



SEROPREVALENCE OF HEPATITIS C VIRUS AMONG HEPATITIS B VIRUS POSITIVE PATIENTS ATTENDING TO TALODI HOSPITAL, SOUTH KORDOFAN, STATE, SUDAN.

Ikram Mustafa Altyeib Fadul^{*1}, Wafa Ibrahim Elhag^{**2}, Mokhtar Zakaria Adam Salih^{***3}.

^{*1}-MSc.student – Al Neelain University, Faculty of Medical Laboratory Science, Sudan.

^{**2}-Associate professor, Faculty of Medical Laboratory Sciences, Al Neelain University, Sudan.

^{***3}- Talodi hospital, Laboratory department, South Kordofan, State, Sudan.

Received 05 February 2015; Accepted 23 February 2015

ABSTRACT

Background: Hepatitis B virus (HBV) and hepatitis C virus (HCV) share common mode of transmission and both are able to induce a chronic infection. Dual HBV/HCV chronic infection is a fairly frequent occurrence, especially in high endemic areas and among individuals at high risk of parenterally transmitted infections. The intracellular interplay between HBV and HCV has not yet been sufficiently clarified, also due to the lack of a proper in vitro cellular model. Dual HBV/HCV infection has been associated to a severe course of the liver disease and to a high risk of developing hepatocellular carcinoma. This study aimed to determine seroprevalence of hepatitis C IgG antibodies among known hepatitis B infected patients.

Methods: This is a cross sectional study included HBV infected patients aged between (11-54) years old with mean 30.4 years old conducted in Talodi Hospital, South Kordofan, State, Sudan, during February to May 2014.

We performed HCV serum marker, was detected by using commercially available enzyme-linked immunosorbent assay (ELISA) kit. Generated data were analyzed by using SPSS program (version11)

Results: HCV IgG Ab was detected only in 1 (1.1%) from patient, and he was male. The mean age of the studied group was (30.4) their age range (11-54) years. There were 63 men (70%) and 27 women (30%), (n = 90).

Conclusion: The seropositivity of HCV was low, similar to study done in Umdurman Maternity Hospital.

Key words: Seroprevalence, Anti HCV, ELISA, Talodi hospital

INTRODUCTION:

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are the two major causes of chronic liver disease worldwide. Both viruses are hepatotropic, but not directly cytopathic and elicit progressive liver injuries resulting in the end-stage liver disease unless effectively eradicated⁽¹⁾⁽²⁾. In Sudan, the incidence of hepatocellular carcinoma (HCC) is high and increasing, in one study conducted in 1996-1998 among 150 HCC patients, indicated that HBV and HCV are important risk factors of HCC in Sudan⁽³⁾.

Both human viral infections can be transmitted by various routes, i.e. blood and blood products (e.g. blood transfusion), sexual, oral, vertical and horizontal transmission⁽⁴⁾⁽⁵⁾⁽⁶⁾.

The World Health Organization (WHO) estimated that 3% of the world's population are infected with HCV, resulting in a total of 120 to 170 million people⁽⁷⁾⁽⁸⁾. There is a distinct geographical variation in both HBV and HCV prevalence and incidence due to lack of proper health

facilities, poor economical status and less public awareness about the transmission of major communicable diseases⁽⁹⁾.

Suppression of HBV replication by HCV in acutely or chronically infected patients is well-described phenomenon. In vivo study, in chimpanzees, showed that acute HCV super infection in chronic HBV infection resulted in marked reduction in the titer of serum HBsAg⁽¹⁰⁾⁽¹¹⁾.

Other study to assess the contribution of hepatitis C virus (HCV) in liver disease In Taiwan, Antibody to HCV (anti-HCV) was studied by radioimmunoassay in 392 patients with chronic liver disease and in 440 healthy adults and 444 subjects at risk. The anti-HCV prevalence was 0.95% in 420 volunteer blood donors, 90% in 100 hemophiliacs, and 81% in 58 parenteral drug abusers. Anti- HCV was Present in 6 (7.7%) of 78 hepatitis B Surface antigen (HBsAg)-positive And 28 (65%) of 43 HBsAg-negative patients with chronic hepatitis, 3 (10%) of 31 HBsAg-

positive And 13 (43%) of 30 HBsAg-negative cirrhotics and 7 (17%) of 42 HBsAg-positive And 15 (63%) of 24 HBsAg negative patients with hepatocellular carcinoma (HCC).

