromogens are hydrolyzed by the bound HRP conjugate to a blue color product. The blue color 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 wells, and to the sample respectively. Wells contain samples negative for anti HCV remain colorless.

3.3.3 ELISA content

Micro well Plate 96 Tests. The reagents were used:

1. Negative control
2. Positive control
3. Wash buffer concentration
4. Conjugate(horse radish peroxidase)
5. Chromogen A(Tetra methylbenzidine (TMB)

6. Chromogen B(urea peroxide)
7. Assay diluents
8. Stop solution (sulphuric acid)
9. Sample diluents
10. Micro well plate

3.3.4 Assay Procedure

Step1 Reagents preparation: The reagents and samples were allowed to reach room temperature (18-30°C) for at least 15-30minutes. The Wash buffer concentrated was checked for the presence of salt crystals. The stocks wash Buffer was diluted 1 to 20 with distilled water.

Step 2 Numbering Wells: Set the strips needed in strip holder and number sufficient number of wells included three Negative control (e.g. B1, C1, D1), two Positive control(e.g. E1, F1) and one Blank (A1, neither samples nor HRP-Conjugate should be added into the Blank well). If the results will be determined by used dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test.

Step 3 Adding Diluents': 100µl Specimen diluents were added into each well except the blank.

Step 4 Adding Sample: 10µl of Positive control, Negative control, and Specimen were added into their respective wells.

Step 5 Incubating (1): The plate was covered with the plate cover and incubated for 30 minutes at 37°C. Thermostat controlled water tank was used to assure the temperature stability and humidity during the incubation.

Step 6 washing (1): After the end of the 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 blotting paper or cleaned towel, and were tapped to remove any remainders.

Step 7 Adding HRP-Conjugate: 100µl HRP-Conjugate was added to each well except the Blank.

Step 8 HRP-Conjugate Incubating (2): The plate was Covered with the plate cover and incubated for 30 minutes at 37°C.

Step 9 Washing (2): At the end of the incubation, the plate cover was removed and discarded. Washed each well 5 times with diluted Wash buffer as in Step6.

Step 10 Coloring: 50µl of Chromogen A and 50µl Chromogen B solution was dispensed into each well included the Blank and mix by tapping the plate gently. The plate was incubated at 37°C for 15minutes avoided light. The enzymatic reaction between the Chromogen A/B solutions produced blue color in Positive control and anti HCV positive sample wells.

Step 11 Stopping Reaction

Used a multichannel pipette or manually, 50µl Stop Solution was added into each well and mixed by tapping the plate gently. Intensive yellow color developed in Positive control and anti-HCV positive sample wells. **Measured the absorbance:** The plate reader was calibrated with the Blank well and the absorbance was read at 450nm.

3.3.5 Interpretation of Results

Each micro plate should be considered separately when calculating and interpreting results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each sample's optical density (OD) value to the Cut-off value (C.O.) of the plate. The Cut-off reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well OD value from the print report values of samples and controls. .

Calculation of Cut-off value (C.O.) = *Nc + 0.12*Nc = the mean absorbance value.

Quality control range:

The OD value of the blank less than 0.0800 and the OD value of the positive control greater than 0.800 and the OD value of negative control less than 0.100.

Negative Results

Samples giving absorbance less than the Cut-off value are negative for this assay, which indicates that no antibodies to hepatitis C virus have been detected with this anti-HCV ELISA kit. Therefore, the patient is probably not infected with HCV.

Positive Results

samples giving an absorbance greater than, or equal to the Cut-off value are considered initially reactive, which Indicates that antibodies to hepatitis C virus have probably been detected using this anti-HCV ELISA kit.

3.3.6 Diagnostic specifity

It is defined as the probability of assay to identify correctly those who do not have the disease, which is true negative; the diagnostic specifity was 99.55%.

3.3.7 Diagnostic sensitivity

The ability of test to identify correctly those who have the disease which true positive .the diagnostic sensitivity was 100%.

3.4 Data analysis and interpretation: The data were analyzed using statistical packages of social science (SPSS) soft program.

CHAPTER FOUR

RESULTS

CHAPTER FOUR

RESULTS

During the period from March to October 2017, a total of 90 blood specimens were collected from the person who lived in Elobeid City to detection of HCV infection. According to their sex the majority of participants were males 67 (73.4%) and females 23 (25.6%) (Table 1).The age range from 18-65 the majority with age range from 26-35 years old (Table 9).The history of surgery was found in 14 (15.6 %) of participants (Table 2), hemodialysis 2 (2.2%) (Table 3), jaundice 26 (28.9 %) (Table 4), blood transfusion 12 (13.3%) (Table 5), multiples sex partner 12 (13.3 %) (Table 6), intravenous drug 3 (3.3%) (Table 7) and other risk factor 51 (56.7%) (Table 8). Study on detection of HCV Elobeid City was revealed that all blood samples were negative for IgG antibodies, this mean the sero prevalence of (HCV) in Elobeid City is zero.

