Scientia Africana, Vol. 20 (No. 3), December, 2021. Pp 21-30 https://dx.doi.org/10.4314/sa.v20i3.3
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SEROLOGIC EVIDENCE OF EXPOSURE TO HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 AND DENGUE VIRUS AMONG PATIENTS ATTENDING DIAGNOSTIC LABORATORY, LAGOS STATE, NIGERIA

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Received: 23-08-2021
Accepted: 02-11-2021

ABSTRACT

Reportedly, in co-infection, dengue viral (DENV) replication negatively impacts human immunodeficiency virus type 1 (HIV-1). Reports on this or on people having dual HIV-1/DENV are however, scarce in Nigeria, where both HIV-1 and DENV are endemic. As a preliminary investigation in southwest Nigeria, we hypothesized that a high proportion of individuals presented for laboratory tests have serologic evidence of dual HIV-1/DENV infection. This is a cross-sectional Research Institute-based serologic study; patients attending a specific Clinical Diagnostic Laboratory in Lagos State were enrolled. Plasma samples (n=150) of consenting asymptomatic patients were serially screened for evidence of HIV-1 antibodies using DETERMINE™ and UNI-GOLD™ HIV-1/-2 rapid test kits. Only the samples (n=54) confirmed HIV-1 reactive by the latter (and some randomly selected non-reactive samples [n=37]) were tested with ELISA kit for anti-DENV immunoglobulin G (IgG) antibodies. Microsoft Excel and Statistical Package for the Social Sciences were used for statistical analysis. Some participants had detectable HIV-1/DENV antibodies with dual positivity rate of 16.5% [as group-specific point-prevalence rate]. This was independently associated (p=0.04) with marital status as participants who were neither single nor married (categorized as 'others') had more than 11 times (OR: 11.3) likelihood of being dual seropositive. Mono-positivity of HIV-1 antibody among the 91 participants tested for dual HIV-1/DENV seropositivity was 59.3%; corresponding rate for DENV antibody was 20.9%. As per mono-positivity rates, the likelihood of participants testing positive to HIV-1 antibody was more than 21 times (Odds ratio [OR]: 21.3) that of DENV antibody. In conclusion, 15 participants (16.5%) of this specific group had serologic evidence of dual HIV-1/DENV infection; this we considered low given the endemicity of Nigeria for both viruses. As a subpopulation with unusually high HIV-1 mono-positivity; the low DENV mono-positivity might be responsible for the low dual HIV-1/DENV positivity.

Keywords: HIV-1, Dengue virus, dual seropositivity, marital status, Nigeria.

INTRODUCTION

Dengue virus (DENV) has four immunologically related but distinct serotypes, DENV-1 to -4; it is a positive-sense single-stranded RNA virus of the genus *Flavivirus* in the family *Flaviviridae*. Most of the viruses in this genus are transmitted by arthropods (mosquitoes or ticks) and are therefore referred to as arthropod-borne viruses (arboviruses) (Gould and Solomon, 2008). DENVs are the

etiological agents for both dengue fever (DF) and dengue haemorrhagic fever (DHF). They are transmitted primarily by *Aedes* mosquitoes, particularly *Aedes aegypti* (WHO, 2009); these typically bite during the day, commonly in the early hours of the morning and in the evening (WHO, 2012). Other *Aedes* species that transmit DENVs include *A. albopictus*, *A. polynesiensis* and *A. scutellaris* (WHO, 2009). *Aedes* mosquitoes are present in Nigeria (Sule and Oluwayelu, 2016; Chimaeze *et al*., 2018).

There is another virus, human immunodeficiency virus, it is of two types (HIV-1 and-2), that infects humans, but they are not spread by mosquitoes. They are rather transmitted during unprotected sexual intercourse; contact with or transfusion of infected blood / blood products, pre-ejaculate, semen and vagina fluids (Mabuka *et al*., 2012). Non-sexual transmission also occur *in-utero*, during birth and through infected breast milk (Mead, 2008). HIV-1 is a Lentivirus (a group of retroviruses), that more commonly causes HIV infection, and if untreated, leads to acquired immunodeficiency syndrome (AIDS) (Douek *et al*., 2009).

