بسم الله الرحمن الرحيم

Sudan University of science and Technology
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Sero-prevalence of Hepatitis C Virus among Refugees in Khartoum State

الإنتشار المصلي لفيروس إلتهاب الكبد (ج) لدى اللاجئين في ولاية الخرطوم

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

BY

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الآية

قَالَ تَعَالَىٰ:

﴿ وَيَسۡـَٔلُونَكَ عَنِ ٱلرُّوحِۖ قُلِ ٱلرُّوحُ مِنۡ أَمۡرِ رَبِّي وَمَآ أُوتِيتُم مِّنَ ٱلۡعِلۡمِ إِلَّا قَلِيلٗا ٨٥ ﴾

سورة الاسراء الآية (85)

DEDECATION

To my parents who always support me
To my teachers
To my mentors
To my brothers
To my sisters
To my colleagues
To my friends

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Firstly, thanks to Almighty Allah who gave me the health and power to carry out this research.

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ABSTRACT

Hepatitis C virus (HCV) infection is a serious global health threat with an estimated 150–170 million individuals chronically infected worldwide, resulting in 350,000 deaths each year due to associated cirrhosis and hepatocellular carcinoma (HCC).

The objectives of this study were to determine the prevalence of HCV infection among refugees in Khartoum State as well as to determine the relationship between the presence of HCV and certain factors such as gender, age, career, social status, education and history of blood transfusion. The study was done during period between February and May 2017.

Ninety blood specimens were collected from refugees resident in a refugees camp in the west of Khartoum State. The participants were informed about the purpose of the research before sample collection and verbal or sign agreement was obtained from each of them. The blood specimens were left for 20 minutes then centrifuged at 3000 round per minute to obtain the serum and freezed in -80ºC until the time of the detection of the virus. The sera were analyzed for the presence of Anti-HCV by Enzyme Linked Immunosorbent Assay (ELISA).

Of the total blood specimens investigated 42 (46.7%) specimens from males and 48 (53.3%) blood specimens from females, four (4.4%) were HCV positive. Of them three (3.3%) from females and one (1.1%) from males. Seven (7) participants have a history of blood transfusion. Of them one male and one female has infection with HCV. All participants who

infected with HCV are married. One infected participant has a primary education and it is female. Two infected females have no education. One infected male has a secondary education.

One positive case in each age groups (18-29, 29-39, 51-61 and 62-72) and there is no infection among these age groups (40-50 and 73-83).

It is concluded that the occurrence of HCV was moderate among refugees.

Further studies with large sample size and advanced techniques are needed to validate the results of this study.

المستخلص

تعد عدوى فيروس الالتهاب الكبدي الوبائي (ج) خطرا على الصحة العالمية الخطيرة، حيث يقدر عدد المصابين بالإصابة بأمراض مزمنة في جميع أنحاء العالم بنحو 150 إلى 170 مليون شخص، مما يؤدي إلى وفاة 350.000 شخص سنويا بسبب تشمع الكبد المصاحب وسرطان الكبد.

وكانت أهداف هذه الدراسة هي تحديد مدى انتشار عدوى فيروس التهاب الكبد الوبائي بين اللاجئين في ولاية الخرطوم وكذلك لتحديد العلاقة بين وجود فيروس التهاب الكبد الوبائي وبعض العوامل مثل الجنس والعمر والوظيفي والوضع الاجتماعي والتعليم وتاريخ نقل الدم . وقد أجريت الدراسة خلال الفترة ما بين شباط / فبراير وأيار / مايو 2017.

تم جمع تسعين عينة دم من اللاجئين المقيمين في مخيم للاجئين في غرب ولاية الخرطوم. وأبلغ المشاركون عن الغرض من البحث قبل جمع العينات والشفهية أو توقيع اتفاق تم الحصول عليها من كل واحد منهم. تم طرد عينات الدم في 3000 دورة في الدقيقة للحصول على المصل وتجميد في -80 درجة مئوية حتى وقت الكشف عن الفيروس. تم تحليل المصل لوجود مضاد للفيروس عن طريق الفحص المناعي المرتبط بالانزيم.

من مجموع عينات الدم حققت 42 عينة (46.7٪) من الذكور و 48 (53.3٪) عينات دم من الإناث، وأربعة (4.4٪) كانت إيجابية. منهم 3 (3.3٪) من الإناث و (1.1٪) من الذكور. من بينهم 7 مشاركين لديهم تاريخ من نقل الدم واحد من الذكور وانثى واحدة لديهم عدوى بفيروس التهاب الكبد الوبائي. جميع المشاركين المصابين بمرض الالتهاب الكبدي متزوجون. وهناك مشارك واحد مصاب به و هو متعلم تعليم ابتدائي وهي أنثى. وهناك اثنان من المشاركين المصابين لم يتلقوا تعليما وهم من الإناث. ولدى مشارك واحد مصاب تعليم ثانوي وهو من الذكور.

هناك حالة إيجابية واحدة في كل فئة عمرية (18-29، 29-39، 51-61، 62-72) ولا توجد إصابة بين هذه الفئات العمرية (40-50 و 73-83).

ويستنتج أن انتشار فيروس التهاب الكبد الوبائي كان معتدلا بين اللاجئين.

وهناك حاجة إلى مزيد من الدراسات مع حجم العينة الكبيرة والتقنيات المتقدمة للتحقق من صحة نتائج هذه الدراسة.

TABLE OF CONTENTS

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

CHAPTER TWO

LITERUTURE REVIEW

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER FOUR

RESULTS

CHAPTER FIVE

DISCUSSION

LIST OF TABLES

LIST OF ABBREVIATIONS

HCV	Hepatitis C virus.
HCC	Hepatocellular Carcinoma.
CDC	Centre for Disease Control and Prevention.
USPTF	Preventive Services Task Force.
NANBH	Non-A, non- B hepatitis.
HBV	Hpatitis B Virus.
HAV	Hepatitis A Virus.
HDV	Hepatitis D Virus.
HEV	Hepatitis E Virus.
HIV	Human Immunodeficiency Virus.
HBsAg	Hepatitis B surface Antigen.
WHO	World Health Organization.
PAT	Parenteral Antischistosomal Therapy.
PCR	Polymerase Chain Reaction.
TMA	transcription-mediated amplification.
DNA	Deoxyribonucleic acid.
RNA	Ribonucleic acid.

