

Survey of malaria and anti-dengue virus IgG among febrile HIV-infected patients attending a tertiary hospital in Abuja, Nigeria

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Background: Dengue and malaria are infections, of great public health concern, especially in sub-Saharan Africa where the burden of HIV infection is high. This study was conducted to determine the seroprevalence of dengue virus IgG antibodies and dengue/malaria coinfection among febrile HIV-infected patients attending the University of Abuja Teaching Hospital, Gwagwalada, Abuja.

Methods: In this cross-sectional study, blood samples from 178 consenting HIV-infected patients receiving antiretroviral therapy were collected and tested for plasmodiasis and anti-Dengue virus IgG using malaria microscopy and ELISA, respectively. Interviewer-based questionnaires were used to assess subjects' sociodemographic variables and dengue risk factors.

Results: Of the 178 screened participants, 44.4% were seropositive for dengue virus IgG antibody, whereas 29.2% were positive for *Plasmodium falciparum*. About 44.2% were positive for both dengue virus and *P. falciparum*. There was a statistical association between anti-dengue IgG and occupation ($p=0.03$) but not with age, residential area, educational level and patients' gender ($p>0.05$). Seroprevalence of anti-dengue specific IgG was relatively higher in participants who adopted protective measures. There was a statistical association between seroprevalence of anti-dengue IgG and adoption of preventive measures ($p<0.05$).

Conclusion: The high prevalence of malaria and dengue virus IgG indicates the need to strengthen vector control and dengue surveillance programs.

Keywords: coinfection, seroprevalence, flavivirus, syndemism

Introduction

Dengue is the most common arthropod-borne and neglected tropical viral disease which affects over 2.5 billion people at risk of infection worldwide.¹ Dengue is an infection caused by positive sense, single-stranded RNA viruses of the Flaviviridae family.^{2,3} The dengue virus serotypes 1–4 (DENV 1–4), which are the causative strains in the pathogenesis of dengue,² are transmitted by *Aedes* mosquitoes, mainly by *Aedes aegypti* and *Aedes albopictus*.³ Malaria is another mosquito-borne disease with severe effects in HIV-infected persons. The clinical manifestations of dengue have several similitudes with those of malaria.⁴ In malaria and dengue coinfection cases, they abruptly lead to a syndemic outcome. However, in people living with HIV, milder pathology is noticed.

Dengue is endemic in tropical and subtropical parts of the world where there is high prevalence of HIV infection.² Although several studies have shown varying prevalences in different parts of Nigeria, there is paucity in the number of reported cases in Nigeria due to a dearth of information about the infection by health care

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professionals and lack of prioritization as well as apathy toward investing in dengue research by relevant stakeholders in the public health sector.⁵

The dengue virus has been considered an important flavivirus that exhibits a protective role against HIV by transiently inhibiting its replication. Coincidentally, all four serotypes of DENV circulate in Nigeria. DENV and HIV coinfection would be an important factor for delayed progression to AIS. In DENV infection, responses by the immune system result in protection against the virus and pathogenesis of the severe form of the disease.⁶ Several cytokines (tumor necrosis factor, IL-1, IL-21, interferon) in innate immunity, produced by CD4+ T cells, have been associated with the pathogenesis of DENV infection, which is suggestive of their protective role in the acute stage of secondary DENV infection and the convalescence stage of primary DENV infection. Immunity against DENV infection is conferred by activating innate and adaptive immune cells, chemotaxis and inhibition of viral replication.⁶ However, high levels of cytokine response also lead to apoptosis and necrosis, endothelial activation, vascular leak and shock.

According to Gonzalez et al,⁷ patients with DENV-HIV coinfection are likely to have acute clinical illnesses with no increase in dengue hemorrhagic fever (DHF) and dengue shock syndrome. Watt et al⁸ suggested that DENV could attribute to a protective role against HIV-1 by transiently inhibiting its replication during acute DENV infection. McLinden et al⁹ and Xiang et al¹⁰ speculated that the suppressed replication of HIV in HIV-DENV-coinfecting patients could be due to the role of the DENV NS5 protein downregulating the HIV coreceptor (CXCR4) expression and elevated synthesis of stromal cell-derived factor 1 (SDF-1). Nevertheless, these cases cannot be used to conclude that there will not be an increased risk of severe disease in DENV-HIV-coinfecting patients.

Dengue fever is clinically difficult to diagnose, especially in developing countries with no established dengue diagnostic reagents/equipment, and could easily be mistaken for malaria, typhoid or pyrexia of unknown origin due to frequent nonspecific illnesses exhibited by HIV-infected individuals.⁴ However, there is paucity of research on the clinical presentations and outcomes of dengue infection in HIV-infected persons. This study was conducted to determine the seroprevalence of dengue virus IgG antibodies and dengue/malaria coinfection among febrile HIV-infected patients attending a tertiary hospital, Gwagwalada, Abuja.

Materials and methods

Study area and design

This was a cross-sectional study conducted at the University of Abuja Teaching Hospital (UATH), Gwagwalada, Federal Capital Territory (FCT)-Abuja, Nigeria. Gwagwalada is about 45 km from the FCT. It is one of the six area council headquarters of the FCT. The town lies downstream of River Usuma and is located between latitudes 8°55' and 9°00'N and longitudes 7°00' and 7°05'E.

