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Co-Infection Status of Enterovirus among Hepatitis (A, B, and C) Positive Individual Living within Abuja Internally Displaced Persons Camps

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Abstract

The Enterovirus (EV-71) co-infection among Hepatitis A, B and C virus has become a global co-mortality. This research determined enteroviruses (EV-71) co-infection profile among HAV, HBV and HCV positive individuals within internally displaced persons camps Abuja, Nigeria. A descriptive cross sectional research design was adopted where blood samples from 450 respondents were collected within the three camps and screened for the presence of Hepatitis A, B and C. Positive samples were further assayed for co-infection with Enterovirus-71 using Rapid Strip RT-PCR Amplification of 16sRNA for Enterovirus 71(EV71). The data were analyzed using the SPSS Version 22.1 Software. The results revealed that out of the 450 studied subjects 11 (2.4%) were HAV positive, 42 (9.3%) were HBV positive and 33 (7.3%) were HCV positive. Only 1 (0.2%) subject was found to be co-infected with HAV/EV-71. The prevalence of hepatitis among the studied subjects was found to differ significantly among males and females (P=0.001), among various age groups (P=0.001) and among various occupation (P=0.001). The study reports low rate of Enterovirus-71 among hepatitis (A, B, and C) positive individuals living within Abuja internally displaced persons camps. The study recommends routine screening of internally displaced individuals for the presence of hepatitis virus and EV-71 for early diagnosis and possible adoption of management and control measures that may include vaccination of infected individuals.

Keywords: Co-infection, Enterovirus, Hepatitis, Internally Displaced Persons (IDPs)

INTRODUCTION

Enterovirus is a virus that belongs to the genus of positive-sense single-stranded RNA (+sssRNA) viruses found associated with several human and as well as animal diseases (Nolte *et al.*, 2017). The enteroviruses are named by their mode of infection via the intestine (i.e. enteric which implies intestinal). Diverse serological investigations have differentiated and grouped EV-71 as a human virus of enteroviral serotypes base on their antibody stability test. A lot of antigenic viral particles of enterovirus have been identified among diverse serotypes base on their deduced or nonreversible cross-stability among different viral species as well as the strains (Renaud *et al.*, 2011: WHO, 2021). The enterovirus were basically classified into four major groups base on their mechanisms of actions to their host including; the polioviruses, echoviruse and coxsackie A and B viruses (CA and CB), with an instant realization of overlapping on the biological properties of the viruses within the diverse groups. The recent isolation of enteroviruses from different source force naming system with consecutive numbering from EV68 and the numbering have already reaches 71(EV68, EV69, EV70 and EV71) (Doitsh *et al.*, 2014: Yang *et al.*, 2019; Huang *et al.*, 2021).

Enteroviruses affect millions of people worldwide each year and are often found in respiratory secretions (e.g saliva, sputum, or nasal mucus) and stool of an infected person. Historically, poliomyelitis was the most significant disease caused by an enterovirus, named poliovirus. There are 64 non-polio enteroviruses that can cause disease in humans; 23 coxsackie A viruses, 6 coxsackie B viruses, 28 echoviruses and 5 other enteroviruses. Poliovirus as well as coxsackie and echovirus spread through the fecal-oral route (Poritz *et al.*, 2011; Yang *et al.*, 2019; Huang *et al.*, 2021). Infection can result in a wide variety of symptoms, including those of mild respiratory illness (common cold), hand, and foot and mouth disease. Acute hemorrhagic conjunctivitis, aseptic meningitis, myocarditis, severe neonatal sepsis-like disease, acute flaccid paralysis and the related acute flaccid myelitis (Garcia *et al.*, 2018).

Hepatitis A, B and C have assumed a serious concern in public health especially in Sub-Saharan Africa. The three viral infections are not only endemic in the region they equally share similar routes of transmission such as injection drug use, sexual contact or from mother to child during pregnancy or birth as well as oral route. Furthermore, there are reports suggesting a more rapid progression of viral hepatitis caused by Hepatitis A, B and C viruses to end-stage liver disease and death in infected patients (Nielsen *et al.,* 2020). Hepatitis A virus (HAV), hepatitis B virus (HBV) and hepatitis C virus (HCV) are equally endemic across African continent and are the leading infection among IDPs Camps, Nigeria inclusive (Mohammed and Bekele, 2014; Huang *et al.*, 2021). The prevalence of mono-infection for hepatitis B infection in there general population ranges from 9-39% and more than 7% chronic carrier rate considered hyper endemic. The prevalence rate of HAV in Nigeria IDPs is also considerably high ranging from 5.8-12.3% (Garg *et al.,* 2012). Due to the endemic nature of these viruses in the sub-Saharan region and the shared routes of transmission and co-infections profile of EV-71 to HAV. The prevalence of co-infection varies depending on the population studied and the location of IDPs. Prevalence of HAV, HBV and HCV co-infections among Nigerian prison inmates and some studied IDPs was reported as 2.7% and 0.7%, respectively (Mohammed and Bekele, 2014). The difference in prevalence is thought to be due to the differential efficacies of these viruses to the types of exposures found in the various IDPs and geographical regions (Hussain *et al.,* 2012). Expert guidelines developed in the United States and Europe recommends screening of all persons for infection with HCV, HBV and HAV for appropriate management of those found to be chronically infected (Benhamou, 2018). This research aimed to determine the enteroviruses (EV-71) co-infection status among HAV, HBV and HCV positive individuals within internally displaced persons camps Abuja, Nigeria.