Since dengue is a tropical infection and HIV is prevalent in sub-Saharan Africa, co-infection is expected as previously observed (Joob and Wiwanitkit, 2014; Kharsany and Karim, 2016). While co-infection (DENV type 3/HIV-1) has been reported in Cuba (Gonzalez *et al*., 2009); only one such report (HIV-1 and DENV) is available in Nigeria (Mustapha *et al*, 2017).

The dual or co-infection becomes intriguing as DENV infection transiently inhibited HIV-1 replication (Watt *et al*., 2003). Possibly, DENV infection in HIV-1 positive patients may delay progression to AIDS and might have a protective or beneficial effect in HIV-1 exposed individuals. In similar studies, McLinden *et al.* (2008) and Xiang *et al.* (2009) observed and speculated that the suppressed replication of HIV in HIV-1/DENV co-infected patients could be due to the role of DENV non-structural protein-5 (NS5) that down-regulates expression of HIV-1 co-receptor (CXCR-4) and elevates synthesis of stromal cell-derived factor 1 (SDF-1).

Detection of antibodies to HIV-1 and DENV serves as markers of exposure to the viruses (Qaisar *et al*., 2020). While detectable anti-HIV-1 antibody may not signify protection against HIV; detectable DENV-specific IgG antibody signifies past exposure, seroconversion and protection against DENV. Immunoglobulin M (IgM) antibody to DENV however, indicates recent/ongoing infection. With these assays, seroepidemiologic studies have been widely reported for both viruses. The prevalence rates of Dengue and HIV-1 infections among humans in Nigeria vary from one geographical location to another, and subject to factors such as age, gender, educational background, among others (Nigeria National Agency for the Control of AIDS [NACA], 2012; Awofala and Ogundele, 2018; Sule *et al*., 2019). In a study carried out in Osun State, Nigeria, by Adeleke *et al*. (2016), of the 100 participants screened, 77.0% were sero-positive for DENV IgG antibody. A similar study by Sule *et al*. (2019) observed DENV IgM and IgG positivity rates of 41.6% and 33.7% respectively among undergraduates in Osun State.

Since Nigeria is endemic for both HIV-1 and DENV (Larson *et al*., 2011; Joob and

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Wiwanitkit, 2014; Pang *et al*., 2015; Mustapha *et al.*, 2017; Sule *et al*., 2019), we hypothesized that a high proportion of individuals presenting for laboratory tests have serologic evidence of dual HIV-1/DENV infection. This was also with the view to identifying participants' variables associated with the dual seropositivity.

MATERIALS AND METHODS

Study Area and Population

The Nigerian Institute of Medical Research (NIMR) is located at Muritala Muhammed Way, Yaba, Lagos State, Southwest Nigeria. NIMR is a national research institute established by the Federal Government of Nigeria through the research institute establishment act of 1977, to promote national health and developments. The Institute receives human samples from different parts of Nigeria for research and diagnostic purposes.

Study Design

This is a retrospective cross-sectional study of plasma samples for serologic evidence of exposure to both HIV-1 and DENV. Ethical approval to conduct the study was received from the Health Research Ethics Committee, College of Health Sciences, Osun State University, Osogbo. Study protocols were explained to prospective participants and only consenting participants were enrolled. Relevant socio-demographic and clinical data were collected from participants using interviewer - administered questionnaire forms. Apparently healthy consenting patients attending NIMR Clinical Diagnostic Laboratory were consecutively selected for the study as they were a group of people presenting for some laboratory tests similar to ours (lab tests for HIV-1 and DENV). Criteria for selection included attendance at the Clinical Diagnostic Laboratory Unit of the Institute, being adult and provision of completely filled-in interviewer-administered questionnaire and informed consent forms to participate in the study. Individuals < 10 years of age were excluded.

