Surveillance of Viral Hemorrhagic Fever Viruses in Lassa Fever Suspects in Ondo State, Nigeria

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ABSTRACT

Lassa Fever (LF) continues to be an endemic acute viral hemorrhagic fever (VHF) illness in Nigeria. Many suspected cases of LF infection have subsequently been confirmed negative and raises concerns as to what the diagnosis of such patients could be. Hence this study was to determine the causative agents of unconfirmed LF among initially suspected cases in South Western Nigeria.

In this retrospective study, blood samples originally collected from 233 suspected cases of a LF outbreak response at Owo and Ose LGAs of Ondo State, were transported in triple level packaging and stored at -80°C. All samples were screened for LF IgM and IgG markers and LF PCR. Forty-five out of the stored plasma samples were randomly retrieved and analyzed for presence of IgM for seven other VHF viruses; Chikungunya (CHIK), West Nile (WN), Rift Valley fever (RVF), Yellow fever (YF), Dengue fever (DEN), Zika and Crimean-Congo hemorrhagic fever (CCHF).

Out of 45 samples screened, 1 (2.2%) was positive for YF IgM antibody. The same sample was previously confirmed LF positive by PCR. This LF and YF co-infection was from a male, 23-year old individual.

The presence of co-infections of LF and YF draw to limelight the need to be broad minded in exploring for the presence of other VHF viruses in outbreaks. Further studies are needed to decipher the diagnosis of LF suspected cases.

Keywords: Lassa, viral hemorrhagic fever, yellow fever virus.

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I. INTRODUCTION

Lassa fever (LF) is a viral hemorrhagic fever (VHF) caused by the Lassa virus (LASV), a member of the virus family “ Arenaviridae” and transmitted by the multimammate rat Mastomys natalensis (M. natalensis). It was initially discovered in 1969 in Nigeria after the demise of two missionary nurses in Lassa Town, Borno State [1]. The disease is endemic in the West African countries of Sierra-Leone, Liberia, Guinea and Nigeria, with about 400,000 cases occurring annually and approximately 5000 deaths [2]. Cases have also been reported in Central African Republic, Democratic Republic of Congo, Mali and Senegal [2]. The sero-prevalence of LF in Nigeria is about 21% [3]. LF cases are difficult to differentiate from other febrile illnesses and if not well managed could result in high fatality rates.

In Nigeria, outbreaks have almost become an annual occurrence. An outbreak of LF occurred in 2018 and spread to 18 states: the largest on record [4]-[6]. In that year there were 1081 suspected cases and 90 reported deaths; 317 of the cases and 72 deaths were confirmed as LF [7]. The total cases in Nigeria in 2019 was 810 with 167 deaths, the largest on record [4]. In 2020, the epidemic began in the second week of January and by week 10 the total number of cases rose to 855 with 144 reported deaths, resulting in a 16.8% case fatality rate [9].

The burden of LF disease is uncertain and only a few cases with clinical suspicion of LF infection are laboratory confirmed. What is responsible for unconfirmed cases of LF in Nigeria is not known. Many suspected cases of LF infection have subsequently been confirmed negative and raises concerns as to what the diagnosis of such patients could be.

VHFs are a diverse group of human and animal illnesses majorly characterized with fever and hemorrhage initiated by viral infection. VHFs are caused by five families of RNA viruses: Filoviridae, Flaviviridae, Rhabdoviridae, and several members families of the Bunyavirales order such as Arenaviridae, and Hantaviridae. All types of VHFs are distinguished by fever and bleeding disorders, progressing to high fever, shock and death in many cases. Some VHF agents cause relatively mild illnesses, such as the Scandinavian nephropathia epidemica (a hantavirus), while others, such as Ebola virus, often lead to severe, life-threatening disease. Signs and symptoms of VHF viruses include flushing of the face and chest, small red or purple spots, bleeding, swelling caused by edema, low blood pressure (hypotension), and circulatory shock. Malaise, muscle pain, headache, vomiting, and diarrhea occur frequently [10].

Different VHFs infect the body differently, causing varying symptoms. In most VHFs, several mechanisms may contribute to symptoms, including liver damage, disseminated intravascular coagulation (DIC), and bone marrow dysfunction. Reasons for variation among patients infected with the same virus are unknown but stem from a complex system of virus-host interactions [11].