SVR	Sustained Virological Rate.
ELISA	Enzyme Linked Immunosorbent Assay.
HRP	Horseradish Peroxidase.
TMB	Tetramethyl benzidine.
PBS	Phosphate Buffer Saline.
OD	Optical Density.
C.O	Cut-Of value.
RIBA	Recombinant Immunoblot Assay.
WB	Western Blot.
SPSS	Statistical Package for Social Science.

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1.1. Introduction

Hepatitis C virus (HCV) infection is a serious global health threat with an estimated 150–170 million individuals chronically infected worldwide, resulting in 350,000 deaths each year due to associated cirrhosis and hepatocellular carcinoma (HCC) (Lavanchy., 2011). Mortality due to HCC has increased over the past four decades in many countries and in part is due to chronic HCV (Perz *et al.*, 2006). Chronic HCV has also resulted in an enormous economic burden and lost productivity (El Khoury *et al.*, 2012).

HCV infected individuals often remain asymptomatic for 30 years or more until liver disease is advanced (Hajarizadeh *et al.*, 2013). Early detection therefore is critical as treatment usually leads to viral eradication, prevents progression of liver disease, and decreases all-cause mortality (Lam *et al.*, 2015). The recent development of safer, more tolerable and highly effective direct acting antiviral combinations offers the real possibility of cure for all HCV infected patients (Feeney *et al.*, 2014). This provides a clear and compelling rationale for identifying and screening groups at risk to avert the projected individual and economic burden from HCV (Greenway C *et al*., 2015).

The traditional approach to HCV control in most low prevalence countries is to screen groups with behavioral risk factors for exposure to infected blood, such as through intravenous drug use or receipt of blood

products prior to routine screening. In spite of these programs, the majority of individuals with HCV (45–80%) in these countries remain undiagnosed and unaware of their infection until they develop chronic liver disease (Smith *et al.*, 2012). To address this issue in the US, the Centre for Disease Control and Prevention (CDC) and the U.S. Preventive Services Task Force (USPTF) recently recommended a one-time HCV birth cohort screening program in addition to risk factor based screening programs (Moyer *et al*, 2013).

Immigrants born in intermediate and high HCV prevalence countries who live in low HCV prevalence countries are likely to be at increased risk for HCV due to exposure in their countries of origin (Greenaway *et al.*, 2011). Unlike low HCV prevalence countries where the primary mode of transmission is through intravenous drug use, most infections in intermediate and high HCV endemic countries are acquired iatrogenically through contaminated needles, medical procedures or receipt of unscreened contaminated blood products (Pepin *et al.*, 2014). Most migrants are therefore unlikely to be detected in current HCV screening programs. Furthermore they have not been identified as a group that should be targeted for HCV screening with the exception of recent UK and Canadian guidelines (National Institute for Health and Clinical Excellence, 2012). This is primarily due to the fact that the HCV burden in this population has not been adequately quantified (Greenway C *et al*., 2015).

1.2. Rationale

Infection with HCV pose serious health problem, especially in developing countries. There is no doubt that migration can change the map of infectious disease and there bilateral effect on the host and moved population.

Periodically identifying the prevalence of HCV infection in a large population of refugees is important for assessing the burden of disease and for providing clear plan of prevention and treatment programs. Due to changes in the patterns of refugees admissions closely linked with changing world events, period prevalence estimates from large aggregate populations of refugees reflect a better picture of the burden of disease in this population.

1.3. Objectives

1.3.1. General objective

To investigate sero-frequency of hepatitis C virus among refugees in Khartoum State.

1.3.2. Specific objectives

1.3.2.1. To detect HCV infection among refugees in Khartoum State by using ELISA.

1.3.2.2. To determine the relationship between the presence of HCV and certain factors such as gender, age, career, social status, education and history of blood transfusion.

CHAPTER TWO

LITERATURE REVIEW

2.1. Hepatitis

Hepatitis is a descriptor for specific histopathologic patterns of hepatocyte injury associated with inflammation and, with chronic, with scarring. Caused by viruses hepatitis (A, B, C, D, E), cytomegalovirus, Epstein-Barr virus, yellow fever virus, Herpes simplex virus, autoimmune and drug induced hepatitis (Kumar *et al*., 2013).

2.1.1. Viral hepatitis

Viral hepatitis is a systemic disease primarily involving the liver. At least six viruses, A through E and a newly discovered G, are considered hepatitis viruses. Hepatitis A virus (HAV) and Hepatitis B Virus (HBV) are the best known, but three non-A, non- B hepatitis (NANBH) viruses (C, G, and E) have been described, as has hepatitis D virus (HDV), the delta agent (Kumar., 2016).

2.1.1.1. Hepatitis A Virus (Infectious hepatitis)

The virus causes infectious hepatitis and it is spread by the fecal–oral route. It is a subacute disease of global distribution, affecting mainly children and young adults. Its RNA virus belong to picornavirus (Kumar., 2016).

2.1.1.2. Hepatitis B and D viruses (HBV and HDV)

The virus is a member of the Hepadnaviridae family of viruses, and has a double-stranded circular DNA and a DNA polymerase enzyme. It has two major proteins: hepatitis B surface antigen (HBs Ag), which is an outer protein expressed in excess when the virus replicates in the liver; and

hepatitis B core antigen, an inner protein, which is expressed only within hepatocytes in the liver. A third protein, hepatitis B e antigen (HBe Ag), is also shed in the blood when the virus replicates, and its presence is associated with high infectivity. HDV is a defective RNA virus, which cannot replicate in humans in the absence of HBV. Patients can be co-infected with HBV and HDV, or HBV infected patients can be super-infected with HDV.

Route of spread: The routes of transmission are parenteral (blood exposure), sexual and vertical (from mother to baby) (Kudesia and Wreghitt., 2009).

