Prevalence of SEN Virus Infection among Volunteer Blood Donors in Nyala Teaching Hospital, Southern Darfur State, Sudan

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Abstract: More than 80% of hepatitis cases are caused by five types of hepatitis viruses (A-E), which known as hepatotropic viruses, The remaining of the 20% cases and also 10% transfusion associated hepatitis are tested negative for all viral hepatitis, that suggest the existence of other hepatitis agents. The latest novel virus proposed as a cause of this kind of hepatitis (non-A-E) is a SEN virus (SENV). This virus was discovered in the serum of an injection drug user (IDU) infected with a human immunodeficiency virus (HIV). And was found subsequently in a large percentage of IDUs and polytransfused patients. This study was designed to search the prevalence of SEN-V among blood donors in Nyala city. SENV DNA was determined by polymerase chain reaction in serum samples from 100 healthy individuals attended to the blood bank of the Nyala teaching hospital. SENV was detected in 19 from 100 (19%) of healthy blood donors. SENV can be transmitted via blood transfusion.

Keywords: SEN -virus (SENV); polymerase chain reaction (PCR).

INTRODUCTION

More than 80% of hepatitis cases are caused by five types of hepatitis viruses (A-E), which known as hepatotropic viruses [1]. The remaining of the 20% cases and also 10% transfusion associated hepatitis are tested negative for all viral hepatitis, that suggest the existence of other hepatitis agents [2, 3].

The latest novel virus proposed as a cause of this kind of hepatitis (non-A-E) is a SEN virus (SENV) [4].

This virus was discovered in the serum of an injection drug user (IDU) infected with a human immunodeficiency virus (HIV) and was found subsequently in a large percentage of IDUs and polytransfused patients [5].

SENV is a single stranded DNA genome, non-enveloped, round, icosahedral symmetry [6]. Belong to Circoviridae family, Circo virus genus and Circo indicates the circular shape of the virus genome [7], that consist of approximately 3,800 nucleotides in length and about 26 nm in size and in these viruses at least three open reading frames (ORF) have been identified [8], this family also includes TT virus (TTV), SANBAN, TUS01 and YONBAN, The characteristic of SENV parallel with the TTV is that the viruses may share a common ancestor [9].This SENV had been sub grouped into 9 genotypes (A to I) [10].

The modes of transmission of SENV infection mostly parenteral, e.g. blood transfusion, hemodialysis and organ or hematological progenitor cell transplantation and drug addiction by injection uses [11]. There was no significant difference between the rates of SENV- blood donors and positive patients with acute hepatitis [12]. SENV was found out at the same frequency in patients with and without liver disease. SENV does not seem to contribute either to the pathogenesis of liver disease or to the improvement of hepatocellular carcinoma from chronic liver disease.
It is not known whether SENV may be an etiological agent in some cryptogenic hepatitis. The aim of this study is to determine the prevalence of SENV in healthy blood donors in Southern Darfur, western Sudan is using conventional polymerase chain reaction.

MATERIAL AND METHODS

Hundred blood samples were obtained from blood donors were involved in this descriptive cross sectional study. All the blood donors were healthy volunteers who met all standard eligibility criteria for donation (i.e., negative test results for hepatitis B surface antigen (HBsAg) and for antibodies to HCV and HIV) in Nyala teaching hospital will be randomly selected, the Data collected through Interview using a self administered questionnaire .Two milliliter blood was taken from each donor and then centrifuged at 3500 g at room temperature. Sera were separated and transferred into fresh tubes. The serum samples were kept at -20°C until the day of examination. All samples were tested for SENV by polymerase chain reaction (PCR) during (October 2016 to July 2017). This study performed by Permission from College of graduate studies and permission from the managers of Nyala teaching hospital and patients.

Viral DNA extraction from serum:

500 μl Serum was mixed with 250 μl of 0.5% SDS. 20 μl of 10 mg/ml proteinase K solution was added and incubated at 56°C for 2 hours, then place the samples at 95°C for 10 minutes to deactivate proteinase K. Protein was precipitated with two phenol: chloroform: isoamyl alcohol (25:24:1) solution and followed by only chloroform treatment. The cold absolute ethanol (100%) and (70%) ethanol were used for DNA precipitation, then precipitate was dissolved in 250 μl of distilled de ionized water and preserved at – 20°C [15].