Signs and symptoms of VHFs vary by disease. Generally, early signs and symptoms can include: fever, fatigue, weakness or general feeling of being unwell, dizziness, muscle, bone or joint aches, nausea and vomiting and diarrhea [12]. Symptoms of Lassa fever similar to other VHFs typically include fever, weakness, headaches, vomiting, and muscle pains. Less commonly there may be bleeding from the mouth or gastrointestinal tract. The risk of death once infected is about 1% and often occurs within two weeks of the onset of symptoms. Of those who survive, about a quarter have hearing loss. Lassa fever symptoms are similar to other VHF symptoms.

Laboratory diagnosis of VHFs take place in highly specialized laboratories, classified with biosafety levels (BSL) ranging from 2 to 4. The categorization is based on laboratory design, containment facility, and handling of biological agents. Reverse transcription PCR (RT-PCR) has become the basis for molecular diagnosis, and RT-PCR assays have been designed for most VHF-associated viruses [13]-[16]. Multiplex assays were developed as it became apparent that detecting one pathogen at a time using RT-PCR has limitations where more than one pathogen is endemic. Multiplex assays for VHF were first described in 2002 with the ability to detect Ebola virus, Marburg virus (MARV), LASV, CCHFV, RVFV, DENV, and yellow fever virus (YFV) [16]. Multiplex assays have now been developed for a range of VHFs with sensitivity and specificity comparable to other RT-PCR assays; with limit of detection ranging from $1 \times 10^2$ to $1 \times 10^6$ copies/ml [16]. Challenges in making timely diagnosis of LF arise from late presentation of patients to centers which have the capability of managing LF cases, patients presenting with nonspecific symptoms and length of time required to get laboratory diagnosis.

This present study is sequel to a response to an outbreak of LF virus in Ondo state South Western Nigeria. The research is intended to describe the prevalence or occurrence of other VHF viruses in LF virus endemic regions of Nigeria. The objective was to investigate other likely VHF viruses in LF suspected cases, as there is limited information on the role of other VHF viruses that have similar clinical presentations in suspected cases of LF infection.

II. MATERIALS AND METHODS

A. Study Design

This was a retrospective study involving retrieval of stored samples, collected between March 2018 and October 2019, in Owo and Ose LGAs of Ondo State, Nigeria.

B. Ethical Consideration

Ethical approval for this study was obtained from the Institutional Review Board (IRB) of Nigerian Institute of Medical Research (NIMR) (IRB/18/018). An additional ethical approval was obtained from the Health Research and Ethics Committee of Federal Medical Centre, Owo, Ondo State. Samples were collected only after obtaining documented signed consent from the participants.

C. Study Samples

Blood samples were originally collected from 233 individuals comprising 102 health workers, 22 LF suspected infected cases and 109 contacts of infected cases at Owo and Ose Local Government Areas, and transported in triple level packaging to NIMR for storage at -80 °C. Suspected cases were defined as those that had febrile symptoms presumptive...
of LF. The samples were previously screened for LF by PCR. This involved extraction of RNA from samples using QiaAmp Viral RNA mini-Kit (Qiagen, GmbH Germany) and analyzed for LASV RNA on real time PCR platform, using RealStar® Lassa Virus RT-PCR kit 2.0 (Altona Diagnostics, Germany) [16].

All 233 samples were also previously screened for IgG and IgM using the ReLASV® Pan-Lassa NP IgG/IgM ELISA Kit (Zalgen Labs, USA) [17]. This kit consists of a mixture of LASV nucleoprotein specific antigen against the three most prevalent lineages of LASV (lineage II, III in Nigeria and lineage IV in Sierra Leone, Guinea, Liberia, and Mali). The assay was performed in accordance with manufacturer’s instructions. Forty-five of the 233 stored samples were randomly retrieved for this study.

