ORIGINAL ARTICLE

The sensitivity and specificity of Lassa virus IgM by ELISA as screening tool at early phase of Lassa fever infection

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ABSTRACT

Background: Early diagnosis, prompt treatment, and disease containment are vital measures in the management of Lassa fever (LF), a lethal and contagious arenaviral hemorrhagic disease prevalent in West Africa. Lassa Virus (LAV)-specific Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) test, the gold standard for diagnosis, is unavailable in most centers. Serologic detection of LAV IgM is a more accessible tool and this work was to investigate its adequacy as an early marker for LF. **Patients and Methods:** A prospective case–control study conducted July 2007-March 2011 in a tertiary referral health center in Nigeria. Blood samples for test and control were evaluated for Lassa specific antigens and IgM using RT-PCR (primers S36+ and LVS 339) and indirect ELISA (Lassa Nucleo-protein (NP)-Antigen) respectively. RT-PCR outcome was used as standard to test for the sensitivity and specificity of IgM. **Results:** Of the 37 confirmed cases of LF infection by RT-PCR, 21 (57%) were IgM positive. Amongst the 35 confirmed negative cases (control group), eight were IgM positive. The diagnostic sensitivity and specificity of the IgM assay were 57% and 77% respectively. The negative and positive predictive values of the IgM serological assay were 63% and 72%, respectively, while the efficiency of the test was 67%. **Conclusion:** The specificity and sensitivity of IgM as a screening tool for early detection of LF appear weak and, hence, the need for a reliable LF "rapid screening kit" since RT-PCR is unavailable in most centers. In the interim, "high clinical index of suspicion," irrespective of IgM status, requires urgent referral to confirmatory centers.

Key words: Immunoglobulin M, Lassa fever, reverse transcriptase polymerase chain reaction test, serology

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INTRODUCTION

Lassa fever (LF), a severe acute hemorrhagic fever caused by the arenavirus, is a disease of public health concern in West Africa. The disease, which was first described in Sierra Leone in the 1950s, is, however, named after the town of Lassa in Borno State, northeastern Nigeria, where the causative virus was identified and the first cases were, in practical terms, isolated in 1969.[1,2]

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This lethal contagious disease transmitted via the excreta of the mastomysnatalensis (carrier rats) and body fluids of infected humans is endemic in the West African sub-region with countries such as Nigeria, Sierra Leone, Liberia, and Guinea particularly affected.[3]

It is also of note that LF virus has a nosocomial mode of infection with outbreaks characterized by high mortality having been documented.[4,5]

The annual morbidity/mortality rate is high, about 0.1 million cases and 5000 deaths in West Africa[6] and 0.25 to 1 million morbidity with 10,000 mortalities across the globe.[7] The case mortalities in some parts of Nigeria is as high as 28%.[8]

Survivors stand the risk of developing severe neurological sequeale like sensorineural hearing losses in both acute and convalescent stages of the infection,[9] seizures, and encephalitis.[10]

Early detection of the illness, prompt treatment with ribavirin anti-viral drug, and supportive treatment are vital measures to survival, given the morbidity and mortality indices. However, early clinical presentations can be non-specific and may mimic some tropical diseases like malaria, typhoid fever, yellow fever, and other hemorrhagic fevers.[3,7] Therefore, early diagnosis depends on high index of suspicion confirmed via laboratory investigations.

The critical importance of early intervention in LF therapy underscores the need for accurate, inexpensive, and rapid laboratory diagnostic methods.

LF infection is generally diagnosed by the use of viral isolation methods or detection of Lassa antigen and/or antibodies by serological assays and molecular techniques.[7,11]

Viral isolation technique, which carries high sensitivity, is basically used as a research method and thus has limited clinical applicability.[3]

The reverse transcriptase polymerase chain reaction (RT-PCR) test on viral antigen isolate by immunoflourescence has proven a reliable diagnostic tool for LF; specificity and sensitivity of near 100% compared with enzyme-linked immunosorbent assays (ELISA) 88% and 90%, respectively.[12] This has been dubbed the "gold standard" in some studies,[3,12] but this biosafety level IV laboratory facility is absent in most hospitals in the developing countries, and by extension the endemic areas of the illness.

