1. INTRODUCTION

1.1 Introduction

Schistosomiasis has been described as re-emerging disease due to increased incidence in previously un-reported regions. Approximately 200 million people are infected, and 652 million people in tropical and sub-tropical regions of the world are at risk. It is estimated that 280,000 annual deaths are attributable to schistosomiasis occur each year in Africa, Middle East, Asia and South America (EL-kholy *et al*., 2013).

In the Sudan it is estimated that, 7-million people are infected as projected by the Director of the National Schistosomiasis Control Program, 2009(personal communication) , it is distributed all over the Sudan including areas like Khartoum State, which was not known previously to be endemic (WHO, 2002).

Schistosomiasis and Hepatitis C Virus HCV co-infection is common in developing countries. Patients coinfected with HCV and schistosomiasis exhibit a unique clinical, virological, and histological pattern (Kama *et al*., 2001).

The two diseases shistosomiasis and viral hepatitis are often associated in developing countries and both lead to chronic liver inflammation (Mudawi, 2008). Schistosomiasis may down regulate the stimulatory effect of hepatitis C virus on Th1 cytokines and this may lead to chronicity of hepatitis C infection in co-infected patients. Dual infection of schitosomiasis and HCV display significant influence on host immune reaction including cytokines shift pattern alteration, cytotoxic T lymphocytes response and other impaired immunologic function with diminished capacity to clear the virus (El Kady *et al*. 2004).

The possible synergistic mechanisms present in the association between schistosomiasis and hepatitis C, and leading to severe hepatic disease remains unclear (Kamal *et al*,. 2004).

Some studies have established a correlation between TNF-α serum levels in hepatitis C patients and the degree of liver inflammation while TGF-β serum levels were correlated with liver fibrosis (Neuman *et al.*, 2002).

Hepatitis C virus (HCV) represents another important public health problem with the highest prevalence in the world, ranging from 6%-28% with average 13.8% in the general population. Co-infection with HCV and *Schistosoma spp* is associated with accelerated deterioration of hepatic function.

Hepatitis C virus (HCV) infection is a major worldwide public health problem. The World Health Organization (WHO) estimates that 3% of the world's populations are chronically infected with HCV, most of these cases occur in Africa, which is reported to have the highest HCV prevalence rate. Although, direct percutaneous inoculation is the most efficient mode of transmission of HCV, several studies have demonstrated that sexual, household, occupational, and vertical transmission of HCV may also be of importance (ElShiekh *et al.*, 2007)

The hepatitis C virus (HCV) is a leading cause of chronic liver disease. It also a major cause of liver fibrosis, which is associated with significant morbidity and mortality. The presence of both HCV and *Schistosoma* spp. is of significant concern as patients with coinfections have been shown to have higher HCV RNA titers, increased histological activity, greater incidence of cirrhosis/hepatocellular carcinoma, and higher mortality rates than patients suffering from single infections. In addition, patients diagnosed with hepatosplenic schistosomiasis have increased opportunities for additional infections and medical abnormalities. These may include up to a 10-fold opportunity for coinfection with hepatitis B virus (HBV) (compared to healthy counterparts), chronic hepatitis on liver biopsy, persistent antigenemia, and increased frequency of liver failure (Abd-Alrahman *et al.*, 2013).

Hepatitis C virus (HCV) infection often is characterized by a protracted clinical course with viral persistence, which leads to liver cirrhosis and hepatocellular carcinoma in

some individuals. Mechanisms underlying viral persistence and liver damage in chronic HCV are not yet clarified. The host immune response probably plays a critical role in the control of both HCV replication and liver injury (Kama *et al.*, 2001).