D. Serological Testing of Other VHF

At the WHO Collaborating Centre for Arboviruses and Haemorrhagic Fevers, of Institute Pasteur Dakar (IPD), serological testing for other VHF were carried out. The first step consisted of sample inactivation performed by heating at 56°C for 30 minutes. The presence of IgG antibodies against Chikungunya (CHIK), West Nile (WN), Rift Valley fever (RVF), Yellow fever (YF), Dengue fever (DEN), Zika and Crimean-Congo hemorrhagic fever (CCHF) viruses were assessed in inactivated samples using IPD in-house ELISA methods, with antigens and immune ascites produced in mice [18]-[19]. Briefly, indirect ELISA testing was performed on 96-well Maxisorp ELISA microplates. After a coating step at 4°C overnight with virus mouse hyperimmune ascitic fluids, followed by specific viral antigen capture, samples and controls were added to the wells, and specific antibody-antigen complexes were revealed using anti-human IgG antibodies conjugated with horseradish peroxidase (KPL, USA) [20]. The specific substrate was added to the wells, and the reaction was stopped with sulphuric acid. Optical densities (ODs) were measured using an ELISA microplate reader (Biotek, Agilent Technologies, CA, USA). A sample was considered as positive if the OD was >0.20 and the ratio (R) between the sample and the negative control was >2 [21]-[22].

III. RESULTS

The 45 samples consisted of 24 PCR LF positive and 21 LF negative samples. Previously, 24/45 (53.3%) were confirmed LF positive cases by PCR while 20/45 (44.4%) and 14/45 (31.1%) were positive for LF IgM and IgG respectively. Out of the 45 samples screened for IgM for CHIK, WN, RVF, YF, DEN, Zika and CCHF viruses, only 1 (2.2%) was positive for YF IgM antibody as shown in Table I. The YF IgM antibody positive sample was also LF PCR and IgG confirmed. This LF and YF co-infected sample was from a male, 23 years old individual, a contact of a LF infected case. A demographic description of the 45 LF suspected cases from which samples were drawn showed a mean age of 39.7 (SD±17.7) years, ranging from 3 to 86 years, with a male: female ratio of 1:1(23 female and 22 male).

<table>
<thead>
<tr>
<th>VHF</th>
<th>IgM Positive N (%)</th>
<th>IgM Negative N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow Fever</td>
<td>1 (2.2)</td>
<td>44 (97.8)</td>
</tr>
<tr>
<td>Crimean Congo</td>
<td>0 (0)</td>
<td>45 (100)</td>
</tr>
<tr>
<td>Dengue Fever</td>
<td>0 (0)</td>
<td>45 (100)</td>
</tr>
<tr>
<td>Zika</td>
<td>0 (0)</td>
<td>45 (100)</td>
</tr>
<tr>
<td>West Nile</td>
<td>0 (0)</td>
<td>45 (100)</td>
</tr>
<tr>
<td>Rift Valley</td>
<td>0 (0)</td>
<td>45 (100)</td>
</tr>
<tr>
<td>Chikungunya</td>
<td>0 (0)</td>
<td>45 (100)</td>
</tr>
</tbody>
</table>

IV. DISCUSSION

Screening for VHF's in Nigeria is of serious importance as many of these viruses have common modes of transmission and similar symptoms. This study was intended to further probe the outcome of a LF outbreak. Due to the low positivity rates of the cases, the negative LF samples were investigated for possibility of harboring other VHF's. YF and LF are both zoonotic diseases (mosquito borne flavivirus and rodent borne arenavirus, respectively) and classified as VHF viruses because of their common clinical presentations – especially fevers and bleeding during the terminal stages of the diseases. In this study, 45 samples composed of suspected and confirmed cases of LF were screened for seven other common VHF's. Among these, a positive YF IgM result was obtained. The positive YF is may be a co-infection (however unconfirmed), as it was found among the PCR positive LF samples. The sample was from a confirmed LF PCR positive individual, a contact to a confirmed case at Owo, Ondo state. The presence of co-infection of LF and YF virus in an individual has rarely been reported, necessitating that health care workers do not rule out other VHF's in LF suspected cases. Co-infection of VHF's can bring about more severe symptomatology and could lead to poor prognosis [23]. Clinical diagnosis is difficult due to the fact that signs and symptoms often cannot be differentiated from common tropical diseases such as typhoid fever, malaria, YF, and other VHF's [23]. This brings to the forefront the importance of constantly screening for various VHF's in endemic societies and settings. It is recommended that further studies to determine the burden of VHF's in endemic societies be conducted.

In conclusion screening for VHF viruses is very important especially as the mosquito vector Aedes aegypti, multimammate rat Mastomys natalensis and other reservoirs of VHF's are dominant in tropical regions of the world. The likely presence of co-infections of LF and YF draw to limelight the need to be broad minded in exploring for the presence of other VHF viruses in LF suspected cases. Further studies are needed to decipher the diagnosis of the LF negatives obtained in this study.

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DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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