Sample Size

The size of study participants was determined using established protocol (WHO, 2004; Naing *et al*., 2006) thus: Minimum sample size (n) = ($[pqz^2]/d^2$](using 77.0% DENV antibody prevalence rate reported in Osun State, southwest Nigeria [Adeleke *et al*., 2016]). This was approximately 272 samples; however, due to logistics, 150 plasma samples were collected for the study.

Blood Sample Collection, Plasma Preparation and Serology

Blood samples (about 5ml) were aseptically collected by venipuncture from each participant into EDTA-treated sample tubes. After about 25 minutes, the blood samples were spun at 3,500 revolution per minute for 5 minutes with a centrifuge. The plasma from each sample was dispensed into correspondingly labelled new cryovial tube, stored at -20ºC until tested for the presence of HIV-1 and DENV-specific antibodies.

In a serial testing algorithm and as per manufacturers' instructions, 150 plasma samples were first screened for the presence of anti-HIV-1 antibodies using DETERMINE^{TM} HIV-1/-2 rapid test kit. The plasma samples reactive to the latter were tested with UNI-GOLD^{TM} HIV-1-2 rapid test kit. Some plasma samples that were non-reactive (6 of them) to

DETERMINE™ test kit were randomly selected and included in the UNI-GOLD™ rapid kit testing. With this HIV testing algorithm, plasma samples correspondingly reactive to both rapid test kits were acceptably confirmed seropositive for HIV (Tegbaru *et al*., 2004; FMoH, 2016; Oguh *et al*., 2021). The confirmed seropositive plasma samples and some randomly selected negative samples (54 and 37 samples, respectively) were thereafter tested for the presence of anti-DENV IgG antibody using ELISA kit [DIA.PRO Diagnostic Bioprobes S.r.l].

Data Analysis

The results of this study were presented with descriptive statistics (mean and proportion). Association between variables of participants and dual HIV-1/DENV seropositivity rate was analysed using Chi-squared test and binary logistic regression. Statistical analysis was done using Microsoft Excel and Statistical Package for the Social Sciences (SPSS) software (version 21.0). Statistical association or difference was indicated by $p \leq 0.05$.

RESULTS

Demographic Data of the Participants

Out of the 91 participants tested for dual positivity, 33 (36.3%) were males while 58 (63.7%) were females. Participants within the age group of 15-49 years were 71 while participants within the age group of 50-74 years were 20. Most of the study participants were within the age group of 15-49 years (78.0%).

Result of DETERMINE™ Rapid HIV-1/2 Test

Of the total 150 plasma samples tested for presence of HIV-1 antibody, 54 were reactive, giving a DETERMINE™ -specific prevalence rate of 36.0%. However, with respect to the 91 samples tested for presence of both HIV-1 and DENV antibodies, the prevalence rate of HIV-1 antibody was 59.3%.

Result of UNI-GOLD™ Rapid HIV-1/2 Test

Of the 54 DETERMINE™ -reactive and the 6 randomly selected non-reactive samples (i.e. 60 plasma samples) tested for presence of HIV-1 antibody, the corresponding 54 (90%) reactive to DETERMINE™ were found reactive to UNI-GOLD™, while the 6 (10%) non-reactive still turned out non-reactive.

Concordance Rates between DETERMINE™ and UNI-GOLD™ HIV Test kits

Plasma samples that were reactive with DETERMINE™ Rapid HIV-1/2 test kit were correspondingly reactive to UNI-GOLD™ Rapid HIV-1/2 test kit. The 6 samples negative to DETERMINE™ Rapid kit were also found negative to UNI-GOLD™ HIV-1/2 Rapid kit. These results therefore showed a 100% concordance rate between the results of the two rapid test kits.

Results of DENV IgG Antibody ELISA

Of the 91 plasma samples tested for anti-DENV IgG antibodies, 19 (20.9%) were sero-positive while 72 (79.1%) were sero-negative. Table 1 shows the inter-relationship between results of HIV-1 antibody and DENV IgG ELISA. Of the 91 participants screened, 15 (16.5%) were positive for both DENV IgG and HIV-1 antibody.