As hepatotropic viruses, that is, hepatitis B virus (HBV) and hepatitis C virus (HCV), cause liver cirrhosis during chronic infections, the synergistic exacerbation of hepatic pathology is expectable outcome of concurrent infections of HBV/HCV and schistosomes. Because of a lack of suitable animal models for infections of these hepatotropic viruses, major findings in schistosome HBV/HCV coinfections have been obtained from epidemiological studies. Regarding HBV, schistosomiasis (especially the severe hepatosplenic form) was correlated with a higher frequency of HBV infection. In contrast to the controversial effects on HBV infections, detrimental effects of schistosomes on HCV infections have been clearly demonstrated, that is, schistosomes weaken anti- HCV immune responses and worsen liver disease. According to studies in Egypt, patients with coinfections were characterized by a more advanced liver pathology, greater viral burden, higher levels of anti-HCV antibodies, and progression to chronic hepatitis. Moreover, schistosomiasis was shown to be inversely correlated with HCV-specific CD4+ T cells, CD8+ T cells, and/or Th1 cytokine responses. In addition to the modulatory effects of schistosomes on HCV-specific immune responses, SEA of *S. mansoni* and *S. haematobium* were shown to enhance *in vitro* viral replication in a hepatoblastoma cell line (HepG2) and peripheral blood mononuclear cells (PBMCs), respectively (Yoshio and Tamotsu, 2011).

1.2 Rationale:

Hepaitis C Virus causes chronic liver disease. it is the major cause of liver fibrosis and leads to development of hepatocellular carcinoma.

World Health Organization (WHO) estimate that 3% of world population chronically infected with HCV most of these cases occur in Africa.

Schitosome infection is worldwide, WHO estimate that 200 million pepole are infected of whom three-quarter live in Africa.

Schistosomiasis infect the childern under 14 year of age.

Many studies have addressed the issue of co-infection with HCV and *Schistosoma* in children. Due to the above facts the current studty was designed to determine the prevalence of HCV among children.

1.3 Objectives

1.3.1 General objective

To determine the prevalence of HCV and the rate of co-infection with *Schistosoma* spp in Khartoum State.

1.3.2 Specific objectives

1. To determine the presence of *Schistosoma spp* in School childern.
2. To detect the presence of anti-HCV antibodies in patient.
3. To determine the rate of co-infection with both HCV and Schistosomiasis

2. LITERATURE REVIEW

2.1 Hepatitis C Virus

Hepatitis C virus (HCV) is a positive strand RNA virus and the only member of the genus hepacivirus within the flavivirus family (Alter *et al*., 1992).

HCV was first identified in 1989 by researchers at the Centers for Disease Control and Prevention in the United States, when it was determined to be the primary cause of non-A, non-B hepatitis (Ballester *et al*., 2005).

2.1.1. Genotypes

There are 6 genotypes of HCV, 52 subtypes within these genotypes, as well as diverse population of quasispecies within each infected individual. The source of this variation, like that of other RNA viruses, is the high mutation rate of its error prone RNA polymerase (Timm and Roggendorf, 2007).

2.1.2. Virus structure and replication

The genome is approximately 9.6 kb and encods an approximately 3000 amino acid polyprotein. It is flanked by 50 and 30 untranslated regions (UTRs), that are required for replication and the initiation of Translation.

The 50 UTR contains extensive secondary structure such as an internal ribosome entry site (IRES) that directs translation, binds to ribosomal protein (Buratti *et al*., 1998).

In addition, other sequences in the 50 UTR are required for replication of the negative strand. A liver specific microRNA, miRNA-122, with binding sites in the 50 UTR has been shown facilitate HCV replication (Jopling *et al*., 2006). The 30 UTR also contains extensive secondary structure and is required for replication. There is evidence for long-range RNA/RNA interactions between the 50 and 30 UTRs as well as between the 50 UTR and RNA sequences at the C-terminus of NS5B.

These interactions are essential for replication and strongly enhance translation from the HCV IRES(5-7. The 50 and 30 UTR's may also be required for encapsidation as they both interact with the core protein (Yu *et al*., 2009).

After translation on the rough endoplasmic reticulum (ER), the polyprotein precursor is cleaved into 10 proteins by a combination of host and viral proteases. These proteins then associate with the ER and modify cellular membranes producing the membranous web upon which viral replication occurs (Yu *et al*., 2009) and (Lai *et al*., 2008).

Virus maturation and assembly occurs in association with lipid droplets and appears to hijack the VLDL secretion machinery for viral egress from the cell (Gastaminza, *et al*., 2008).

The p7 protein is a viroporin that may perform a function similar to the M2 protein of influenza, it is dispensable for replication but essential for assembly and release of infectious virions (Brohm, *et al*., 2009).

The structural proteins, core, E1, E2, and the viral porin p7 are processed by host signal peptidase (Hijikata *et al*., 1991). The core protein is further processed by host signal peptide peptidase to yield its mature form that can associate with lipid droplets (Yu *et al*., 2009).

