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Detection of West Nile Virus in Hospitalized Children with Acute Meningitis using Reverse Transcription Loop-Mediated Isothermal Amplification in Khartoum state, Sudan

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ABSTRACT

Background: This study was carried out to detect West Nile virus (WNV) among hospitalized children with acute aseptic meningitis in Khartoum State, Sudan.

Methods: Cerebrospinal fluid specimens were collected from 70 aseptic meningitis patients from Mohammed Alamin Hamid Hospital during the period January to May 2017.

Out of these 70 patients, 41 (59%) were males and 29 (41%) were females with age that ranged between 1 and 24 months.

The collected CSF specimens were subjected to Real time LAMP for detection of WNV RNA.

Results: Out of 70 patients, 14 (20 %) were positive using Real time LAMP to WNV RNA,, 9 from them were males and the other 5 were females.

Conclusions: In Sudan, the existence of WNV in aseptic meningitis patients was confirmed through detection of WNV RNA. Further study using various diagnostic methods should be considered to determine the prevalence of WNV at the national level.

Keywords : West Nile Virus, aseptic meningitis, Real Time RT-LAMP Sudan

I. INTRODUCTION

West Nile virus (WNV) is an arthropod-borne virus that is taxonomically classified within the family *Flaviviridae*, genus *Flavivirus*, and is member of the Japanese encephalitis (JE) virus serocomplex. WNV circulates in natural transmission cycle involving primarily Culex species mosquito and birds, and humans are incidental hosts [(1)]. WN virus may also be spread through blood transfusion and organ transplants [(2,3)]. It is also possible for the virus to spread from an infected mother to her child through breast milk [(4)].

The genetic material of WNV is a positive-sense, single strand of RNA, which is between 11,000 and 12,000 nucleotides long; that encodes seven non structural proteins and three structural proteins [(5)].

WNV infection is usually asymptomatic. Clinical symptoms are present in 20-40% of infected patients

[6,7]. The most common symptoms are fever, headache, and macular rash. In 1 to 150 cases the virus affects central nervous system causing meningitis, encephalitis or flaccid paralysis [8,9].Neuroinvasive disease is fatal in approximately 10% of patients. Old age is a risk factor for the occurrence of West Nile neuroinvasive disease (WNND) and most deaths due to WNV infection occur in elderly patients [10] .However, WNND and death have been also reported in children, in whom the disease seems to be under-diagnosed [11,12] .

Two lineages of WN strains have been identified: viruses from lineage I caused the main recent epidemics with human encephalitis and are distributed worldwide (Africa, India, Europe, Asia, the Middle East, North America), while viruses from lineage II have been identified only in Sub-Saharan Africa [13] .

The present study aimed to identify West Nile virus infection in cases of aseptic meningitis in Khartoum State, Sudan during the period January to May 2017. This was accomplished by subjecting cerebrospinal fluid samples (CSF) taken from aseptic meningitis patient's to Real Time RT-LAMP.

The LAMP assay is a novel approach to nucleic acid amplification that amplifies DNA with high specificity, selectivity, and rapidity under isothermal conditions, thereby obviating the need for a thermal cycler. The LAMP assay originally described by Notomi et al. [14]is based on the principle of autocycling strand displacement DNA synthesis. The reaction is performed by a DNA polymerase with high strand displacement activity and a set of two specially designed inner primers and two outer primers [14]. LAMP is highly specific for the target sequence because of the recognition of the target sequence by six independent sequences in the initial stage and by four independent sequences during the later stages of the LAMP reaction [15].

II. Materials and Methods

Data collection:

Ethical approval was obtained from Ministry of Health for collection and examination of the samples. The collected data included gender, age and date of sample collection

Study period:

A total of 70 CSF specimens were collected from different patient groups from Mohammad Alamin Hamid Hospital during January to May 2017.

Specimen collection:

The samples were collected from patients with aseptic meningitis. Each specimen that met the inclusion criteria of having a negative result for bacterial meningitis was included in this study. CSF samples were collected under aseptic conditions by experienced healthy workers from the arachnoid space using a sterile wide born needle inserted between fourth and fifth lumber vertebra.The CSF was allowed to drip into dry sterile container, then the samples were stored at-80 C until used .

RNA extraction:

Viral RNA was extracted from the CSF using the QIAamp viral RNA Mini kit (Qiagen, Germany) according to manufacturer's instructions.