Table 1. Frequency and percentage of participants according to their sex

Sex	Frequency	Percent
Males	67	74.4
Females	23	25.6
Total	90	100.0

Table 2. Frequency and percentage of participants according to previous surgery

Surgery	Frequency	Percent
Yes	14	15.6
NO	76	84.4
Total	90	100.0

Table 3. Frequency and percentage of participants according to previous hemodialysis

Hemodialysis	Frequency	Percent
Yes	2	2.2
NO	88	97.8
Total	90	100.0

Table 4. Frequency and percentage of participants according to the history of jaundice

Jaundice	Frequency	Percent
Yes	26	28.9
NO	64	71.1
Total	90	100.0

Table 5. Frequency and percentage of participants according to the history of Blood transfusion

blood transfusion		Frequency	Percent
	Yes	12	13.3
	NO	78	86.7
	Total	90	100.0

Table 6. Frequency and percentage of participants according to the history of multiple sex partners

Multiple sex partner		Frequency	Percent
	Yes	12	13.3
	NO	78	86.7
	Total	90	100.0

Table 7. Frequency and percentage of participants according to the history of intravenous drug

Intravenous drug	Frequency	Percent
Yes	3	3.3
NO	87	96.7
Total	90	100.0

Table 8. Frequency and percentage of participants according to the history of other risk factor

Other risk factor		Frequency	Percent
	Yes	51	56.7
	NO	39	43.3
	Total	90	100.0

Table 9. Frequency and percentage of participants according to the Age group

Age group	Frequency	Percent
18-25	26	28.9
26-35	33	36.7
36-45	14	15.6
46-55	9	10.0
56-65	8	8.9
Total	90	100.0

Table 10 .Frequency and percentage of participants according to the finding of IgG antibody

IgG antibody		Frequency	Percent
	Positive	0	0%
	Negative	90	100%
Total		90	100.0

Table 11 . Frequency and percentage of participants according to the risk factor and result of lab finding

Risk factor	HCV (-ve)			
	Frequency		Percent	
	Yes	NO	YES	NO
Surgery	14	76	15.6%	84.4%
jaundice	26	64	28.9%	71.1%
hemodilaysis	2	88	2.2%	97..8%
Blood transfusion	12	78	13.3%	86.7%
multiple sex partner	12	78	13.3%	86.7%
Intravenous drug	3	87	3.3%	96..3%
Other risk factor	51	39	56.7	43.3%

CHAPTER FIVE

DISCUSSION

CHAPTER FIVE

DISCUSSION

Infections due to Hepatitis C viruses (HCV) are significant health problems around the globe. The prevalence of HCV in the general population in Africa ranges between 0.1% and 17.5%, depending on the country. The countries with the highest prevalence include Egypt (17.5%), Cameroon (13.8%) and Burundi (11.3%). The countries with the lowest prevalence include Zambia, Kenya, Malawi and South Africa (all with a prevalence <1%) (Mercy and Abraham, 2013). In my study, the frequency of Hepatitis C virus infections among general population in Elobeid City is 0% this was approximately similar to the study which was conducted from Bahir Dar health institutions was 0.6% (Yohannes, 2015), In contrast the prevalence of HCV in our finding was less than the studies conducted from Nigeria (3.6%) (Ugbebor *et al*., 2011), Cameron (1.9%) (Njouom *et al*., 2003), Egypt (6.4%) (Kamal *et al*., 2010) and Gondar, Ethiopia (1.3%) (Tiruneh, 2008). These discrepancies might not be disparate with the fact that some of the studies were not from the same risk group and some of the them were done with the detection of both hepatitis C virus RNA and anti HCV antibody, which was Anti HCV antibody detection only in case of our study. This may loss the acute infections before antibody production. None of the expected risk factors (history of blood transfusion, surgery, dental manipulations, tattooing circumcision etc. and other socio-demographic factors) for sero positivity of HCV had been identified in the study. This might be due to the small sample size we used. Moreover in similar studies reported at Nigeria, these expected risk factors were not associated for the positivity of HCV .The explanations for such observations needs to be addressed in the future.

CONCLUSION

The frequency of hepatitis C virus in this study was low. None of the expected risk factors for sero-positivity of HCV had been also identified in the study. There is a need of further study on large sample size and using both antibody and RNA detection of HCV.

RECOMMENDATIONS

1. Due to the great diversity in prevalence and modes of transmission, accurate epidemiological information on HCV infections is urgently needed to guide national and regional plans for prevention, treatment, and reduction of complications of the infection.

2. More specific assay should be made such as QRT-PCR.

3. Further studies with large sample size are needed to determine the rate of spreading of HCV among general population in Elobeid City.

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