Recently, following the lack of specificity of the traditional indirect fluorescent antibody (IFA) technique for diagnosis of LF, especially in non-endemic populations,[13] ELISA for LAV antigen, and LAV immunoglobulins have become widely used.[14-16]

Reports have also suggested that the elaboration of acute antibodies to LF infection do not commence immediately following the infection and, therefore, a "window period" for serological Lassa IgM may hamper early diagnosis. However, it is known that given our attitude to seeking healthcare in the developing world, patients may not present so promptly to warrant a significant miss of earliest possible diagnosis with IgM. The question remains to what extent can this affordable method of detection be relied upon for earliest diagnosis in our environment because early detection and commencement of therapy is extremely important for good clinical outcome. Thus, this work was set to investigate the adequacy of Lassa virus-specific IgM antibody as an early marker in LF diagnosis in our environment. The LAV-specific RT–PCR for LF antigen was set as the "gold standard."

MATERIALS AND METHODS

A prospective case–control study of LF patients was conducted between July 2007 to April 2009 and March 2011 at the Irrua Specialist Teaching Hospital, Irrua, Edo State Nigeria (Referral Center for diagnosis and management of LF in Nigeria). The data was obtained from a larger study titled "Lassa and hearing loss," which received approval from the Institution Review/Ethical Committee of Irrua Specialist Teaching Hospital Nigeria in accordance with the Helsinski declaration. The inclusion criterion involves suspected cases within the first 4 weeks of suggestive symptoms.

Some relevant sociodemographic and clinical information was obtained on admission. The initial diagnosis of acute LF was made based on the suggestive clinical features.[17,18] Furthermore, blood samples were taken for the LAV-specific RT–PCR test for LF antigen. The positive samples for the RT-PCR (between July 2007 and April 2009) were further screened for IgM antibodies.[12] Then, consecutive samples from referred suspected cases (through March 2011), which turned out negative for RT-PCR (controls) were also screened for Lassa IgM. The PCR used was based on the primers S36+ and LVS 339 whereas immunoglobulin M (IgM) antibodies to LF virus were assayed by an indirect ELISA method using Lassa Nucleo–protein (NP)-antigen.

All confirmed cases were given standard treatment in accordance with ISTH protocol (Barrier nursing, Ribavirin antiviral agent, and supportive therapy).

A comparative analysis of sensitivity and specificity of IgM in early detection of LF infection was made using the RT-PCR as standard. The results were presented in tables and figures whereas the statistical analysis was done with the Statistical package for social sciences (SPSS) version 15 and relevant correlation analytical packages used. The level of statistical significance was set at $P<0.05$.

RESULTS

Thirty-seven patients were confirmed as having acute LF disease (positive RT-PCR). They were 16 (43.2%) males and 21 (56.8%) females with mean age 35.3±14.1 years (range 11-61 years). Another subset of 35 patients confirmed negative for acute LF disease with RT-PCR served as matched controls. They comprised 19 (54.3%) males and 16 (45.7%) females with mean age 35±12.8 years (range 24–48 years). The majority (60%) of the patients belonged to the low socioeconomic class (Oladeji classes 3-5) and the remaining 40% were of the high socio-economic class (Oladeji classes 1 and 2).[19]

Out of the 37 confirmed cases of LF infection by RT-PCR, 21 (57%) were IgM positive whereas the rest were negative [Table 1]. Of the 35 confirmed negative cases (control group), eight were IgM positive and the rest were negative [Table 2].