MATERIAL AND METHODS

Study Design

This study adopted a descriptive cross sectional design to assess the level of co-infection among HAV, HBV and HCV positive individual in selected internally displaced persons camps Abuja, Nigeria.

Population

The study population comprised of male and female within the ages of (0-45 years) living in Durumi, New Kuchigoro and Karumajiji IDPs camp Abuja.

Sample Size

There are 40,969 internally displaced persons in Abuja IDP camps. A random sample of adults and Children between the aged of 0-45 years were selected from three selected internally displaced persons camp. According to the international organization for migration (IOM, 2016).

Sample size, $n = \frac{N}{1+(e)^2}$

Where n = corrected sample size, N = population size (40,969) and e = margin of error (0.05)

Therefore $n = \frac{40,969}{1+40969(0.05)^2} = \frac{40969}{02.42} = 400.8$

Four hundred and fifty (450) respondents were recruited for the study with a response rate of 100%.

Sampling Techniques

The study employed multistage sampling technique. All the camps in Abuja were first clustered from which three (3) camps were selected through balloting system. The respondents were finally selected through systematic sampling techniques.

Sample Collection and Transportation

A total of four hundred and fifty (450) blood samples were collected using 1ml volume vacutainer syringes; 150 from the participants of the three camps respectively. The samples were packaged in an ice packed cooler at a temperature of 4^{o}C and was immediately transported to the Department of Microbiology Laboratory Faculty of Sciences, Kaduna State University and stored at -70^{0}C until assayed. The Rapid Diagnostic Test (RDT) kits, vacutainer tubes and syringe, cryo-vials, HAV, HBV and HCV test strips were purchased from Scientific and Medical Stores and used according to manufacturers Protocols (Bristol Scientific).

Assay Procedure for Enterovirus 71 using Rapid Strip

The presence of Enterovirus 71 in the samples was detected according to manufacturer's instructions (Bristol Scientific, UK). The specimen was aclimatize and allowed to come to the temperature of the laboratory. 1 drop of 15µl of each blood serum was placed into a sample dilution tube and thoroughly mixed, 80 µl of each diluted sample was pipetted and dropped on the window of the cassette. The result was read and recorded after 30 minutes of incubation period.

RNA Extraction of Enterovirus 71

The RNA Extraction kit (QIAamp Viral RNA kit) was used. A volume of 0.01 carrier RNA was added and dissolved into the bottle of AVL Buffer and mixed thoroughly by inverting the bottle to avoid foaming. A carrier RNA with 560 µl of AVL Buffer was added into 1.5ml tubes and labeled. The labeled tubes containing 140µl and 560µl of AVL was mixed by a vortex for 15 seconds and incubated at room temperature for 10 minutes. The tube was labeled and spinned for 10 seconds. About 560µl of ethanol (96%) was added to the tube and mixed by pulse-vortexing for 15 seconds. The tube was centrifuged for 10 seconds to separate drops from the inside of the lid. The cap of the tubes was closed and centrifuged at 6,000 x*g* for 1minute. The QIAamp Mini was spinned into a new 2ml collection tube, and the old tube containing the filtrate was discarded. The blood serum sample was centrifuged for 1 minute. The QIAamp Mini was spinned and 60µl of AVL Buffer was added and maintained at room temperature. The cap was closed and incubated at room temperature for 1 minute and centrifuged at 6,000 x*g* for another 1 minute. The QIAamp Mini spinned was discarded. The extracted RNA (25µl) was pipetted and put into new-labeled tubes and stored at -80° C until use.

RT-PCR Amplification of Gene for Enterovirus 71(EV-71)

Table 1: Specific Primers used for VPI Gene of Enterovirus 71 (EV-71).