The core protein, which forms the viral capsid, has been shown to interact with both the 50 and 30 UTRs(8, as well as with the envelope glycoprotein E1. This interaction is dependent on oligomerization of core (Nakai *et al*., 2006).

In addition, since mutations in core can be rescued by compensatory mutations in NS2 and p7, it has been proposed that they too interact with core (Murray *et al*., 2007).

The two envelope glycoproteins form heterodimers on the ER (Deleersnyder et al., 1997) and are glycosylated there, but not further modified by Golgi enzymes indicating that they are retained in the ER (De Beeck *et al*., 2004).

2.1.3. Stability

HCV is inactivated by exposure to lipid solvents or detergents, heating at 60°C for 10 h or 100°C for 2 min in aqueous solution, formaldehyde (1:2000) at 37°C for 72 h- ß-propriolactone and UV irradiation

HCV is relatively unstable to storage at room temperature and repeated freezing and thawing (WHO, 2013).

2.1.4. Pathogenesis induced by HCV

Systemic "omics" approaches are beginning to unravel the host cell networks that are involved in HCV pathogenesis. It has long been known that HCV infection in patients leads to apoptosis, which stimulates both Kupffer and stellate cells to produce TGF-b leading to activation of stellate cells and the deposition of collagen. These repeated cycles of liver damage (apoptosis) and collagen deposition eventually leads to fibrosis, cirrhosis and liver failure. The apoptosis of hepatocytes was thought to be mediated by the adaptive immune response to HCV infected cells **(**Nelson, 1998).

However, two recent papers point to a greater role for the hepatocyte in both apoptosis and fibrosis. it was shown that apoptosis is induced by HCV infection in mice with chimeric mouse/human livers lacking an adaptive immune system (Joyce, *et al*. 2009).

Moreover, the apoptosis is specific to HCV infected cells. This effect was mediated by a combination of induction of ER and oxidative stress and the down-regulation of anti-apoptotic proteins NF-kB and Bcl-xl in infected hepatocytes.

It was hypothesized that the initial apoptotic signal came from Kupffer or Natural Killer cells, and that this is the initial step in liver damage which precedes the

activation of stellate cells and fibrosis. Using global transcriptional profiling of infected Huh 7.5 cells, it has been shown that in addition to induction of oxidative stress, and apoptosis markers, genes associated with TGF-b signaling were induced, in the absence of other cell types (Walters *et al*., 2009).

2.1.5. Diagnosis

This disease is often a precursor to potentially fatal diseases, such as cirrhosis and hepatocellular carcinoma. Consequently, the United States National Institutes of Health (NIH) recommends testing for hepatitis C in humans with a history of transfusion of blood or blood-products before 1990, on hemodialysis, who have had multiple sexual partners, who are spouses or household members of HCV patients, or who inject drugs or share instruments for intranasal drug administration. Testing is important because humans with hepatitis C are typically asymptomatic or have only minor, non-specific symptoms. The first recommended test is the enzyme immune assay that detects HCV antibodies (anti-HCV). This test is sensitive and specific, is reproducible and inexpensive, and thus is appropriate for screening at-risk populations. HCV RNA assays may be used to confirm the diagnosis. Testing for serum ALT is inexpensive and noninvasive but is less sensitive for determining disease status. A problem is that about 30% of patients with chronic HCV have normal ALT. Repeated ALT testing over time may allow a better assessment of liver injury, but this has not been clearly documented. A liver biopsy cannot serve to diagnose HCV infection, although it can provide useful histological information on liver injury. According to the NIH Consensus Statement

(A Struthers PhD *University of the Sciences in Philadelphia, 2007).*

2.1.6. Treatment

Treatment for chronic hepatitis C should start when patients have 3 indicators of HCV:

- Abnormal ALT for over 6 months
- Positive HCV RNA
- Liver biopsy has shown fibrosis and signs of necrosis and inflammation.

Vaccination against hepatitis A and B is firstly required and then second, pegylated interferon plus the antiviral ribavirin, for 24 to 48 weeks, depending on the genotype of the HCV.

A 48-week treatment regimen including a standard dosage of ribavirin was reported most effective against genotype 1.