2.1.1.3. Hepatitis E

Hepatitis E virus (genus *Hepevirus*) was formerly considered a calicivirus based on structure and size (27–34 nm), but now it is placed in its own family, *Hepeviridae*. Hepatitis E virus infects the liver and causes hepatitis E, also known as *enteric hepatitis*. Hepatitis E is fatal to 20% of infected pregnant women. Doctors do not prescribe any specific medicine to treat hapatitis E, usually merely recommending rest, plenty of fluids, and good nutrition. The disease is self-limiting; that is, patients recover on their own. Prevention involves interrupting the fecal-oral route of infection with good personal hygiene, water purification, and sewage treatment.

So far we have considered naked, positive ssRNA viruses in the families *Picornaviridae, Caliciviridae, Astroviridae* and *Hepeviridae.* Now we turn our attention to enveloped, positive ssRNA viruses (Bauman., 2014).

2.1.1.4. Hepatitis C virus (HCV)

Hepatitis C virus is a single-stranded RNA virus belonging to the family Flaviviridae, to which flaviruses such as dengue and yellow fever viruses also belong. There is one serotype but at least 6 different genotypes (1 to 6). Some of the genotypes are further divided into subtypes. For example there are two subtypes to HCV genotypes 1 and 3 (e.g. 1a, 1b and 3a, 3b). The genotypes are important because the treatment response depends upon the infecting genotype. Furthermore, genotypes and subtypes are important epidemiological tools as some are geographically limited in their distribution. In the UK most of the infections are due to genotype 1a, 1b, 2 and 3. In Egypt genotype 4 predominates (Kudesia and Wreghitt., 2009).

Route of spread As for hepatitis B, exposure to infected blood and secretions contaminated with infected blood is the main route of transmission, through the following: Blood and blood product transfusion. A particular tragedy was the transmission of the virus to >90% of haemophiliacs through contaminated factor VIII prior to the introduction of screening of blood for HCV. An outbreak of HCV also occurred in Ireland related to a batch of contaminated immunoglobulin (Kudesia and Wreghitt., 2009). Intravenous drug use In the UK, intravenous substance use (drug use) accounts for most of the infected cases and up to 50 % of all intravenous drug users (IVDUs) have evidence of HCV infection. Sharing of contaminated equipment is the main cause. Iatrogenic (through medical treatment) Reuse of needles, syringes and sharp instruments without proper sterilization for medical treatment in the developing countries has been responsible for the spread of the virus. Egypt has a high rate of HCV infection because of the reported reuse of needles during the national vaccination campaign to eliminate schistosomiasis (bilharzia), a parasitic

infection. There are case reports of infection being passed on to patients from infected healthcare workers (HCWs) during surgical treatment (performance of exposure prone procedures) (Kudesia and Wreghitt., 2009).

2.2. The liver

The liver is the largest gland in the body, weighing between 1 and 2.3 kg. It is situated in the upper part of the abdominal cavity occupying the greater part of the right hypochondriac region, part of the epigastric region and extending into the left hypochondriac region. Its upper and anterior surfaces are smooth and curved to fit the under surface of the diaphragm its posterior surface is irregular in outline (Waugh and Grant., 2014).

2.3. Hepatitis C virus

2.3.1. Background

Hepatitis C virus (HCV) is a RNA virus known to infect humans and chimpanzees, causing similar disease in these 2 species. HCV is most often transmitted parenterally but is also transmitted vertically and sexually (Karoney and Siika., 2013). HCV is up to 4 times more infectious than Human Immunodeficiency Virus (HIV). It also requires less exposure that HIV to cause infection (Te and Jensen., 2010).

HCV is a leading cause of chronic liver disease in the world (Williams, 2006). The World Health Organization (WHO) estimates that 170 million people are infected with HCV globally and 3-4 million new infections occur each year (Madhava *et al.*, 2002), making it one of the leading public health problems in the world. With a prevalence of 5.3% and an

estimated 32 million people infected with HCV, Sub Saharan Africa has the highest burden of the disease in the world (Karoney and Siika., 2013). Other WHO regions with a high prevalence of HCV include Eastern Mediterranean (prevalence 4.6%) and Western Pacific (prevalence 3.9%) (Karoney and Siika., 2013).

Despite its high prevalence and highly infectious nature, HCV remains under-diagnosed and underreported in Africa (with the exception of Egypt). Most of the available data on HCV in Africa are old and outdated. Because of such paucity in available data, little attention has been given to HCV in Africa. We therefore set out to review available medical literature on HCV in Africa with a view to determining the prevalence, disease burden and common transmission modes. In addition we draw attention to diagnosis, treatment and prevention of HCV (Karoney and Siika., 2013).

2.3.2. Disease burden and distribution

The estimated prevalence of HCV in Africa is 5.3% (Pybus *et al.*, 2003). Egypt has the highest worldwide prevalence (17.5%). Egypt unusually high prevalence is attributable to the history of unsterile injection equipment use for mass treatment of the general population with parenteral antischistosomal therapy (PAT) from the 1920s to the 1980s (Global Burden Of Hepatitis C Working Group, 2004). The prevalence of HCV increases with age, with the highest rate being reported in the age group older than 40 years. No data were available on HCV morbidity and mortality in Africa. However, based on the general trends for most other diseases, it is possible that these indicators may be worse than the WHO reports of 75% of HCV-infected individuals developing chronic liver disease. Of those HCV-infected patients who develop chronic liver disease

1.6% progress to Hepatocellular carcinoma (HCC), a condition with a mortality rate >80% (Maheshwari and Thuluvath., 2010).

2.3.3. Transmission

The routes of transmission of HCV are:

A. Blood, blood products.

B. Tissue and organs

C. Unsafe medical procedure

D. Healthcare exposure e.g. needle stick injury (Xia *et al.*, 2008);

E. Intravenous drug use (Tohme and Holmberg., 2010)

F. Sexual transmission (Jafari *et al.*, 2010)

G. Body piercings (Lam *et al.*, 2010)

H. Vertical transmission (Owusu-Ofori *et al.*, 2005).

In Africa, only 19% of blood is screened for HCV (anti HCV antibodies). The main reason for this low screen rate is the prohibitive cost of the laboratory tests (Karoney and Siika., 2013). Also, inconsistent screening procedures for blood donors make blood transfusion a major means of acquisition of HCV infection. This is evidenced by a high HCV prevalence in sickle cell patients (17%) who have received multiple blood transfusions (Touzet *et al.*, 2000). While reported prevalence of HCV in intravenous drug users in the developed world is as high as 80%, little is known about the prevalence of similar risk groups in Africa (Karoney and Siika., 2013). However, Madhava et al found drug use to be an uncommon means of HCV transmission in Africa (Karoney and Siika., 2013). While there is significant variation between countries, WHO estimates that in sub Saharan Africa, approximately 18% of injections are given with reused syringes or unsterilized needles thus increasing risk of transmission through unsafe injection practices (Gibb *et al.*, 2000). Vertical

transmission is low but significant in the setting of co-infection with HIV, a condition that is of pandemic proportions in Africa (Alter, 2007).

