

**INTERNATIONAL JOURNAL OF ADVANCES IN
PHARMACY, BIOLOGY AND CHEMISTRY**

Research Article

**Serofrequency of Hepatitis E Virus among
Hemodialysis Patients in Omdurman Military
Hospital Khartoum-State**

**ALIA QURASHI MOHAMMED AHMED,¹ Wafa Ibrahim Elhag^{2*}, Huda Ali
Mohamed Ali Ahmed³**

¹Faculty of Medical Laboratory Sciences, Al Neelain University, Sudan

²Microbiology Department, Faculty of Medical Laboratory Sciences,
Al-Neelain University, Sudan

³Microbiology Department, Faculty of Medical Laboratory Sciences,
Al Neelain University, Sudan.

ABSTRACT

A total of 90 participants (45 hemodialysis patients as case and 45 healthy individuals as control group) who attending medical checkup in Military hospital during the period from March to April 2015, were enrolled in this study. Their age ranged from (25 — 55) years with 40 years mean. The aim of this study was to detect Hepatitis E virus IgG antibodies among patients and control group, and to detect the relation between seropositivity and to correlate between seropositivity and age, duration of dialysis.

90 serum specimens were collected and analyzed by ELISA technique.

The results showed that 8(8.9%), 5(5.6%) were positive for IgG antibody among patients and control respectively, and 37 (41.1%), 40(44.4%) were negative for IgG patient and control respectively, of the total, 13(14.4%) were positive and 77(85.6%) was negative.

Statistical analysis showed that there was insignificant correlation between age, duration of dialysis, and present of HEV IgG.

Large-scale studies in different settings and studies in Sudan are required.

Keywords: Serofrequency, HEV, IgG- Hemodialysis -ELISA-Khartoum-Sudan.

INTRODUCTION

Hepatitis E is a self-limiting acute hepatitis caused by hepatitis E virus (HEV), which can occur both in sporadic or epidemics forms ⁽¹⁾. In term of clinical symptoms, hepatitis E cannot be differentiated from other viral hepatitis cases. The spectrum of symptom range from asymptomatic to fulminate disease, most patients suffer from jaundice, pain, abdominal tenderness, nausea, vomiting, and fever. Hepatitis E does not become chronic and often show no recurrence. The most important complication is a

sever fulminate and fetal hepatitis in pregnant women and people with chronic liver disease ⁽²⁾. A large outbreak of Hepatitis E was reported in June 2004 in the internally displaced population camps of Darfur ⁽³⁾. The infection represents an important public health concern in many developing countries, where it is often responsible for epidemics outbreaks ⁽³⁾. The infection effects primarily young adults and is generally mild; however the mortality rate is higher among women especially in second and third

trimester of pregnancy. In Sudan the fatality ratio of 17.8% was found in an outbreak in Darfur⁽³⁾.

Hepatitis E virus (HEV) is spherical, non-enveloped; single stranded of positive-sense RNA virus⁽⁴⁾ that belongs to the new genus, *Hepevirus* of the family *Hepeviridae*⁽⁵⁾. Patient on dialysis are highly susceptible to infection because they often are immunocompromised are exposed routinely to invasive technique and devices. This pathogen is responsible for at least 50% of acute non A non B hepatitis in developing countries. HEV infection is a major cause of human viral disease with clinical and pathological feature of acute hepatitis; in related study Takehiro, reported prevalence rate of anti-HEV IgG (9.4%)⁽⁶⁾.

Most studies addressing risk factor for HEV in non-endemic area come from developed area such as North America and Europe. In this study they evaluated seroprevalence for HEV in 88 patient on dialysis in Argentina. They found a significantly higher seroprevalence of HEV IgG in those undergoing dialysis compared with health controls

(10.2% and 4.3% respectively, $p = 0.03$).⁽⁷⁾

This study aimed to detect seroprevalence of hepatitis E virus among hemodialysis patients.

MATERIALS AND METHODS

This was descriptive- cross sectional study which had been conducted in Omdurman military hospital during period April to June 2015, 45 patients as case and 45 healthy individuals as control were enrolled. Data was collected by using direct interviewing questionnaire, and ethical clearance was obtained from Research Ethical Committee of faculty of Graduate Studies and Ministry of Health Khartoum State.