Scientia Africana, Vol. 20 (No. 3), December, 2021. Pp 21-30 *https://dx.doi.org/10.4314/sa.v20i3.3*
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Table 1: Inter-relationship between Results of HIV-1 Antibody and DENV IgG ELISA among the 91 study participants

DENV IgG Test Results	HIV-1 Antibody Test Result	
	No. Positive (%)	No. Negative (%)
No. Positive (%)	15 (16.5)	4 (4.4)
No. Negative (%)	39 (42.9)	33 (36.3)

Prevalence Rates of HIV-1 Antibody, Dengue Virus IgG Antibody and Dual HIV-1/DENV Seropositivity in Relation to Age of the Participants

Of the participants within the age group of 15-49 years, 40 (56.3%) were positive for HIV-1, 14 (19.7%) were positive for DENV IgG and 11 (15.5%) had dual Positivity for HIV-1/DENV Antibody. In addition, of the participants within the age group of 50-74 years, 14 (70.0%) were positive for HIV-1, 5 (25.0%) were positive for DENV IgG and 4 (20.0%) had dual Positivity for HIV-1/DENV Antibody.

Mono-positivity of HIV-1 Antibody and DENV IgG, and Dual Positivity of HIV-1/DENV Antibodies among the Study Participants

Of the 91 participants tested for dual HIV-1/DENV antibodies, 59.3% and 20.9 % were respectively positive for HIV-1 and DENV antibodies. From the single positivity rates, the likelihood of participants testing positive to HIV-1 antibody was more than 21 times (Odds ratio [OR]: 21.3) that of DENV antibody (Data not shown).

The dual positivity of HIV-1/DENV antibody was 16.5% (15/91) (Tables 1 and 2). As shown in Table 2, the prevalence rates of dual HIV-1/DENV seropositivity was higher among female participants (20.7%). Only marital status however, showed independent association ($p = 0.04$) with dual seropositivity with participants in 'others' category having more than 11 times (Odds Ratio: 11.3) higher likelihood of being dual seropositive compared to 'singles' (Table 2). The remaining variables showed no statistical association with dual HIV-1/DENV seropositivity.

Table 2: Prevalence Rates of Dual HIV-1/DENV Seropositivity according to subgroups among the Study Participants

Variables	No. of participants tested	No. dual positive for HIV-1/DENV Antibodies (%)	p-value
Age Range (years)			
15-49	71	11(15.5)	
50-74	20	4 (20.0)	0.63
Gender			
male	33	3 (9.1)	
female	58	12 (20.7)	0.15
Educational Status			
primary	3	0 (0.0)	
secondary	40	9 (22.5)	
tertiary	48	6 (12.5)	invalid*
Marital Status			
single	36	2 (5.5)	1
married	50	11 (22.0)	0.05
others	5	2 (40.0)	0.04
Ever Received Blood Transfusion?			
yes	8	1 (12.5)	
no	83	14 (16.9)	0.75
Vaccination against Yellow Fever			
yes	13	1 (7.7)	
no	78	14 (17.9)	0.36
Presently Experiencing Fever?			
yes	9	3 (33.3)	
no	82	12 (14.6)	0.15
Presently Experiencing Headache			
yes	22	4 (18.2)	
no	69	11 (15.9)	0.81
Presently Experiencing Skin Rash			
yes	6	1 (16.7)	
no	85	14 (16.5)	0.99
Presently Experiencing Joint Pains			
yes	37	7 (18.9)	
no	54	8 (14.8)	0.60
Presently Experiencing Muscle Pains			
yes	25	6 (24.0)	
no	66	9 (13.6)	0.23

* = statistical analysis was invalid due to zero value for primary school variable

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DISCUSSION

With the previous observations that DENV infection negatively impacts HIV-1 infection (Watt *et al*., 2003; McLinden *et al.* 2008; Xiang *et al.*, 2009); and since only one study had documented DENV infection in HIV-1 patients in Nigeria (Mustapha *et al.*, 2017), we preliminarily sought to establish dual infection of humans with both viruses in southwest Nigeria. Contrary to studying the general population, we purposively enrolled patients visiting the Clinical Diagnostic Laboratory for lab tests in NIMR, Lagos State, Nigeria.