2.3.4. Prevalence

Hepatitis C virus is prevalent worldwide; it is estimated that there are about 200 million carriers in the world. The prevalence in the general population varies from about 20% in Egypt to 1–5% in Mediterranean countries, Africa, South East Asia and the USA. In the UK, the prevalence is low at 0.5–1%. The prevalence of HCV is not uniform throughout the population and varies according to the risk and lifestyle of the population (Kudesia and Wreghitt., 2009).

2.3.5. Risk groups

These include intravenous drug users, healthcare workers, people who have received blood and blood products (although in the developed countries where blood is now routinely screened for HCV this is no longer a risk), hospitalized patients (because of iatrogenic spread), patients at risk due to their lifestyle (body piercing, tattooing, multiple sexual partners), sexual contacts and babies born to HCV infected mothers (Kudesia and Wreghitt., 2009).

2.3.6. Genotypes

There are 11 HCV genotypes: 1-11, with many subtypes: a, b, c, and about 100 different strains: 1, 2, 3 based on the sequence of the HCV genome (Williams., 2006). Genotypes 1-3 are widely distributed globally, with genotypes 1a and 1 b accounting for 60% of infections worldwide. Genotype 4 is characteristic for the Middle East, Egypt and Central Africa (Pearlman., 2006).

2.3.7. Disease progression

Few data are available on natural history and progression of HCV infection in Africa. However studies done on African Americans show higher rates of chronic HCV infection compared to whites (Lauer and Walker., 2001). Acute infections and less advanced stages of chronic disease are clinically silent (Alberti *et al*., 2002) and only about half of the viremic patients exhibit elevated Alanine Aminotransferase (ALT) activity (Fried *et al.*, 2002). HCV is often first diagnosed in late stage when the therapeutic options are already limited. Due to slow and silent onset, many patients are unaware of their infection and at least 40% cases remain undetected (Te and Jensen., 2010).

Chronic hepatitis C is difficult to assess, because it is frequently subclinical. Patients with chronic hepatitis C are at risk of cirrhosis and hepatocellular carcinoma and their contacts at risk of acquiring the infection through exposure to the virus (Friedrich-Rust *et al.*, 2007). The risk of developing cirrhosis ranges from 5% to 25% over periods of 25 to 30 years (Seeff., 2002).

2.3.8. Diagnosis

HCV testing is recommended among persons with high risk of getting infected and patients with unexplained high ALT levels (Karoney and Siika., 2013). Highly sensitive and specific rapid tests for diagnosis of HCV are available. HCV RNA can be detected in the blood using amplification techniques such as polymerase chain reaction (PCR) or transcription-mediated amplification (TMA) (Karoney and Siika., 2013). Quantitative HCV RNA should be determined before initiating treatment. Follow-up HCV RNA is useful in monitoring success of HCV treatment (Karoney and Siika., 2013).

Although genotyping does not predict the outcome of infection (Friedrich-Rust *et al.*, 2007), it is useful in predicting the likelihood of treatment response and determines the duration of treatment in many cases as discussed in the Treatment section below(Karoney and Siika, 2013).

2.3.9. Treatment indications

All patients with chronic hepatitis C infection should be considered potential candidates for drug therapy (Kim and Saab., 2005). Treatment is recommended for patients who are at risk of developing cirrhosis, generally defined by a measurable hepatitis C RNA level and liver biopsy showing portal or ridging fibrosis along with moderate inflammation and necrosis (Ghany *et al.*, 2009). Treatment is also recommended for patients with elevated serum ALT levels who meet the following criteria (Sandeep and Dhawan., 2012):

A. Age >18 years.

B. Positive HCV antibody and serum HCV RNA test results.

C. Compensated liver disease (e.g., no hepatic encephalopathy or ascites).

D. Acceptable hematologic and biochemical indices (hemoglobin at least 13 g/dL for men and 12 g/dL for women; neutrophil count >1500/mm3, serum creatinine < 1.5 mg/dL).

E. Willingness to be treated and to adhere to treatment requirements.

F. No contraindications for treatment.

A pretreatment liver biopsy is not mandatory but may be helpful in patients with normal transaminase levels, particularly those with a history of alcohol dependence, in whom little correlation may exist between liver enzyme levels and histologic findings (Karoney and Siika., 2013).

2.3.9.1. Recommended treatment regimens

Spontaneous resolution of hepatitis C virus is common and waiting 2-4 months before initiation of therapy is recommended (Karoney and Siika., 2013). The objective of therapy is to eradicate the virus and prevent potential complications from chronic HCV infection. If detected early, progression of chronic hepatitis to severe liver disease can be prevented in 54-63% of patients through antiviral treatment (Friedrich-Rust *et al.*, 2007). Efficacy of treatment is assessed by measuring Hepatitis C RNA viral load. The goal is to achieve a Sustained Virological Rate (SVR), defined by the continued absence of hepatitis C RNA 6 months after the completion of treatment (Ghany *et al.*, 2009). Treatment for chronic HCV infection has evolved from interferon monotherapy, which results in an SVR of 10 to 20% (McHutchison *et al.*, 2001) to combination therapy with interferon plus ribavirin, which is associated with a higher SVR rate of nearly 40% (Richter., 2002).