EXPERIMENTAL WORK

Collection of Specimens:

Blood specimens were collected from 90 participants, under direct medical supervision by medial vein puncture using 5 ml syringe into plain tube to obtain serum by centrifugation at 5000 rpm for 10 min. serum was kept in -20°C till serological study was performed.

Specimens were processed by Enzyme linked immune sorbent assay (ELISA) (3rd generation ELISA) (Weka- China) for detection of IgG

Enzyme linked immune sorbent assay for detection anti Hepatitis E virus IgG.

All reagents and samples were allowed to reach room temperature for 15 minutes before use.

Washing buffer was prepared 1:9 from buffer concentrate with distilled water.

Patient's specimens were diluted 1:101 in sample buffer.

One hundred (100 μl) of the calibrator, positive and negative controls or diluted patient sample were pipetted into the individual microplate wells, then plate was covered and incubated for 30 minutes at (25°C). Plate was taken out and wash buffer was added to each microplate wells (Washing 1) and aspirated off after 20 seconds. This step was repeated for 3 times until each well become dry.

One hundred (100 μl) of HRP-Conjugate Reagent was added in to each micro plate wells, the plate was mixed well and covered with the plate cover and incubated for 30 min at (25°C).

The plate cover was removed and discarded. The liquid was aspirated and each well was rinsed in wash buffer (Washing 2) and aspirated off after 20 second. This step was repeated for 3 times until each well become dry.

One hundred (100 μl) of substrate solution was added in to each wells and mixed by tapping the plate gently. The plate was incubated at (25°C) for 15 min.

One hundred (100 μl) Stop solution was pipetted and added into each micro plate wells and mixed gently.

Photometric measurement of the color intensity was made at wavelength of 450 nm within 30 min of adding stop solution. Prior to measuring slightly shake the micro plate to ensure a homogeneous distribution of the solution.

The results can be evaluated by calculating a ratio of the extinction value of the control or patient sample over extinction value of calibrator 3. Calculated the ratio according to the following formula:

$$\frac{\text{Extinction of control or patient sample}}{\text{Extinction of calibrator 3}} = \text{Ratio}$$

Interpretation of Results:

Ratio < 0.8: negative

Ratio > 0.8 to 1.1: borderline

Ratio > 1.1: positive

Data analysis: Data was analyzed by SPSS (Statistical Package of Social Science) software program version 16

RESULTS

A total of 90 participants (45 healthy individual as control and 45 hemodialysis patients as case) were enrolled in this study.

The result revealed that sero-positivity of hepatitis E virus IgG of control and patients were shown on (Table 1), The sero-positivity of case was 8 (8.9%),

and control 5 (5.6) ,(p-value insignificant 0.3). The study population age ranged from 25-55 years with 40 years mean (Table 2), most of sero-positivity was observed among case group 8(17.8%), and control 5 (11.1%),(p-value insignificant(0.3). And the duration of hemodialysis patient were shown on (Table 3) most of sero-positivity of them in 3 years and more than 3 years 4(8.9%) (P-value insignificant (0.5). Statistical analysis showed that there was insignificant correlation (P-value more than 0.05) between age, duration of dialysis and presence of hepatitis E virus IgG.

DISCUSSION

HEV infection is major cause of human viral disease of acute hepatitis; this study presented the most recent data on the Serofrequency of hepatitis E virus IgG in the hemodialysis patients in terms of gender, age groups, and duration of dialysis.

The present study results revealed that 8(17.8%), were positive among patients for IgG, when compared with others finding, in Iran it was similar study results revealed that (19.2%), were seropositive. Seropositive patients were not significantly different from seronegative patients, with regard to age, sex, duration and frequency of hemodialysis. agreed with their result in insignificant Similar study was reported the duration of dialysis ($p = 0.87$), and age ($p = 0.3$)⁽⁸⁾.

patients on dialysis are highly susceptible to infection because they often are immunocompromised are exposed routinely to invasive technique and devices .The possibility that HEV might be transmitted during dialysis , underscores the necessity for dialysis facilitates to strictly adhere to proper infection control at all time and must be all blood line attachment to the dialysis machine were disposable and discard after each dialysis session .Unfortunately HEV vaccine have not been mass produced and are not available for public use⁽⁹⁾.