An Outbreak of non-A, non-B hepatitis revealed 18% of 57 patients to be positive for anti-HCV, and in 29 Patients with post transfusion hepatitis prospectively followed, 7 (24%) developed anti-HCV. Thus, HCV Infection appears to play a relatively minor role in HBsAg-positive Liver disease in Taiwan but is strongly associated with HBsAg-negative Chronic liver disease and HCC. The Infection is extremely common in hemophiliacs and parenteral drug abusers.⁽¹²⁾

MATERIALS AND METHODS:

Design:

This is across sectional study included HBV infected patients aged between (11-54) years old with mean 30.4 years old conducted Talodi hospital, South Kordofan, State, Sudan, during February to May 2014. The data was collected by structured questionnaire. Ethical approval was taken from Al Neelain University research ethical board and from patients verbally.

Experimental work:

Serum specimens were collected from known HBsAg positive patients, and screened for hepatitis C IgG antibodies using enzyme linked immune sorbent assay (ELISA) technique at research laboratory \AL Neelain University.

Collection of specimens and processing:

Three millilitres of blood were collected under aseptic technique into plain container, the sera obtained after centrifugation was kept at -20°C. The serum samples were tested for the presence of HCV IgG antibodies, using ELISA kit (Biorex Diagnostics, UK), (4th generations), All reagents and Samples were allowed to reach the room temperature for at least 15-30 minutes. The washer buffer concentrate was checked for the presence of salt crystals. The washer buffer diluted 1 to 20 with distilled water.

The strips were set in strip-holder and the wells were labelled including 3 for negative controls, 1 for positive control and 1 for blank.

100ul of diluents was added to each well except the blank. Then 10µl of negative controls and positive controls and specimens were added into their respective wells, plate was covered with the plate cover and incubated for 30 minutes at 37°C.

At the end of incubation the plate cover was removed and each well was washed 5 times with diluted wash buffer. After final washing cycle the plate was blotted onto clean towel to remove any remaining liquid.

100ul of HRP-conjugate was added to each well except blank then covered with plate covered and incubated for

30 minutes at 37°C. Plate covered was removed and each well washed 5 times with diluted wash buffer, then blotted. 50µl of chromogen A and 50µl of chromogen B were added into each well including blank, plate was covered with plate cover and incubated for 30 minutes at 37°C. The enzymatic reaction between the chromogen solution and HRP-conjugate produced blue colour in positive control and positive sample wells.

The plate cover removed and 50µl of stop solution were added into each well, intensive yellow colour was developed in positive control and positive sample wells.

Measuring the Absorbance

The plate reader was calibrated with blank well and the absorbance was read at 450nm.

Results were calculated by relating each sample optical density (OD) value to the Cut off value of plate.

Calculation of Cut off (C.O) value.

C.O=*Nc+0.12

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

Interpretation of the Results

Negative results:

Samples giving absorbance less than Cut-off value are negative for this assay.

Positive result: sample giving absorbance equal to or greater than Cut-off considered initially reactive.

Borderline:

Sample with absorbance to Cut-off value are considered borderline and retesting of these samples in duplicate is recommended to confirm the results.

Data Analysis

Data was analyzed by SPSS (Statistical Package of Social Science) software version 16.

RESULTS:

A total of 90 HBsAg positive patients, attending Talodi hospital, South Kordofan State, Sudan, during February to May 2014, were enrolled in this study, their mean age range 30.4 years old, of them 63 (70%) were males and 27 (30%) were females .The positive of HCV IgG Abs detected only in 1 (1.1%) and he was male and had hepatitis B for one month and less than 1 year duration .regarding risk factors , statistical analysis showed significant relation between HCV seropositivity and duration of hepatitis B ,while it was in significant with other factors including positive family history, cupping, surgery, and dental surgery.