LAMP PCR

Primers mix that contains six primers (F3, B3, FIB (F1c +F2), BIP (B1c +B2), LF, LB) which were derived from the sequence of the WNV E gene(Reference?). Final concentration of FIP and BIP was 40 pmol each, FLP and BLP was 20 pmol each, F3 and B3 5 pmol each. The sequences of these primers are shown in (Table 1) [16].

LAMP primer preparation mixture

The LAMP primer mixture (PM) included 5ul (F3) ,5ul (B3) ,40ul (FIB) , 40ul (PIP) , 20ul (LF) , 20ul (LB) , ddH2O 70ul ,with total 200ul .

LAMP condition

The reaction was performed in a final volume of 25 µl using LAMP regents (Mast, Reinfeld, Germany)

which contained 12.5 µl 2x LAMP reaction buffer, 1 µl of Bst DNA polymerase, 2 µl primer mix (PM), µl fluorescence dye (FD), 1 µl reverse transcriptase enzyme , 5.5 µl H2O and 2 µl of target RNA. The mixture was incubated in a real-time PCR at 64oC for 60 minutes and visualized the results using FAM channel.

Table 1: Details of oligonucleotide primers used for reverse transcription loop-mediated isothermal amplification of E gene of WestNile virus.

F3	TGGATTTGGTTCTCGAAGG
B3	GGTCAGCACGTTTGTCATT
FIP	TTGGCCGCCTCCATATTCATCATTTTCA GCTGCGTGACTATCATGT
BIP	TGCTATTTGGCTACCGTCAGCGTTTTT GAGCTTCTCCCATGGTCG
Loop F	CATCGATGGTAGGCTTGTC
Loop B	TCTCCACCAAAGCTGCGT

III. Results

The results of RT-LAMP for diagnosis of WNV in CSF collected form hospitalized Children with acute meningitis in Khartoum State are shown in Tables 2, 3 and 4.

Table (1): Frequency of WNV-RNA among children with aseptic meningitis

Test	Positive	Negative	Total tested
RNA_WNV	14(20%)	56(80%)	70

Table (2): Distribution of WNV- RNA according to gender.

Gender	Positive	Negative	Total
Male	9(22%)	32(78%)	41
Female	5(17%)	24(83%)	29

Table (3): Distribution of WNV- RNA according to age group

Age group	positive	Negative	Total
1-12 months	9(20%)	35(80%)	44
13-14 months	5(19%)	21(81%)	26

Discussion:

WNV has recently emerged as a significant viral pathogen, which poses considerable human health risk across the globe. WNV infection can progress to encephalitis, meningitis, and acute flaccid paralysis and, in some cases, especially in aged and immune-compromised patients; WNV infection can be fatal or develop into serious long-term consequence [(17)]. No specific treatment or vaccines are currently available. The main preventive measures are aimed at informing the at-risk human population, reducing exposure to mosquito bites and exclusion of blood donations from donors visiting or living in affected areas [(17)]. Human cases of WNV infection have been reported from several countries since the 1960s, but it appears that the frequency of outbreaks may have accelerated over the past 15 years [(18)]. The difficulty in isolating the virus from clinical specimens has also necessitated the development of rapid and reliable virus detection assay systems[(15)]

Our current study in Sudan is one of the first to report directly-measured rates of WNV-associated with hospitalized children with acute meningitis. The study revealed that WNV is circulating in Sudan, and was detectable in 20% of the patients tested. A study in Iran reported using RT-PCR reported a much lower overall prevalence rate (1.2%)compared to ours (20%) but similar to our findings the infection was more frequent in males than in females [(19)].

On the other hand a study from Egypt study reported that WNV was detected using haemagglutination inhibition test in 14.6% of the children admitted to the hospital with a febrile illness [(20)] .

In Sudan WNV antibodies were documented as early as 1956 in children aged up to 14 years invarious

regions of the Nuba Mountainsbeing 23% in Kadugli, 68% in Um Dorein and 29% in Talodi[21].

Recently, WNV outbreak occurred in 2002 in Ngorban County, in the Nuba Mountains of Sudan that involved 31 children aged up to 12 years [22] .

The results obtained here in indicated the importance of WNV in the etiology of aseptic meningitis of children in Sudan. However these results need to be confirmed using other techniques and nucleotide sequencing. The results should also call for wider surveillance at the national level in order to fully elucidate the true status and epidemiology of WNV viruses in Sudan.

IV. CONCLUSIONS

Existence and incidence of West Nile virus in Sudan were documented through detection of WNV RNA in CSF samples indicating infection among pediatric meningitis patients in Sudan. Moreover, the WNV detection using Real Time RT-LAMP was established. Generally, these findings are useful for further studies since there is little available information about WNV infection in Sudan.

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