The duration of standard interferon plus ribavirin therapy has been based on the viral genotype and the pre-treatment viral load (Karoney and Siika., 2013). The SVR rates for patients infected with genotype 2 or 3 are essentially the same for 24 and 48 weeks of therapy, showing no benefit for the longer course of therapy (Lee *et al.*, 2002). For patients infected with genotype 1 isolates, 48 weeks of interferon plus ribavirin therapy is recommended for those with a high viral load (>800,000 IU/ml) and only 24 weeks of therapy for patients with those with a low pre-treatment viral load (Poordad *et al.*, 2011).

Liver transplant is the only therapeutic option for patients with end stage liver disease (Linney., 2011). The drugs used to treat Hepatitis C cost approximately $30,000 for 48 weeks. The cost of treating side effects of

these drugs further increase the cost of treating hepatitis C. Future hepatitis C drugs are expected to be more expensive (Daw and Dau., 2012).

2.3.10. Prevention strategies

Primary prevention activities include: screening and testing of blood, plasma, tissue, organ and semen donors; virus inactivation of plasma derived products; risk reduction counseling services and implementation of infection-control practices. Secondary prevention activities includes identification and testing of persons at risk and management of infected persons (Karoney and Siika., 2013).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

3.1.1. Type of study

This is a descriptive cross sectional study.

3.1.2. Study area

This study was done at waiting point back refugees camp about 2 hours west of Khartoum State.

3.1.3. Study duration

This study was carried out during the period from 13th February to 13th may.

3.1.4. Study population

Refugees came from South Sudan.

3.2. Inclusion criteria

Every person who came from South Sudan and resident in the refugees camp.

3.3. Exclusion criteria

People who are not resident in refugees camp.

3.4. Ethical consideration

The present study was proved by the College Board of, Medical

Laboratory Science, Sudan University of Science and Technology. The participants were informed about the purpose of the research before sample collection and verbal or sign agreement was obtained from each of them.

3.5. Sample size

Blood specimens were collected from ninety (90) refugees.

3.6. Data collection

The information related to the study such as age, gender, marital status, signs and symptoms were collected using structured questionnaire.

3.7. Experimental work

3.7.1. Specimen collection, transport and storage

3.7.1.1. Specimen Collection: Blood was collected by venipuncture, the patient swabbed by alcohol pad then the syringe was injected and withdraw the blood. The blood were allowed to clot naturally and completely. The sera was separated from the clotted blood by centrifugation at 3000 RPM for 10 minutes at room temperature and freezed in -80º C.

3.7.1.2. Transportation and Storage: The sera transported in ice bag from the camp to the hospital and freezed then transported from hospital to the research laboratory within 30 minutes.

3.8. Detection of HCV

3.8.1. Principle

This kit employs solid phase, indirect ELISA method for detection of antibodies to HCV in two-step incubation procedure. Polystyrene microwell strips are pre-coated with recombinant, highly immunoreactive antigens corresponding to the core and the non-structural regions of HCV (Fourth generation HCV ELISA). During the first incubation step, anti-HCV specific antibodies, if present, will be bound to the solid phase pre-coated HCV antigens. The wells are washed to remove unbound serum proteins, and rabbit anti-human IgG antibodies (anti IgG) conjugated to horseradish peroxidase (HRP Conjugate) are added. During the second incubation step, these HRP-conjugated antibodies will be bound to any antigen-antibody (IgG) complexes previously formed and the unbound HRP conjugate is then removed by washing. Chromogen solutions containing Tetramethyl benzidine (TMB) and urea peroxide are added to the wells and in presence of the antigen-antibody-anti-IgG (HRP) immunocomplex, the colourless Chromogens are hydrolyzed by the bound HRP conjugate to a bluecoloured product. The blue colour turns yellow after stopping the reaction with sulfuric acid. The amount of colour intensity can be measured and is proportional to the amount of antibody captured in the wells, and to the sample respectively. Wells containing samples negative for anti-HCV remain colourless.

3.9. Kit content

3.9.1. Microwell plate

The plate is sealed in aluminium pouch with desiccant. 8×12/12×8-well strips per plate. Each well contains recombinant HCV antigens. The microwell strips can be broken to be used separately. Place unused wells or strips in the plastic sealable storage bag together with the desiccant and return to 2~8°C.

3.9.2. Negative control

Blue-coloured liquid filled in a vial with white screw cap. 1ml per vial.

Protein-stabilized buffer tested non-reactive for HCV antibodies. Preservatives: 0.1% ProClin 300. Ready to use as supplied. Once open, stable for one month at 2-8°C.

3.9.3. Positive control

Red-coloured liquid filled in a vial with white screw cap. 1ml per vial. anti-HCV antibodies diluted in protein-stabilized buffer Preservatives: 0.1% ProClin 300. Ready to use as supplied. Once open, stable for one month at 2-8°C.

3.9.4. Specimen diluents

Blue liquid filled in a white bottle with white screw cap. 65ml per bottle. Protein-stabilized buffer, casein, and sucrose solution. Ready to use as supplied. Once open, stable for one month at 2-8°C.

3.9.5. Horseradish peroxidase-conjugate (HRP-CONJUGATE) REAGENT

Red-coloured liquid filled in a white vial with white screw cap. 65ml per bottle. Horseradish peroxidase-conjugated rabbit antihuman IgG antibodies. Ready to use as supplied. Once open, stable for one month at 2-8°C.

3.9.6. Stock wash buffer

Colourless liquid filled in a white bottle with white screw cap. 50 ml per bottle. PH 7.4, 20 × PBS (Contains Tween-20 as a detergent) The concentrate must be diluted 1 to 20 with distilled/deionized water before use. Once diluted, stable for one week at room temperature, or for two weeks at 2-8°C.

3.9.7. Chromogen solution A

Colourless liquid filled in a white vial with green screw cap. 7ml per bottle. Urea peroxide solution. Ready to use as supplied. Once open, stable for one month at 2-8°C.

3.9.8. Chromogen solution B

Colourless liquid filled in a black vial with black screw cap. 7ml per bottle. TMB solution(Tetramethyl benzidine dissolved in citric acid). Ready to use as supplied. Once open, stable for one month at 2-8°C.

3.9.9. Stop solution

Colourless liquid filled in a white vial with yellow screw cap. 7 ml per bottle. Diluted sulfuric acid solution (2.0M H_2SO_4). Ready to use as

supplied.

3.9.9. Cardboard plate cover

The plate was covered during incubation to prevent evaporation or contamination of the wells.