Discussion:

Hepatitis B virus (HBV) and Hepatitis C virus (HCV) are common causes of liver disease globally⁽¹³⁾.

This study aimed to estimate seroprevalence of HCV among HBsAg patients, attending to Talodi Hospital, South Kordofan, State, Sudan it included 90 HBsAg

positive patients were investigated for HCV IgG. The overall, seropositive of HCV IgG among HBsAg patients was only 1(1.1%), similar to the study conducted by Elshekh *et al* (2007), in Sudan who found anti-HCV 3(0.6%) out of 423 pregnant by using ELISA⁽¹⁴⁾. Other studies Emad Aldin, *et, al* (2012), on prevalence of HCV infection was lower in various regions of Sudan than that of HBV infection. It was reported 1.82% anti-HCV seropositivity which is closer to other previous studies in Sudan⁽¹³⁾ A study by Mudawi *et al.* (2007), who detected 2.2% prevalence of anti-HCV antibodies from total of 410 in Gezira State⁽¹⁵⁾. Also, in a southern Sudanese population McCarthy *et al* (1994), 666 out-patients attending 6 public clinics in the city of Juba were enrolled in a serosurvey, was found only 21 (3%) of the 666 samples were positive for anti-HCV using a second generation immunoblot assay (RIBA-2). None of the anti-HCV-positive subjects reported receiving a prior blood transfusion and only 5 subjects reported a history of jaundice.⁽¹⁶⁾ The variation may be due to sample size technique use for analysis. Compared to study conducted in Egypt by El-Sayed *et al* (1997), who was found 10.3% anti HCV, while 5% were positive for both hepatitis B and C in 506⁽¹⁷⁾.

In this study the seropositivity of HCV shown statistical significant relationship with duration of hepatitis B infection, while no relationship between risk factors (cupping, surgery, dental surgery, blood transfusion), and seropositivity of HCV.

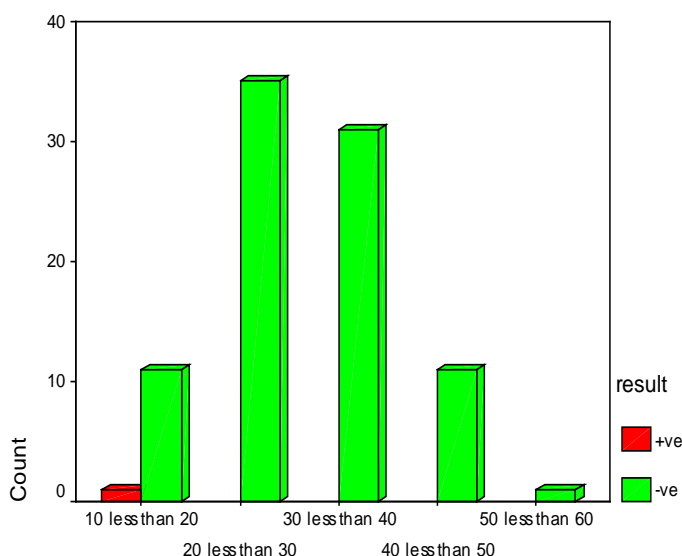


Figure 1: Serofrequency of HCV IgG Abs among studied population.

Acknowledgements:

Our thanks to Hamza Abdallah, Ministry of Health, Virology lab, Khartoum, State, Sudan and staff of medical microbiologist Al. Neelain University, Sudan.