Treatment with pegylated interferon and ribavirin can cause significant side effects, including symptoms similar to influenza, abnormalities in the blood, and neuropsychiatric effects. Depression is common among humans with HCV and also a side effect of interferon.

Some of the most common problems are fatigue, headache, myalgia, shaking and fever, and pyrexia (A Struthers PhD *University of the Sciences in Philadelphia, 2007).*

2.2. Schistosoma

Among the world's serious parasitic diseases Schistosomiasis ranks second only to malaria in the number of people infected, and the extent of the areas where the disease is endemic. Currently, some 74 countries are endemic for Shistosomiasis, it is estimated that, out of 779 million people at risk of schistosome infection worldwide, 200 million are infected, of whom three-quarters live in Africa and 20 million suffer from severe sequel (WHO, 2002).

Prevalence is highest (47.4%) among school children. However, the Global Burden of Disease Study, attributes a disability weight of 0·06 and an annual mortality of 14 000 deaths per year to Shistosomiasis (Lopez *et al*, 2006).

The transmission of shistosomiasis takes place only in the place where fresh water snail vector is present and where there is contact between the population and infested water. Children who practice swimming are particularly at high risk, because of their prolonged and complete body exposure. These endemic areas are often characterized by low socioeconomic condition and poor sanitary facilities, erroneous habitats of the people as regards urination and defecation in canal water, and the exposure to this polluted water by bathing, swimming, washing clothes, walking bare_foot during irrigation in agriculture or fishing (Sibomana, 2009).

Diagnosis is by finding the eggs of the parasite in a person's urine or stool. It can also be confirmed by finding antibodies against the disease in the blood (WHO, 2014).

2.2.1. Signs and symptoms

Skin symptoms: At the start of infection, mild itching and a papular dermatitis of the feet and other parts after swimming in polluted streams containing cercariae (James *et al*., 2006).

Clinical manifestations of schistosomiasis are associated with the species causing the problem and the intensity of infection. Urinary schistosomiasis caused by *Schistosoma haematobium* is characterized by haematuria as a classical sign. In chronic infection, the patient may suffer bladder and uretral fibrosis, sandy patches in the bladder mucosa and hydronephrosis, while bladder cancer is reported as a late stage complication. Similarly, intestinal schistosomiasis caused by *Schistosoma mansoni* or *Schistosoma japonicum* is usually associated with abdominal pain and appearance of blood flakes in the stool. In chronic infection, hepatosplenomegaly is commonly reported with ascites and other signs of portal hypertension (.Al-Zabedi *et al*., 2014).

2.2.2. Epidemiology

The disease is found in tropical countries in Africa, the Caribbean, eastern South America, Southeast Asia and in the Middle East. In these areas as of 2010 it affects approximately 238 million people (Vos *et al*., 2012). 85% of them live in Africa. An estimated 600 million people worldwide are at risk. Worldwide it is estimated that 12,000 to 200,000 people die due to schistosomiasis yearly (Thétiot –Laurent *et al*., 2013).

Among human parasitic diseases, schistosomiasis (sometimes called bilharziasis) ranks second behind malaria in terms of socio-economic and public health importance in tropical and subtropical areas. The disease is endemic in 74-76 developing countries. They live in rural agricultural and peri-urban areas (Oliveira *et al*., 2004). 20 million have severe consequences from the disease. In many areas, schistosomiasis infects a large proportion of children under 14 years of age (Kheir *et al*., 1999).

2.2.3. Treatment

Schistosomiasis is readily treated using a single oral dose of the drug praziquantel annually. The World Health Organization has developed guidelines for community treatment of schistosomiasis based on the impact of the disease on children in endemic villages:

- When a village reports more than 50 percent of children have blood in their urine, everyone in the village receives treatment.
- When 20 to 50 percent of children have bloody urine, only school-age children are treated.
- When fewer than 20 percent of children have symptoms, mass treatment is not implemented (The carter center, 2008).

