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Research Article

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

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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 out breaks (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, Herpesvirus 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 none 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 Serofrequency 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 (100μl) 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 3 times until each well become dry.

One hundred (100μl) 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 (Washing2) and aspirated off after 20 second. This step was repeated for 3 times until each well become dry.

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

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

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 3years
and more than 3years 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).

patients on dialysis are highly susceptible to infection
because they often are immunocompromised are
exposed routinely to invasive technic and devices.
The possibility that HEV might be transmitted
during dialysis, underscores the necessity for dialysis
facilities 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.

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.

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We do know the efforts of Omdurman Medical
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Microbiology in Faculty of Medical Laboratory Al
Neelain University.

<table>
<thead>
<tr>
<th>Anti HEV</th>
<th>Result</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive no (%)</td>
<td>Negative no (%)</td>
</tr>
<tr>
<td>Patients group</td>
<td>8(17.8%)</td>
<td>37(82.2%)</td>
</tr>
<tr>
<td>Healthy group</td>
<td>5(5.6%)</td>
<td>40(88.9%)</td>
</tr>
<tr>
<td>total</td>
<td>13(14.4%)</td>
<td>77(85.6%)</td>
</tr>
</tbody>
</table>

(P-value insignificant 0.3)

<table>
<thead>
<tr>
<th>Age groups in years</th>
<th>Result</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hemodialysis patients</td>
<td>Healthy individuals</td>
</tr>
<tr>
<td></td>
<td>Seropositive of patient no (%)</td>
<td>Negative of patient no (%)</td>
</tr>
<tr>
<td>25-35</td>
<td>2(4.4%)</td>
<td>10(22.2%)</td>
</tr>
<tr>
<td>36-45</td>
<td>5(11.1%)</td>
<td>18(39.9%)</td>
</tr>
<tr>
<td>46-55</td>
<td>1(2.2%)</td>
<td>9(20%)</td>
</tr>
<tr>
<td>Total</td>
<td>8(17.8%)</td>
<td>37(82.2%)</td>
</tr>
</tbody>
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Table 3
Serofrequency of HEV among hemodialysis patient (n=45) according to duration of dialysis

<table>
<thead>
<tr>
<th>Duration of Renal hemodialysis</th>
<th>Result</th>
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<tbody>
<tr>
<td></td>
<td>Seropositive of patient no (%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative of patient no (%)</td>
<td></td>
</tr>
<tr>
<td>1-2 year</td>
<td>0(0%)</td>
<td>4</td>
</tr>
<tr>
<td>3 years</td>
<td>4(8.9%)</td>
<td>23</td>
</tr>
<tr>
<td>More than 3 years</td>
<td>4(8.9%)</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>8(17.8%)</td>
<td>45</td>
</tr>
</tbody>
</table>

REFERENCES
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.