REFERENCES:

- Liang TJ, Rehermann B, Seeff LB, Hoofnagle JH: Pathogenesis, natural history, treatment and prevention of hepatitis C. *Annals of Internal Medicine* 2000, 132:296-305.
- Lok AS, McMahon BJ: Chronic hepatitis B. *Hepatology* 2001, 34:1225-1241.
- Omer RE, Veer Van't P, Kadaru AM, Kampman E, El Khidir IM, Fedail SS, Kok FJ: The role of hepatitis B and hepatitis C viral infections in incidence of hepatocellular carcinoma in Sudan. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2001, 95:487-49.
- Brooks FG, Butel FJ, Morse AS: Hepatitis viruses. In *Medical Microbiology*. 22nd edition. Appleton and Lang, New York; 2001:403.
- Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA: Hepatitis viruses. In *Medical Microbiology*. 4th edition. Mosby, St. Louis; 2002:591.
- Bahatia R, Ichhpujani RI: Viral hepatitis. In *Essentials of Medical Microbiology*. 3rd edition. Jaypee Brothers, Medical Publishers, Ltd, New delhi; 2004:396-406.
- Liu Z, Hou J: Hepatitis B virus (HBV) and hepatitis C virus (HCV) dual infection. *Int J Med Sci* 2006, 3(2):57-62.
- Huo TI, Huang YH, Hsia CY, Su CW, Lin HC, Hsu CY, Lee PC, Lui WY, Loong CC, Chiang JH, Chiou YY, Lee SD: Characteristics and outcome of patients with dual hepatitis B and C-associated hepatocellular carcinoma: are they different from patients with single virus infection. *Liver Int* 2009, 5:767-773.
- Brotman B, Prince AM, Huima T, Pfeifer U: Interference between non-A and non-B hepatitis virus infection in chimpanzees. *J Med Virol* 1983, 11:191-205.
- Bradley DW, Maynard JE, McCaustland KA: Non-A, non-B hepatitis in chimpanzees: interference with acute hepatitis A virus and chronic hepatitis B virus infections. *J Med Virol* 1983, 11:207-213.
- Crespo J, Lozano JL, Carte B, Heras B, de la Cruz E2, Pons-Romero FI: Viral replication in patients with concomitant hepatitis B and C virus infections. *Eur J Clin Microbiol Infect Dis* 1997, 16:445-451.
- George c.Kuo, Juei-Low Sung, -YangLai, Jin-Chuan Sheu, Pei-Jer Chen, Pei-Ming Yang, Hsu-Mei Hsu, Mei-Hwei Chang, Chien-Jen Chen, Liang-Cheng Hahn, Qui-Lim Choo, Teh-Hong Wang, and Michael Houghton. Hepatitis C Virus Infection in an Area Hyperendemic for hepatitis B and Chronic Liver Disease: The Taiwan Experience Ding-Shinn Chen, <http://jid.oxfordjournals.org>

13. EmadAldin Ibrahim Osman*, Nagwa Ahmed Abdulrahman, Osman Abbass ,Waleed Hussein Omer, Hafi Anwer Saad and Muzamil Mahdi Abdel Hamid, Prevalence of Hepatitis B surface antigen and Hepatitis C virus antibodies among presurgery screened patients in Khartoum, Central Sudan Journal of General and Molecular Virology Vol. 4(1), pp. 6-9, October 2012 Available online at <http://www.academicjournals.org/JGMV> DOI: 10.5897/JGMV12.003 ISSN 2141-6648 ©2012 Academic Journals
14. Elsheikh RM, Daak AA, Elsheikh MA, Karsany MS, Adam I (2007). Hepatitis B virus and hepatitis C virus in pregnant Sudanese women. Virol. J. 4:104.
15. Fedail SS (2007c). Epidemiology of HCV infection in Gezira State of central Sudan. J. Med. Viro.79:383385
16. McCarthy MC , el Tigani A , Khalid IO,Hyams KC(1994) . Hepatitis B and C in Juba, southern Sudan: results of a serosurvey. Trans. R. Soc. Trop. Med. Hyg. 88:534-536
17. H.F El-Sayed , S.M Abaza , S Mehanna , P.J Winch . November 1997, The prevalence of hepatitis B and C infections among immigrants to a newly reclaimed area endemic for *Schistosoma mansoni* in Sinai, Egypt, *Acta Tropica*