2.2.4. Evidence that parenteral antischistosomiasis transmitted HCV

Several studies have established that the antischistosomal injections, rather than schistosomiasis itself or some other cause, were the probable cause of the hepatitis C epidemic in Egypt. First, there is evidence that schistosomiasis can exist without leading to HCV. A study in central Sudan, where there is a high prevalence of *S mansoni* infection, found no correlation between the presence of HCV antibodies and *S mansoni* infection, suggesting that the parasite is not the cause of hepatitis C (Mudawi *et al.*, 2007b)

A study of 506 residents of an area in Egypt endemic for *S mansoni* also found that there was no association of *S mansoni* infection with the seroprevalence of hepatitis C as indicated by anti-HCV.(El-Sayed *et al*., 1997)

However, there was a significant association of anti- HCV seropositivity with previous parenteral treatment for schistosomiasis (Strickland *et al*., 2002).

Second, there is evidence that parenteral antischistosomal therapy is closely associated with the prevalence of HCV in Egypt. A 2001 community-based study of 801 humans over 10 years who lived in the Nile River delta identified no current practices that would contribute to the spread of HCV(Darwish *et al*., 2001) It found that the seroprevalence of HCV increased with age - from about 19% in the 10 to 19 year old cohort to about 60% among humans over 30. These findings are consistent with the theory that previous parenteral therapy facilitated HCV transmission.

2.3. Previous studies

A study done in Sudan by Mudawi *et al* (2007) found that anti-HCV prevalence was (4.5%) in hepato-splenic patient (Mudawi *et al*., 2007b).

A study done in Gazira state in central of Sudan HCV infection has low prevalence (91%) had *S. mansoni* (11.2%) had anti-HCV (Mudawi *et al*., 2007a).

In Ethiopia Berthe *et al* (2007) reported (65.9%) were positive for SM (1.3%) was positive or HCV the highest prevalence found among children (Berthe *et al*., 2007).

Adam *et al* (2011) in Taiz, Yemen determine the disease prevalence and it is relationship with hepatitis B and C viruses among 1484 school children aged between 5 and 16 years in five areas endemic for *S. mansoni* (20.76%) and(1.1%) was positive for HCV and *S. haematobium* (7.41%) and (2.86%) was positive for HCV. There was correlation between *S. haematobium* and hepatitis B, but no association between *S. mansoni* infection and hepatitis B and C viruses (Adam *et al*., 2011).

Jose *et al* (2005) in Brazil reported that Anti-HCV marker was assayed by ELISA- and RIBA-II in serum sample obtained from 1228 resident (85.8%). The Anti-HCV (ELISA) was positive in 6(0.5%) individual 8(0.6%) cases were inconclusive and 1214 (98.9%) were negative. However, only one ELISA-II positive serum sample (0.08%) were antibodies confirmed by RIBA-II .In conclusion, no association was observed between HCV and SM in the endemic area studied (Jose *et al*., 2005).

Silva *et al* (1999) observed a (1.2%) prevalence of anti-HCV antibodies in individual from the metropolitan region of Salvador and a (0%) prevalence of anti-HCV in a population study carried out in the rural area of the state of Bahia, Northeast of Brazil (Silva *et al*., 1999).

3. Materials And Methods

3.1 Study area

This study was carried out in Khartoum State Locality of Alhalfaya –Alsamrab area.

3.2 Study design

This is an analytical, descriptive study conducted in the period from August 2013 to May 2014. Included 84 schistosomic basic school children within the age of 9 to 17 years old mean age 12 year ±1.7.

3.3 Inclusion criteria

Basic school children in Khartoum State those are infected with *S. heamatobium* and *S. mansoni.*

3.4 Exclusion criteria

Non shistosomic patient

3.5 Sampling technique

Non-probability sampling method was used (only those who volunteered were involved in sample), then urine and stool analysis were done to diagnose *Schistosoma* infection and blood samples were collected to detect antibodies against HCV.

3.6 Tool of data collection

The data was collected by both, a pretested questionnaire used in a similar previous study and laboratory procedures.

3.7 Data analysis

The collected data was analyzed using the computer program SPSS Statistics *version 15.1.1* (originally, Statistical Package for the Social Sciences, later modified to read Statistical Product and Service Solutions).

3.8 Ethical considerations

1. Local authorities were informed with the goal and aim of study to get their approval in order to be conducted.
2. An agreement of the head master of the basic school of interest was obtained.
3. The agreement of study participant's parents was taken to be included in the study after being informed with detailed objectives of study.
4. All participants were provided with the investigation results.
5. Patients detected with *Schistosoma* were treated under the medical supervision.