Gene target	Primer names	Type	Length	Sequences (5' - 3'),	Ampilicon size (bp)	Reference
VP1	EV71 – F1	Forward	18	CCAGACCATCAATTTCCA	300	Tu *et al.*, 2007
	EV71 –R2	Reverse	18	CCTCAAGTTCTCGAAGTT		
	EV71	Forward	17	GTTCTTAACTCACATAGCA	289	Singh *et al.*, 2010
VPI	EV71	Reverse	17	TTGACAAAAACTGAGGGG		

PCR was run using SYBR green containing master mix.

Master Mix:

The 20µl reaction mixture that contained 9µl of master mix was used, 10µl of tough mix and 0.2µl was used for each Enterovirus 71primer of F1 and R1 (all 10 mM) and 7.6µl of distilled water was used and 2µl of RNA template.

All the PCR components (DNA template, DNA polymerase, primer and buffer) are mixed together and are taken through series of 3 major cyclic reactions conducted in a Thermocycler machine of 30 cycles for 30 minutes. The processes involve; ***Denaturation;*** Initial and final denaturation step involves heating the reaction mixture to 94˚C for 30seconds. During this, the double stranded DNA is denatured to single strands due to breakage in weak hydrogen bonds. ***Annealing;*** the reaction temperature is rapidly lowered to 55˚C for 30 seconds. This allows the primers to bind (anneal) to their complementary sequence in the template DNA. ***Elongation;*** Initial and final primer extension was increased to 75˚C for 4 minute, where polymerase enzyme sequentially adds bases to the 3′ each primer, extending the DNA sequence in the 5′ to 3′ direction, the DNA polymerase amplifies and add up to about 1,000 bp/minute respectively (Huang *et al.*, 2017).

Agarose Gel Electrophoresis

After completion of PCR conditions, 2% of agarose gel was used to electrophorise the PCR product on the bases of Sambrook *et al.* (2009). The amplified PCR product was visualized on Ultra-Violet Transilluminator and Gel Documentation System was used to photograph the visualize product the presence or absence of band at 300bp target gene fragment. The residue of reaction mixture was purified using QIA quick PCR purification kit as instructed by manufacturer (Bristol Scientific-Qiagen) base on Vogelstein and Gillespie (2010) protocol

Data Analysis

The collected data were analyzed using Statistical Package for social sciences (SPSS) version20.00. The responses of the respondents were presented with frequency counts and percentages. The Chi-square (χ^2) statistics were used to examine significant association between the demographic values and the disease effect and P value ≤ 0.05 was considered significant.

RESULTS

The results revealed that out of the 450 studied subjects 11 (2.4%) were HAV positive, 42 (9.3%) were HBV positive and 33 (7.3%) were HCV positive (Table 1). Only 1 (0.2%) subject was found to be co-infected with HAV/EV-71 (Table 1).

The prevalence of hepatitis among the studied subjects was found to differ significantly among males and females (P=0.001), among various age groups (P=0.001) and among various occupation (P=0.001)

Table 1: Occurrence of Hepatitis A, B, C and EV-71 Co-Infection among Individuals Residing in IDPs Camps in Abuja

Variable	No. Screened	HAV No (%)	HBV No (%)	HCV No (%)	HAV/EV-71 No (%)	HAB/EV71 No (%)	HCV/EV71 No (%)	P-value
Sex								
Male	210	4 (1.9)	27 (12.9)	15 (7.1)	1 (0.5)	0	0	0.010
Female	240	7 (2.9)	15 (6.3)	18 (7.5)	0	0	0	
Total	**450**	**11 (2.4)**	**42 (9.3)**	**33 (7.3)**	**1 (0.2)**	**0**	**0**	
Age (years)								
0-15	200	1 (0.5)	19 (9.5)	3 (6.5)	0	0	0	0.001
16-30	150	7 (4.7)	13 (8.7)	10 (6.7)	1 (0.7)	0	0	
31-45	100	3 (30.0)	10 (10)	10 (10)	0	0	0	
Total	**450**	**11 (2.4)**	**42 (9.3)**	**33 (7.3)**	**1 (0.2)**	**0**	**0**	
Occupation								
Student	200	4 (2.0)	12 (6.0)	3 (6.5)	1 (0.5)	0	0	0.001
Farmer	100	2 (2.0)	10 (10.0)	10 (10)	0	0	0	
Civil servant	150	5 (3.3)	20 (13.3)	10 (6.7)	0	0	0	
Total	**450**	**11 (2.4)**	**42 (9.3)**	**33 (7.3)**	**1 (0.2)**	**0**	**0**	

Key: HAV=Hepatitis A Virus, HCV=Hepatitis C Virus, HBV= Hepatitis B Virus, EV-71=Enterovirus 71