Detection of SENV DNA

SENV DNA was detected by polymerase chain reaction (PCR) with SENV-specific primers according to Kojima et al. [14]. By using a primer pair, which detected a 349-bp conserved region for all SENV genotypes (A-I), was used (forward primer AI-1F [5-TWCYCMACGACCAGCTAGACCT-3] and reverse primer AI-1R [5-GTTLTGTGTGAGGAGACAGGA-3]). PCR reaction was carried out with a 25 μl reaction mixture containing 1μl Primer (Forward) AI-1F; 1μl Primer (Reverse) AI-1R, 5μl of Template DNA, 13μl of nuclease free water and all was added to the Master Mix tube. The setting temperature profile was 44 cycles (94°C for 20 seconds, 56°C for 25 seconds and 72°C for 30 seconds in each cycle) with a final extension time for 5 minutes at 72°C in a thermocycler.

Gel Electrophoresis

Tris-borate-EDTA buffer (10 ×) was diluted 10 times to be (1 ×) by adding 100 ml of Tris-borate-EDTA buffer (10 ×) with 900 ml distilled water, and 3 grams of Agarose powder was dissolved in 200 ml of (1 ×) Tris-borate EDTA buffer to a final concentration of 1.5% Agarose buffer the last solution had been boiled in the microwave; after boiling, the solution was cooled at room temperature and adding 5 μl Ethidium bromide was added to stain the gel , poured into the gel tray and the comb was placed to make the well on which the PCR products were loaded.

After amplification, the product was run on (1.5%) agarose gel electrophoresis, in Genei TM, Bangalore, India was used. The PCR products, positive and negative control, and 10µl were loaded in each well according to prepared master chart. 100 base pair ladder (Fermentas, USA) was used for comparison.

STATISTICAL ANALYSIS

Analysis of data was carried out using the available statistical package of SPSS-17 (Statistical Packages for Social Sciences- version 17). Data were presented in simple measures of frequency, percentage, mean, standard deviation, and range. The significance of difference of different percentages (qualitative data) was tested using Pearson Chi-square test Statistical significance was considered whenever the P value for the test of significance was equal or less than 0.05

RESULTS AND DISCUSSION
Fig-1: show pcr detection of SENV. Lanes 1,2,3,5,6,7 show positive samples fragment (349bp). Lane 4 shows 100 bp DNA ladder. Lanes 8 and 9 show negative samples.

Fig-2: Showed the age group distribution of the studied population

Fig-3: Showed the frequency of SENV among the blood donors
Fig-4: Showed the age group distribution of the infected individuals

Table-1: Showed blood group in relation to infection

<table>
<thead>
<tr>
<th>Blood group</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A+ve</td>
<td>3</td>
<td>15.8%</td>
</tr>
<tr>
<td>B+ve</td>
<td>4</td>
<td>21.1%</td>
</tr>
<tr>
<td>O-ve</td>
<td>1</td>
<td>5.3%</td>
</tr>
<tr>
<td>O+ve</td>
<td>11</td>
<td>57.9%</td>
</tr>
</tbody>
</table>

Association between Age group and SIN Virus

No association between the frequency of sen virus infection and age group 16-26 and age group 28-38(Chi-square =0.053, df =1 and P value=0.819).

DISCUSSION

The present study indicates that SEN-V is a relatively common viral infection (19%) among healthy individuals who attended the blood bank center for donation in Nyala teaching Hospital.

The age-specific prevalence rates and tendency toward a younger age for SEN-V–positive was between ages 16-38 years with a mean age of versus SEN-V–negative individuals residing in the community provide some insight as to which routes might be more common. For example, an association with young age would be highlighted in the future. In several countries, researches revealed a nearby prevalence in our study using the same detection procedure. A study carried out in Iran by Zohreh Sharifi showed 23% similar as our results which indicate persist of SENV in other countries [16].

Minoru Shibata et al from Japan recently reported that SENV-H and SENV-D. SENV was detected in (32%) of patients with fulminant hepatitis, (17%) of patients with acute hepatitis, (27%) of patients with chronic hepatitis, (31%) of patients with liver cirrhosis, (33%) of patients with autoimmune hepatitis, (46%) of patients with primary biliary cirrhosis, and (10%) blood donors [17], which most probably is an association of liver disease in that country.

SEN-V was detected in (43%) of thalassemic patients and (10.7%) of blood donors by Bashar et al from Iraq [18] and the high prevalence of SENV among thalassemic patients may be due to multiple transfusion. To our knowledge this is the first study to be carried in Sudan for detection of SENV in blood donors. The limitation of this study, we are not genotyping the Sudanese SENV to look for the specific typing of the viruses.

ACKNOWLEDGMENTS

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Conflict of interests

The authors did not declare any conflict of interest.

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