3.9 Detection of *Schistoma* infection

3.9.1. Formal ether (Formalin-Ethyl Acetate) sedimentation technique

3.9.1.1. Principle

Formalin fixes the eggs, larvae, oocysts, and spores, so that they are no longer infectious, as well as preserves their morphology. Fecal debris is extracted into the ethyl acetate phase of the solution. Parasitic elements are sedimented at the bottom. (Cheesbrough, 1999).

3.9.1.2. Procedure

Ahalf teaspoonful of faeces was transfered in 10 ml of water in a glass container and mix thoroughly.Two layers of gauze was placed in a funnel and strained the contents into a 15 ml centrifuge tube centrifuged for 2 minutes at about 500 g.

The supernatant was discarded and the sediment was resuspended in 10 ml of physiological saline. Centrifuged at 500 g and the supernatant were discarding. The sediment was resuspend in 7 ml of 10% formaldehyde (1 part of 40% formalin in 3 parts of saline). Three ml of ether were added (or ethyl acetate). The tube was closed with a stopper and shaked vigorously to mix. The stopper was removed and centrifuged at 500g for 2 minutes.

The tube was then rested in a stand. Four layers became visible at the top layer consisting of ether, second was a plug of debris, and third were clear layer of formalin and the fourth was the sediment. The plug was removed and the debris poured from the side of the tube with the aid of a glass rod and the liquid was poured off leaving a small amount of formalin for suspension of the sediment.

3.9.2. Urine concentration technique

A 10 ml of fresh *, well mixed urine transferred to conical tube and centrifuged at RCF 500-1000 g to precipate the schistosome egg, the supernatant was removed and the sediment was transferred to a slide, covered with cover glass and the entire sediment is examined microscopically using 10X objective. The egg in sample was counted and reported in number/10ml, if the count of the eggs in specimen is more than 50 eggs/10ml the count is stopped and reported as heavy infection. (Cheesbrough, 1999).

*the urine was examined within 30 minutes, if not it was placed in dark to prevent miracidia hatching.

3.9.3 ELISA for detection of HCV

3.9.3.1 Principle of the assay

This is an ELISA for qualitative detection of antibodies against Hepatitis C virus in human serum or plasma. It is intended for screening blood donors and diagnosing patients infected with hepatitis C virus.

It employs a solid phase, indirect ELISA method for detection of antibodies to HCV in two steps incubation procedure. Polystyrene microwell strips are precoated with recombinant, highly immunoreactive antigens (Ags) corresponding to the core and the non-structural regions of HCV (third generation HCV ELISA). During the first incubation step, anti-HCV specific antibodies, if present, will be bound to the HCV Ags pre-coated solid phase.

The well are washed to remove unbound serum proteins, and rabbit anti-human IgG antibodies (anti-IgG) conjugated to horse reddish peroxidase (HRP-conjugate) are added. During the second incubation step, these HRP-conjugated antibodies will be bound to any antigen-antibodies (IgG) complexs previously formed and the unbound HRP-conjugate is then removed by washing. Chromogen solutions containing Tetramethylbezidine (TMB) and urea peroxide are added to the wells and in presence of 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 antibody captured in the wells, and to the sample respectively. Wells containing samples negative for anti-HCV remain colorless (Appendix 2).

3.9.3.2 Assay procedure

Step 1 Reagent preparation

The reagents and samples were allowed to reach room temperature (18-30 ºC) for 15 minutes. The stock wash buffer had been diluted 1 to 20 with distilled water.

Step 2 Numbering the wells

Three wells were marked as Negative control (B1, C1, and D1), two wells as Positive control (E1, F1) and one blank (A1).

Step 3 Adding diluent

100 µl of diluent were added into each well except the blank.

Step 4 Adding samples

10 µl of negative controls, positive controls and samples were added into their respective wells.

Step 5 Incubation (1)

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

Step 6 Washing (1)

The plate cover was removed and discarded. The wells were washed 5 times using ELISA washer (TECAN washer, Germany).

Step 7 Adding HRP-conjugate

100 µl of HRP-conjugate were added in to each well except the blank.

Step 8 Incubation (2)

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

Step 9 Washing (2)

The plate cover was removed and discarded. The wells were washed 5 times using ELISA washer (TECAN washer, Germany).