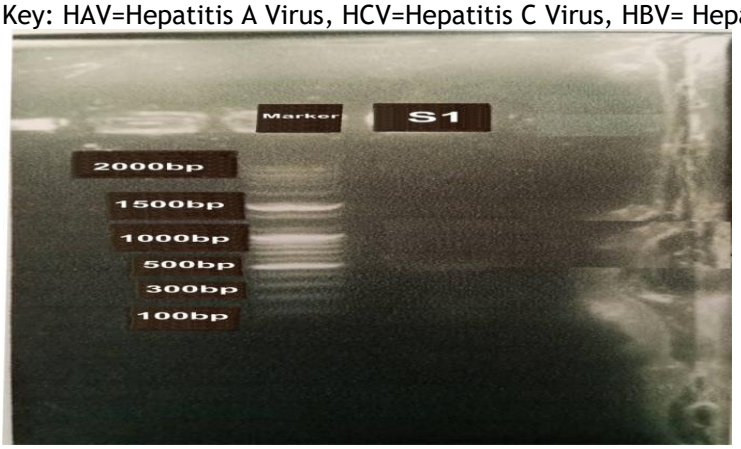

Figure 1: A Negative Gel Electrophoto of EV-71 from HAV Positive Sample.

DISCUSSION

The samples were carefully screened and tested for the positive individual on the IDPs Camps with overall prevalence rates of 19%, where 9.3% and 7.3% positive subjects were screened for HBV and HCV from the whole population with lowest prevalence rate of 2.4% positive individual for HAV. This could be as a result of the vulnerability of the IDPs to the risk factor associated with the cause of viral infection within the camps. The study further detects the co-infection rates in relation to the socio-demographic factors within the internally displaced person's camp. On the bases of sex; male recorded the highest EV-71 co-infection rate among HAV, HBV and HCV infected individual living in the camps as compare to Female with no case of co-infection among HAV, HBV and HCV infected person. This finding is contrary to the work of Yoannes *et al*. (2014) who recorded a higher co-infection rate among females on hepatitis infected persons. The rate of co-infection rate among male may be as a result of their unhygienic practices, lack of portable water and their exposure to an infected individual among different sex within the camps with a statistical difference (P=0.001) between male and female to the hepatitis and co-infection rate.

The rate of co-infection among different age group was observed with the highest EV-71 co-infected individual between the ages of 16-30 among HAV, HBV and HCV positive individuals living in the camps as agreed by the research carried out by Noah *et al*. (2016) who document a highest co-infection rate among HBV and C positive individual.

The highest co-infection rate recorded may be due to the fact that the IDPs within this age group are more likely exposed to the cause of EV71. The lowest co-infection was recorded among HAV, HBV and HCV positive individual between the age of 0-15 and 31-45 with no single EV-71 co-infection. The finding was contrary to the research carried out by Njouom and Tejiokam (2019) who recorded a highest co-infection rate of about 13% of HBV and C among individual of 45 years above; this may be due to the differences in hygienic practice and their level of awareness on the danger of handling contaminated objects among the IDPs living in the camps with a statistical different of (P=0.001) among various age group. Considering the occupation of the study subjects, student recorded the highest EV71 co-infection rate among HAV, HBV and HCV infected individual with civil servant and farmers recording the lowest EV71 co-infection rate among HAV, HBV and HCV infected persons with students co-infected with EV-71 among IDPs infected with HAV. The findings indicated that students are at the high risk of contracting EV71 with the highest number of positive individual. Similar report was documented by UNAIDS (2017); Oti *et al*. (2018) on a research carried out among health workers and opined that the handling of sharp objects and contaminated objects from an infected patient are the root cause of hepatitis infection among the health workers. The highest rate of co-infected individual among students may be as a result of their exposure to infected persons and other factors that predisposes to the infection with a statistical difference of (P=0.001) on different type of hepatitis on the bases of their occupation.

The study further revealed a negative molecular identification of EV-71 using real time-polymerase chain reaction (RT-PCR) on gel electrophoresis at 300 bp fragment as a target band though seropositive for EV-71 among the positive sample on the HAV infected individual, this may be connected to the fact that EV-71 is an RNA virus and are often known for their instability, seroconvertion and contamination during the RNA extraction. This finding is in line with the research documented by Boman *et al*, (2019) who opined that seroconvertion, contamination during the RNA extraction and instability of RNA may lead to a negative result during the molecular identification of EV71. Again, the instability of this RNA virus may be due to sero conversion and heat labile which may be affected by the temperature during the RNA extraction or digestive enzymes as well as the RNA contamination.

CONCLUSION

It's however concluded that the Enterovirus (EV-71) co-infection status investigated in relation to socio-demorgraphic factors was high within Male between the ages of 16-30 who are mostly students with a negative EV-71 gene amplicon among HAV, HBV and HCV positive individuals within the IDPs Camps Abuja. Though, data was reported to show a decrease on the co-infections rates compared to WHO estimations, with a predominance of EV-71/HAV co-infection.

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