3.9.10. Additional materials and instruments required but not provided

1. Freshly distilled or deionized water.
2. Disposable gloves and timer.
3. Appropriate waste containers for potentially contaminated materials.
4. Disposable V-shaped troughs.
5. Dispensing system and/or pipette (single or multichannel), disposable pipette tips.
6. Absorbent tissue or clean towel.
7. Dry incubator or water bath, 37±0.5°C.
8. Microshaker for dissolving and mixing conjugate with samples.
9. Microwell plate reader, single wavelength 450nm or dual wavelength 450nm and 630nm.
10. Microwell aspiration/wash system.

3.10. Assay procedure

3.10.1. Step 1 reagents preparation

The reagents and samples were allowed to reach room temperature (18-30ºC) for at least 15-30minutes. The wash buffer concentrate was checked for the presence of salt crystals. The the stock wash buffer was diluted 1 to 20 with distilled or deionized water.

3.10.2. Step 2 numbering Wells

Set the strips needed in strip holder and number sufficient number of wells including 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 using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test.

3.10.3. Step 3 Adding Diluent

100µl specimen diluents were added into each well except the blank.

3.10.4. Step 4 Adding Sample

10µl of positive control, Negative control, and Specimen were added into their respective wells.

3.10.5. Step 5 incubating (1)

The plate was covered with the plate cover and incubated for 30minutes at 37ºC. Thermostat controlled water tank was used to assure the temperature stability and humidity during the incubation.

3.10.6. Step 6 washing (1)

After the end of the incubation, the plate cover was removed and discarded. Each well was washed 5times with diluted Wash buffer. Each time, the microwells were allloed to soak for 30-60seconds. After the final washing cycle, the strips plate were turned onto blotting paper or clean towel, and was tapped to remove any remainders.

3.10.7. Step 7 adding HRP-conjugate

100µl of HRP Conjugate was added to each well except the blank.

3.10.8. Step 8 HRP-conjugate incubating (2)

The plate was covered with the plate cover and incubated for 30minutes at 37ºC.

3.10.9. Step 9 washing (2)

At the end of the incubation, the plate cover was removed and discarded. Wash each well 5times with diluted Wash buffer as in Step6.

3.10.10. Step 10 colouring

50µl of Chromogen A and 50µl Chromogen B solution was dispensed into each well including the blank and mixed by tapping the plate gently. The

plate was icubated at 37°C for 15minutes avoiding light. The enzymatic reaction between the Chromogen A/B solutions produces blue colour in positive control and anti-HCV positive sample wells.

3.10.11. Step 11 stopping reaction

Using a multichannel pipette or manually, 50µl of stop solution was added into each well and mixed by tapping the plate gently. Intensive yellow colour develops in positive control and anti-HCV positive sample wells.

3.10.12. Step 12 measuring the absorbance

The plate reader was calibrated with the blank well and the absorbance was read at 450nm. (Note: the absorbance was read within 5 minutes after stopping the reaction).

3.11. Interpretation of results and quality control

Each microplate 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. If 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. In case the reading is based on dual filter plate reader, do not subtract the Blank well OD from the print report values of samples and controls.

Calculation of Cut-off value (C.O.)

Cut-off value (C.O.) = *Nc + 0.12

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

Important

If the mean OD value of the negative control is lower than 0.02, take it as 0.02. If higher than 0.02 see the Quality control range.

If one of the Negative control values does not meet the Quality control range specifications, it should be discarded and the mean value is calculated again using the remaining two values. If more than one negative control OD value does not meet the Quality control range specifications, the test is invalid and must be repeated.

3.12. Quality control range

The test results are valid if the Quality Control criteria are verified. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient sample being analyzed.

1. The OD value of the Blank well, which contains only Chromogens and Stop solution, is less than 0.080 at 450 nm.
2. The OD value of the Positive control must be equal to or greater than 0.800 at 450/630nm or at 450nm after blanking.
3. The OD value of the Negative control must be less than 0.100 at 450/630nm or at 450nm after blanking.
4. Interpretations of the results: (S = the individual absorbance (OD) of each specimen)

3.12.1. 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.

3.12.2. 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. Retesting in duplicates of any initially reactive sample is recommended. Repeatedly reactive samples could be considered positive for antibodies to HCV and therefore the patient is probably infected with hepatitis C virus. Blood unit positive for HCV antibodies should be immediately discarded.

3.12.3. Borderline

Samples with absorbance O.D. $\leq$ Cut-off $\times 2$ are considered borderline and retesting of those samples in duplicates is recommended. Repeatedly positive samples could be considered positive for hepatitis C virus infection. Follow-up and supplementary testing of any anti-HCV positive samples with other analytical system (e.g. RIBA, WB) is required to confirm the diagnosis.

3.13. Data analysis

Data were analyzed by the Statistical Package for Social Science (SPSS) software program and presented in form of tables and graphs.

CHAPTER FOUR

RESULTS

A total of 90 blood specimens were collected from refugees people resident in a refuegees camp.

The specimens obtained from forty two (42) males and forty eight (48) females were analyzed for Hepatitis C Virus (Table 1).

Four (4) were HCV positive, giving percentage of HCV among refugees in Khartoum State to 4.4% (Table 2 and fig 1).

One (1) positive obtained from male giving the percentage of HCV among refugees in Khartoum State of males to be 1.1% (Table 3).

Three (3) were HCV positive, giving percentage of HCV among refugees in Khartoum State of females to be 3.3% (Table 3).

Seven (7) participants have a history of blood transfusion, three (3) of them are males and four (4) are females, one (1) male and one (1) female have infected with HCV (Table 4).

The four (4) positive participants are married and there is no infection among single participants (Table 5).

Nine (9) have a history of hepatitis, three (3) males and six females, one (1) female have a positive HCV result and the rest are negative (Table 6).

Twenty six (26) have a surgery in the past, nine (9) of them are males and seventeen (17) are females, two (2) of them have an infection with HCV and are females (Table 7).

Twenty eight (28) have a history of jaundice, twelve (12) of them were males and sixteen (16) are females, one (1) of them have an infection with HCV and is female (Table 8).