Step 10 Coloring

50 µl of chromogen A and 50 µl of chromogen B solution were added into each well including the blank. The plate was incubated at 37 ºC for 15 minutes. Blue color was produce in the positive control.

Step 11 Stopping the reaction

Using a multichannel pipette 50 µl of stop solution was added into each well.

The blue color of positive controls turned yellow.

Step 12 Measuring the absorbance

The absorbance was read at 450 nm using TECAN reader.

Cut of result and quality control validation were also calculate using TECAN reader (Appendix 4).

3.9.3.3 Calculation, QC ranges and Interpretation

Calculation of cut off:

Cut off =NC+0.12

Quality control ranges

The value of the blank well should be less than 0.08 at 450 nm.

The value of positive control must be equal to or greater than 0.08 at 450 nm.

The value of negative controls must be less than 0.10 at 450 nm.

Interpretation

Negative results

Samples giving an absorbance less than cut off value were considered negative which indicated that no antibodies to hepatitis C virus had been detected.

Therefore the patient was probably not infected.

Positive results

Samples giving an absorbance equal to or greater than cut off value were considered initially reactive, which indicates that antibodies to hepatitis C virus had been detected.

Borderline

Samples with absorbance to cut off ratio between 0.9 and 1.1 were considered borderline and retesting was recommended.

4. Results

4. Results

This study was carried out during the period from August 2013 to May 2014. Included 84 basic school children infected with *S. heamatobium* and S. *mansoni* within the age of 9 to 17 years old mean age 12 year ±1.7.

The main exclusion criteria adopted for the selected study was if the person did not live at the study site. People with no history of contaminated water contact and/or with liver disease caused disorders or by Hepatitis viruses A, B, D or with others etiologies were also excluded from this study.

From 84 schistosomic infected children, 80(95.2%) of them were infected with *S. haematobium* while 4(4.8%) were *S. mansoni* infected (Table 1).

Table 2 shows the frequency of HCV among schistosomic children, 5% of children infected with *S. haematobium* have anti-HCV antibodies where was no anti-HCV antibodies detected in children infected with *S. mansoni*.

Table (1) Frequency of *Schistosoma* among school children

Type	Frequency	Percent
S. haematobium	4	4.8
S. mansoni	80	95.2
Total	84	100

Table (2) frequency of HCV among Schistosomic children

	HCV				Total
	positive		negative		
	No.	Percent	No.	Percent	
S.haematobium	4	5%	76	95%	80
S.mansoni	0	0%	4	100%	4
Total	4	4.8	80	95.2%	84

P= 0.647

5. Discussion

5.1 Discussion

HCV infection is a major worldwide public health problem. In this current study, the overall prevalence rate of schistosomiasis infection among school children aged 9 to 17 years in Khartoum State, Al-Halfaya –Al-smrab area was calculated.

A total of 84 *Schistosoma* infected boys were included in this study 80(95.2%) out of them infected with *S. haematobium* and 4(4.8%) with *S. mansoni*.

Adam *et al* (2011) in Taiz Yemen reported that the prevalence of schistosomiaisis infection among children aged from 5 to 16 years was (20.76%) and (7.4%) for *S. mansoni* and *S. haematobium* respectively. Therefore, *S. mansoni* was more prevalent. Furthermore, the prevalence of HCV (4.8%) was higher than the result reported by Adam *et al* (2011) in Taiz, Yemen (2.86%). While, there were no Anti HCV in *S. mansoni* patients comparing with (1.1%) positive reaction in Adam's study.

The study of Mudawi *et al* (2007b) in Sudan shows that prevalence of HCV in *S.mansoni* patients was (4.5%), while in this study the prevalence of *S. mansoni* was (0%). It was also lower than that of Berthe *et al* (2007) in Ethiopia where 65.9% was positive for *S. mansoni* (1.3%) had anti HCV.

Regarding the result reported by Mudawi *et al* (2007a) in Gezira State of Central Sudan shows 91% with *S. mansoni* and (11.9%) had anti HCV, there was a disagreement with the current study.

Statistically, there was no significant different between the *Schistosoma* infection and HCV ($P > 0.05$).

5.2 Conclusion

Shistosomiasis infection is an important public health problem in the world.