Clearly, we observed that 16.5% of the 91 plasma samples tested for dual anti-HIV-1/DENV antibodies were positive. Since Nigeria does not use HIV-1 and DENV vaccines, the detectable antibodies could only be from natural past exposures to the viruses. Though the sample size was rather low, we considered the group-specific dual point-prevalence rate surprisingly low among the study participants in Nigeria, a tropical country where HIV-1 and dengue viral infections are endemic (Larson *et al*., 2011; Joob and Wiwanitkit, 2014; Pang *et al*., 2015; Mustapha *et al.*, 2017; Sule *et al*., 2019). The only study in Nigeria by Mustapha et al. (2017) observed dual positivity of 44.4% which is more than twice our observation. Possible explanations for this could be that they enrolled confirmed HIV-infected with larger sample size. In addition, since mono-positivity of HIV-1 antibody among the participants was unusually high, the low dual seropositivity in our study might be due to the low mono-positive rate of dengue virus IgG.

We studied possible influences of some variables of the participants on the dual positivity; only marital status had independent association with the positivity; the reason for "others" (Table 2) having significantly higher dual positivity could be explained from the fact that "others" in this study refers to the divorced, widows and widowers who were more likely to have multiple sexual partners (a risk factor for HIV-1 positivity) (Dunkel *et al*., 2008; Tenkorang, 2014). In addition, the very small sample size of that sub-group (n=5 for "others" [Table 2]) might be responsible, a future study with larger sample size might be more revealing.

We observed that mono-positivity for HIV-1 antibody was unusually high (36.0% for n=150 and 59.3% for the n=91); this would need to be interpreted with caution and might not be extrapolated to State's or National HIV prevalence rate as our study focused on a specific group of individuals who though, apparently healthy, were visiting the Clinical Diagnostic Lab for some lab tests with the view to detecting evidence of exposure to HIV and dengue viruses. Only study of HIV-1 in antenatal care women or a study like the Nigeria HIV/AIDS Indicator and Impact Survey [NAIIS], 2019 would appropriately extrapolate results to general population regarding HIV epidemiology. As recent studies have documented co-infection of the two viruses with observations that the duo has not been extensively studied (Wiwanitkit, 2017; Delgado-Enciso *et al*., 2017; Hottz *et al*., 2019). Our study results will be useful for future interrogation of inhibitory effects of DENV replication on HIV-1 in co-infection with the two viral pathogens in southwest Nigeria.

Regarding dengue virus, the mono-positivity (dengue virus IgG) was relatively

low (20.9%) compared to reports of Adeleke *et al*. (2016) and Sule *et al*. (2019) in Osun State, a Southwestern state like Lagos State, Nigeria. It was however, comparable to 23.4% prevalence rate reported for DENV IgM by Onoja *et al*. (2016) in Ibadan, Oyo State, Nigeria. Possible reason for the low DENV antibody positivity among the study participants could not be immediately discerned as Nigeria is endemic for dengue with high prevalence rates (Adeleke *et al.,* 2016; Mustapha *et al.,* 2017; Sule *et al*., 2019).

A limitation of our study might be that it was Clinical Diagnostic laboratory-based with relatively low sample size.

In conclusion, this study documents dual HIV-1/ DENV infection among a specific group of people in Lagos State, Nigeria; the dual seropositivity was unexpectedly low for a country endemic for both viral pathogens.

Acknowledgements

The sample collection/storage assistance of staff members in Clinical Diagnostic laboratory, Nigerian Institute of Medical Research (NIMR); and the material provision by laboratory technologists in the Department of Microbiology, Osun State University, Osogbo are acknowledged.

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