Thirty eight (38) suffering from fatigue, sixteen (16) of them are males and twenty two (22) are females, three (3) persons from them have an infection with HCV, one (1) is male and two (2) are females (Table 9).

No one have a self injection with intravenous (IV) drug.

No one have a renal failure.

Twenty five (25) suffering from fever, five (5) are males and twenty (20) are females, one (1) male and one (1) female are infected (Table 10).

Thirty six (36) have abdominal pain, thirteen (13) are males and twenty three (23) are females, one (1) male and one (1) female are infected (Table 11).

Four (4) have sharing razors and shaving machines, one (1) male and three (3) females, and no one have an infection with HCV.

Table 1. Distribution and frequency of specimens according to the gender.

Sex	Frequency	Percent%
Male	42	46.7
female	48	53.3
Total	90	100

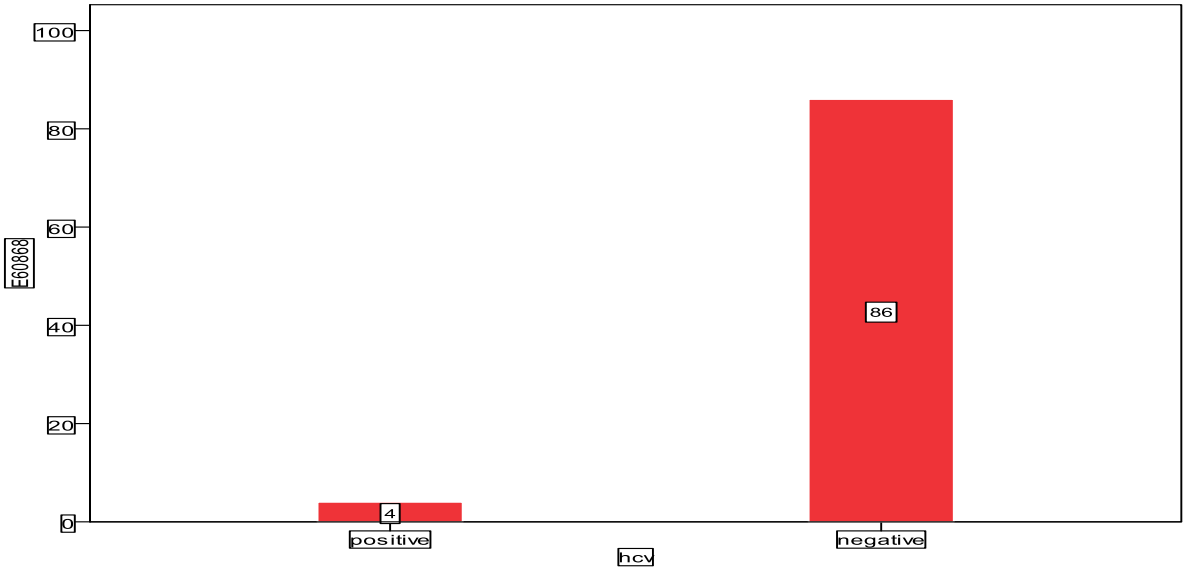

Fig (1): Frequency of the HCV among refugees.

Table (2): Frequency and percentage of HCV among refugees

HCV	Frequency	Percentage%
Positive	4	4.4
Negative	86	95.6
Total	90	100

Table (3): Frequency of HCV according to gender.

Gender	Frequency	Percentage%
Males	1	1.1
Females	3	3.3
Total	4	4.4

Table (4): Frequency of HCV according to history of blood transfusion.

Gender	History of transfusion +ve HCV	History of transfusion -ve HCV	No History of transfusion +ve HCV	No History of transfusion -ve HCV	Total
Male	1	2	0	39	42
Female	1	3	2	42	48
Total	2	5	2	81	90

Table (5): Frequency of HCV according to marital status.

Gender	Married +ve HCV	Married -ve HCV	Single +ve HCV	Single -ve HCV	Total
Male	1	0	0	41	42
Female	3	0	0	45	48
Total	4	0	0	86	90

Table (6): Frequency of HCV according to family history of hepatitis.

Gender	History of hepatitis +ve HCV	History of hepatitis -ve HCV	No History of hepatitis +ve HCV	No History of hepatitis -ve HCV	Total
Male	0	3	1	38	42
Female	1	5	2	40	48
Total	1	8	3	78	90

Table (7): Frequency of HCV according to history of surgery.

Gender	History of surgery +ve HCV	History of surgery -ve HCV	History of surgery +ve HCV	History of surgery -ve HCV	Total
Male	0	9	1	32	42
Female	2	15	1	30	48
Total	2	24	2	62	90

Table (8): Frequency of HCV according to history of jaundice.

Gender	history of jaundice +ve HCV	history of jaundice -ve HCV	history of jaundice +ve HCV	history of jaundice -ve HCV	Total
Male	0	12	1	29	42
Female	1	15	2	30	48
Total	1	27	2	59	90

Table (9): Frequency of HCV among people who have fatigue.

Gender	History of fatigue +ve HCV	History of fatigue -ve HCV	No History of fatigue +ve HCV	No History of fatigue -ve HCV	Total
Male	1	15	0	26	42
Female	2	20	1	25	48
Total	3	35	1	51	90

Table (10): Frequency of HCV among people who have fever.

Gender	Fever +ve HCV	Fever -ve HCV	Fever +ve HCV	Fever -ve HCV	Total
Male	1	4	0	37	42
Female	1	19	2	26	48
Total	2	23	2	63	90

Table (11): Frequency of HCV among people who have abdominal pain.

Gender	Abdominal pain +ve HCV	Abdominal pain -ve HCV	Abdominal pain +ve HCV	Abdominal pain -ve HCV	Total
Male	1	12	0	29	42
Female	1	22	2	23	48
Total	2	34	2	52	90

Table (12): Frequency of HCV according to age group.