1- Both *S. mansoni* and *S. haematobium* were detected, but *S. haematobium* was more prevalent.

2- 4.8% HCV seroprevalence rate among shistosomic school boy infected with *S. haematobium* and no HCV were detected with *S. mansoni* in Khartoum State.

3-There was no association between HCV and *Schistosoma* infection.

5.3 Recommendations

1. More work is needed to find out the relation between shistosomiasis and hepatitis viral infection.

2. Snail control by using suitable methods and the water sources should be treated.

3. I recommend making a regular screening for detection of Anti-HCV in any schistosomal patient and vising versa.

4. Increase sample size in studies especially, patients infected with *S. mansoni*, because it enhances the HCV infection.

5. Further studies should be done to cover the whole country to elucidate the full dimension of the problem to health policy makers to plan and decide for the problem control and refute my results.

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Appendix (1): Questionnaire:

Name:…….……I.D……..…….

Age:…..……..year

Resident:……..…….……..……

Stool analysis :

……

Density of infection

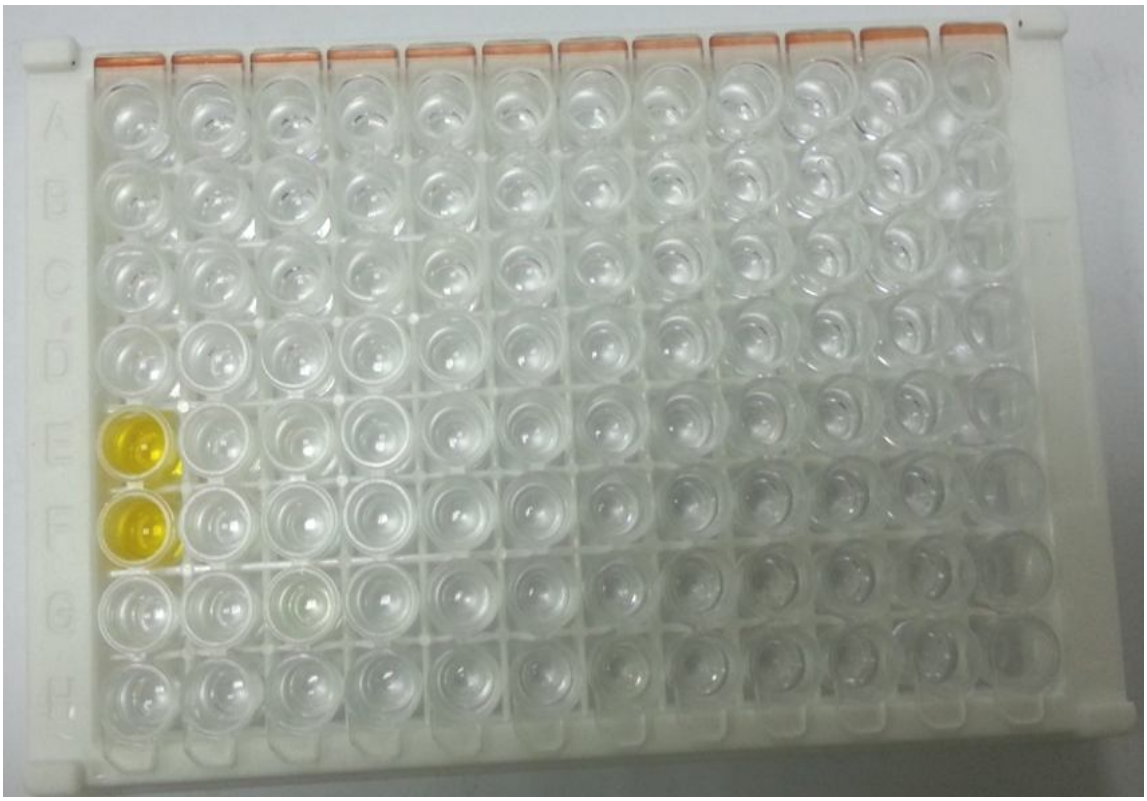
Mild: ……..…... ……..…… sever…….……..…..

Urine analysis:

……..……

Density of infection

Mild: ……..…... ……..…… sever……...……..…..



HCV ELISA Microplate

ELISA KIT

ELISA Washer