CONCLUSION

Prevalence of HEV seromarkers for previous and current infection is high. Facilities for routine diagnosis and vaccination are lacking. Initiation of organized screening and vaccination programs is limited by lack of vaccine. We recommend the patient who started dialysis in the previous year may be how more exposed to the risk of infection.

ACKNOWLEDGEMENT

We do knowledge the efforts of Omdurman Medical Military hospital and also staff of Medical Microbiology in Faculty of Medical Laboratory AL Neelain University.

Table 1
Serofrequency of HEV among hemodialysis(n=45) and control group (n=45)

Anti HEV	Result		Total (%)
	Positive no (%)	Negative no (%)	
Patients group	8(8.9%)	37(41.1%)	45 (50%)
Healthy group	5(5.6%)	40(44.4%)	45 (50%)
total	13(14.4%)	77(85.6%)	90 (100%)

(P-value insignificant 0.3)

Table 2
Serofrequency of HEV according to age among hemodialysis (n=45) and control group (n=45)

Age groups in years	Result				Total
	Hemodialysis patients		Healthy individuals		
	Seropositive of patient no (%)	Negative of patient no (%)	Seropositive of control no (%)	Negative of control no (%)	
25-35	2(4.4%)	10(22.2%)	3(6.7%)	35(77.8%)	50
36-45	5(11.1%)	18(39.9%)	1(2.2%)	1(2.2%)	25
46-55	1(2.2%)	9(20%)	1(2.2%)	4(8.9%)	15
Total	8(17.8%)	37(82.2%)	5(11.1%)	40(88.9%)	90

Table 3
Serofrequency of HEV among hemodialysis patient (n=45) according to duration of dialysis

Duration of Renal hemodialysis	Result		Total
	Seropositive of patient no (%)	Negative of patient no (%)	
1-2 year	0(0%)	4(8.9%)	4
3 years	4(8.9%)	19(42.2%)	23
More than 3 years	4(8.9%)	14(31.1%)	18
Total	8(17.8%)	37(82.2%)	45

REFERENCES

1. Anderson DA. Hepatitis E virus. In mandell GI, Douglas RG, Bennett JE, eds. Principles and practice of infectious diseases, 7th ed .Philadelphia, Churchill Livingstone, 2010; 2411-2421.
2. Koff RS. Hepatitis A and E. in: Zakim D, Boyer TD, eds.hepatology: atextbook of liver disease, 4th ed. Philadelphia, Saunders, 2003; 939-958.
3. Boccia D,Guthmannjp,Klovstad H,Hamid N,Tatay M,Ciglenecki I,et al.High mortality associated with an outbreak of hepatitis E among displaced person in Darfur, Sudan .*Clin Infect Dis.*200; 42(12):1679-1684.
4. Ahmed I, Holla RP, JameelS. Molecular virology of hepatitis E virus. *Virus Res.*2011; 161 (1): 47-58
5. Emerson SU, Purcell RH. Hepatitis E virus. *Rev. Med. Virol.* 2003; 13:145-154.
6. Psychogiou M, Vaindirli E, Tzala E, Voudiclari S, Boletis J, Vosnidis G, Moutafis S, SkoutelisG,Hadjiconstantinou V, Troonen H, Hatzakis A, Hepatitis E Virus Infection in Hemodialysis Patients. The Multicenter Hemodialysis Cohort Study on Viral Hepatitis, *Nephrol Dial Transplant.* 1996 Jun; 11(6): 1093-5.
7. Maria Belen Pisano, Domingo Balderramo, Maribel Martinez Wassaf, Martin Lotto, YaninaCarlino, Viviana Elizabeth Re, Jose D. Debes. Hepatitis E virus infection in patients on dialysis in Argentina, Official Journal of the Virology Division of the international Union of Microbiological Societies. 2016, 0304-8608.
8. Peyman Eini, Mojgan Mamani, Marzieh Javani. Seroprevalence of Hepatitis E among Hemodialysis patients, 2015; 15(5): e26260.
9. Aggarwal R, Naik S. Epidemiology of hepatitis E: current status. *Journal of Gastroenterology and Hepatology*, 2009; 24(9):1484-1493.