Age group	+ve HCV	-ve HCV
18-28	1	7
29-39	1	23
40-50	0	26
51-61	1	20
62-72	1	7
73-83	0	3

CHAPTER FIVE

DISCUSSION

5.1. Discussion

Infections with HBV and HCV pose serious healthcare problem, especially in developing countries. Recently, some of the developing countries started ambitious projects to combat these infections (Hussein., 2015). Also, screening programs have been established for the diagnosis of HBV and HCV. The war in South Sudan obliged population to move to seek safe shelters. More than 10,000 people moved to republic of Sudan Khartoum State. There is no doubt that migration can change the map of infectious diseases and there is a bilateral effects on the host and moved population. Probably, infectious disease is one of the most challenging risks facing both populations (Sharara and Kanj., 2014).

This study aimed to determine the frequency of HCV among refugees in Khartoum state. The results revealed frequency of HCV in refugees was 4.4% (three females and one male).

This may be due to the culture of the human of South Sudan in which the female is work and the male is resting in the home.

All the participants are married and no infection among single people, two have history of blood transfusion which is one of the route of transmission, one have history of hepatitis, two have history of surgery which can transmit virus through contaminated objects on the theater or blood transfusion, one have history of jaundice, three suffer from fatigue which is the one of symptoms, two suffer from fever, two suffer from abdominal

pain, there is no self injection with intravenous drug, renal failure or have needle prick among infected participants.

My study has some limitations. First of all, the sample size was relatively small for such a study. Probably, screening all the refugees was more desirable. However, limited resources were the main obstacles to perform mass screening. Secondly, the risk factor associated with such infections was not studied. It is important to mention that this study should be considered preliminary and more studies are needed to investigate risk factors associated with the infections and other infectious diseases such as tuberculosis and others.

5.2. **Conclusion**

Four participants have infection with HCV, one male and three females. All the participants are married and no infection among single people, most infected participant are females, two have history of blood transfusion, one have history of hepatitis, two have history of surgery, one have history of jaundice, three suffer from fatigue, two suffer from fever, two suffer from abdominal pain, there is no self injection with intravenous drug, renal failure or have needle prick among infected participants.

To conclude, the5 prevalence of HCV was moderate in refugees according to(Hatim M.Y. Mudawi *et al*., 2007) in Sudan.

An immediate action plan is needed to screen all refugees for HCV, to determine HCV infection and treat accordingly and also impose preventive measures to halt the spread of the infection.

5.3. **Recommendations**

This study has limitations such as sample size, duration and doesn’t represent all refugees.

More studies are needed involving all refugees to determine the actual number of HCV infection among refugees.

Further studies with large sample size and advanced techniques are needed to validate the results of this study.

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APPENDICES

Questionnaire

Prevalence of HCV among refugees in Khartoum state

*Age: …….. Sex: ……

*Tribe: ……... Education:…….**.....**

*Occupation: ……..……. ……..

*Head country: …… Date of migration: ……..

*Recent address: ……..... Serial No: …….

*Marital status:

1) Single ( ) 2) Married ( )

*Needle sticks per year:

1) Yes ( ) 2) No ( )

*Sharing Razor and razor blade and shaving machine:

1) Yes ( ) 2) No ( )

*History of blood transfusion:

1) Yes ( ) 2) No ( )

*Family history of hepatitis:

1) Yes ( ) 2) No ( )

*Self injection with I.V drugs:

1) Yes ( ) 2) No ( )

*Haemodialysis:

1) Yes ( ) 2) No ( )

*Surgery:

1) Yes ( ) 2) No ( )

*History of jaundice:

1) Yes ( ) 2) No ( )

*Symptoms:-

*Fatigue:

1) Yes ( ) 2) No ( )

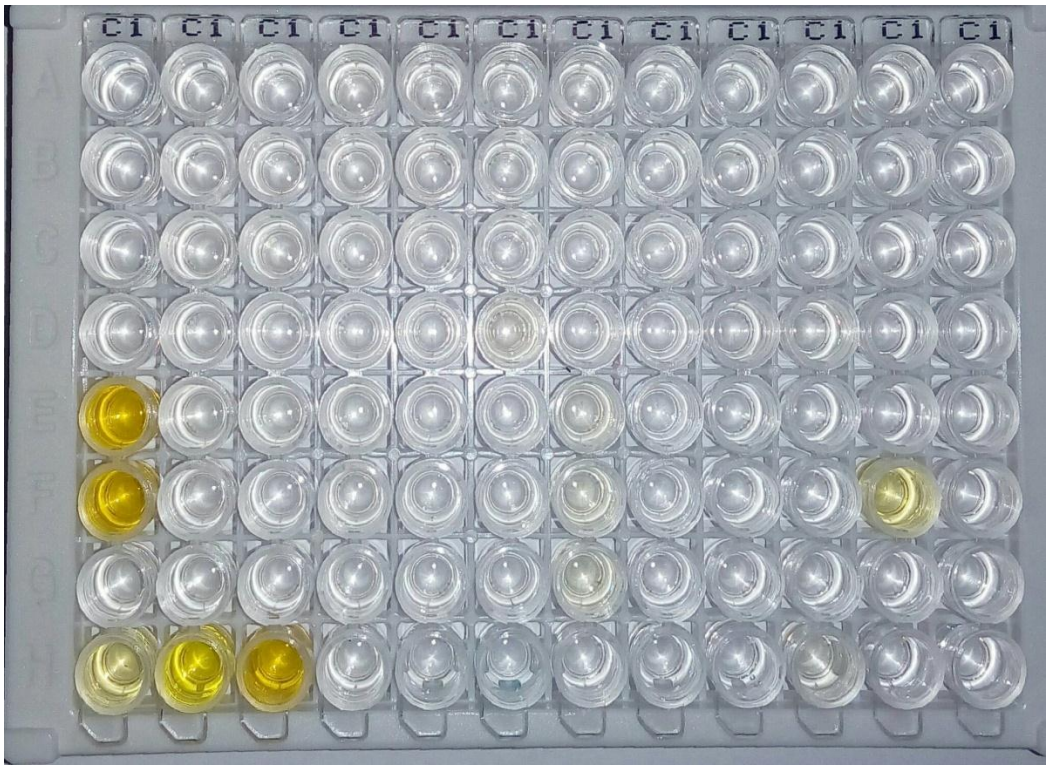
*Fever:

1) Yes ( ) 2) No ( )

*Abdominal pain:

1) Yes ( ) 2) No ( )

*Others: ……



96 Micro titer plate showing the result.