The diagnostic sensitivity and specificity of the IgM assay for LF were 57% and 77%, respectively. The negative and

Table 1: The outcome of IgM screening on confirmed cases of Lassa fever with RT-PCR

	Type of test	
	RT-PCR	IgM
Positive (+ve)	37	21
Negative (-ve)	0	16
Total (no. of patients)	37	37

RT-PCR – Reverse transcriptase polymerase chain reaction

Table 2: Outcome of IgM assay on confirmed negative cases of Lassa fever using RT-PCR

Type of test	Positive (+ve)	Negative (–ve)	Total (no. of patients)
RT-PCR	0	35	35
IgM	8	27	35

RT-PCR – Reverse transcriptase polymerase chain reaction

positive predictive values of the IgM serological assay were 63% and 72%, while the overall efficiency of the test was 67%.

DISCUSSION

LF, classified by the National Institute of Allergy and Infectious disease (NIAD) as category "A" pathogen and Biolevel 4 agent, has no predilection for age, gender, or social class as shown by the demographic data above and collaborated with earlier reports.[9,18,20]

Only 57% of confirmed cases of acute Lassa infections were positive for IgM, thus giving a sensitivity of same when used as a screening tool at early phase of LF infection.[9] This value is very close to that recorded in an earlier study[9] titled "Early-onset sensorineural hearing loss in Lassa fever" in which only 60% of the patients were antibody positive for IgM. An early immunosuppression resulting from the disease appears responsible for the depressed production of IgM at the early phase of the infection and, as a result, some patients failed to elaborate IgM at the time of presentation. Current reports by Branco *et al*.,[21] show that the detection of significant level of IgM response to Glycoprotein1 (GP1) and GP2 only become apparent between 11th and 20th day of infection whereas Bausch *et al*.,[12] had earlier reported that acute phase antibodies to LAV only start appearing from 4th-14th day of infection.

Furthermore, tumour necrotic factor(TNF-α) is sometimes elaborated in high volume following LF infection, which induces endothelial damage via apoptosis and also thrombocytopenia, leading to immunosupression.[22,23] A recent immunohistochemical finding also showed that Lassa Virus Nucleo-protein (LASV NP) suppresses the host innate immunity through its exonuclease activity that digests the double-stranded ribonucleic acid (dsRNA) generated during the replication and transcription phases of immune response.[24] This inhibition can result in unchecked viral replication, failure to initiate an adaptive immune response, and increased morbidity and mortality.[25-27]

The sensitivity of 57% as recorded in this study is poor and the low negative predictive value makes serological IgM assay a likely inefficient screening tool for acute LF infection. This fact is also buttressed by the low value recorded for the overall efficiency of the test (67%). The positive predictive value though higher than the negative predictive value still falls below acceptable norms, given that roughly one out of every three patients will be false positive and thus a candidate for unnecessary treatment for LF.

In view of the fact that a single undetected and untreated case in the community is considered an "epidemic" given the infection transmissibility and fatality rate, a test with a high level of diagnostic sensitivity and specificity is highly desirable.

The fatality rate is placed at about 55% and could be reduced to 5% following the administration of IV Ribavirin within the first week of onset of the symptoms of the disease.[28,29] Beyond this period, survivors are prone to several oto-neurological complications. This underscores the importance of early diagnosis and therapy.

It is also instructive that the IgM assay recorded a high number of false positive results in a LF endemic region like ours. This is a pointer to the need for further studies to elucidate the antibodies that could be showing cross-reactivity with the LF virus IgM antibodies as this could aid in mitigating pitfalls in diagnosis of this disease.

CONCLUSIONS

Following the importance of LF disease, we conclude that the specificity and sensitivity of 57% and 77% for IgM antibody as screening tool for early detection of LF appears weak. Therefore, there is a need for development/use of more reliable "rapid screening kits" for early detection of LF infections since RT-PCR (the gold standard) is not readily available in most centers. In the interim, "high clinical index of suspicion" with or without LAV IgM positivity should warrant urgent referral to equipped hospitals/facilities for confirmation.

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