Informed consent and ethical approval

Ethical approval (FCT/UATH/HREC/PR/571) was obtained from the ethical research committee of the University of Abuja Teaching Hospital, Gwagwalada, FCT-Abuja. For the study, we enrolled patients that provided their written consent to voluntarily participate, were sero-positive for HIV and on antiretroviral therapy and presented with febrile illnesses regardless of onset and duration of illness. We excluded patients who were nonfebrile, critically ill, seronegative for HIV or seropositive for HIV and not on antiretroviral therapy drugs.

Study population

A total of 178 HIV-infected patients seeking medical assistance at the UATH were enrolled in the study and were designated test subjects. The selection of all participants was conducted by physicians and nursing staff of the PEPFAR (President's Emergency Plan for AIDS Relief) clinic, UATH, Abuja. Informed consent was obtained from all participants in accordance with the standards of human experimentation and with the Helsinki Declaration of 1975 as adopted by the World Medical Association.¹¹

Prior to this study, there has never been a similar study in Nigeria; hence, the sample size was determined using the equation derived by Naing et al,¹² and the prevalence was estimated by using the 2012 national HIV prevalence rate, 3.4%.¹³ Therefore, the minimum sample size at 95% confidence level was 51. However, in order to enhance the statistical reliability of the study, the sample size was increased to 178 participants.

Methods

Sample collection and storage

A sample of 3 mL of blood was collected from each participant using a standard venepuncture procedure. The blood was carefully and gently dispensed into sterile, plain, sample containers. The tubes were labelled appropriately with the participants'

identification numbers. Sera from these blood samples were separated by allowing the blood to clot at room temperature before centrifuging at 2500 rpm for 10 min. Thereafter, the retracted sera were dispensed into serum aliquot containers and stored at -10°C pending laboratory analyses.

Detection of malaria parasite and speciation

Thick and thin blood films were made, stained with 10% Giemsa and microscopically examined to detect malaria parasites and identify their species, respectively, as described by McKenzie et al.¹⁴

Determination of IgG antibodies against dengue virus

The IgG to dengue fever virus was detected in the serum using commercial ELISA kits (Anti-Dengue virus EuroimmunTM IgG ELISA Kits, Luebeck, Germany [Catalog Number EQ-266a-9601 G]). The assay was performed based on the manufacturer's instructions.

Result interpretation

Samples with ratios of ≥ 1.1 in relation to the optical density of standard 2 of the kit were reported as positive, while negative samples were those with ratios < 0.8 in relation to the optical density of standard 2.

Data analysis

Results generated from analysis and data obtained from the questionnaire were analyzed using the Statistical Package for Social Sciences (SPSS) version 20 (IBM Corporation, Armonk, NY, USA). The chi-square test was used to determine the level of significance in the occurrence of malaria parasitemia and dengue virus IgG in association with categorized variables at a CI of 95%. *p*-values were reported to be statistically significant at < 0.05 .

Results

Out of 178 samples studied, 79 (prevalence of 44.4%) were seropositive for dengue virus IgG and 23 of the 79 (prevalence of 44.2%) were coinfecting with dengue virus and malaria parasites (Table 1). The prevalence of malaria in this study is 29.2%. All malaria parasites were *P. falciparum*.

Based on demographic factors, 51 of the 79 positive cases for dengue virus IgG were female participants with a prevalence of 49.0% (51/104), which was higher than that of their male counterparts with a seroprevalence of 37.8% (28/74). Seropositivity was observed to increase from participants who were within the age range of 21–30 years (36.8%: 21/57) compared to participants who were ≥ 50 years of age

Table 1 Seroprevalence of dengue virus IgG in association with demographic factors among patients attending UATH, Gwagwalada, FCT-Abuja, Nigeria

Sociodemographic variable	No of subjects tested	No of subjects with anti-DENV IgG positive (%)	<i>p</i> -value
Age range (years)			
≤ 10	21	8 (38.1)	0.536
11–20	40	20 (50.0)	
21–30	57	21 (36.8)	
31–40	28	12 (42.9)	
41–50	22	12 (54.5)	
> 50	10	6 (60.0)	
Gender			
Male	74	28 (37.8)	0.138
Female	104	51 (49.0)	
Residence			
Village	60	26 (43.3)	0.841
Suburb	118	53 (44.9)	
Education			
Primary	37	11 (29.7)	0.152
Secondary	45	21 (46.7)	
Tertiary	40	17 (42.5)	
No formal education	56	30 (53.7)	
Occupation			
Farmer	10	9 (90.0)	0.030*
Driver	18	7 (38.9)	
Housewife	35	16 (45.7)	
Civil servant	68	31 (45.6)	
Unemployment	47	16 (34.0)	

Note: *Significant for $p < 0.05$.

Abbreviations: DENV, dengue virus serotypes; FCT, Federal Capital Territory; UATH, University of Abuja Teaching Hospital.

(60.0%: 6/10). Regarding occupation, IgG to dengue virus was detected in the highest prevalence of 90.0% (9/10) among farmers, while the lowest prevalence of 34.0% (16/47) was recorded among the unemployed participants. Unlike other risk factors, statistically significant differences were observed between occupation and the presence of IgG to dengue virus ($\chi^2=10.75$; $df=4$, $p=0.030$) (Table 2).

Based on risk factors, 53 out of 138 participants who resided close to refuse dump sites or water collection spots were positive for IgG to dengue virus with a prevalence of 38.4%, while 26 out of 40 participants who resided within a hygienic zone of beyond 500 m from the waste dump sites were positive for IgG to dengue virus with a prevalence of 65.0%. There was a statistically significant difference between the proximity to waste dump sites and the presence of IgG to dengue virus ($\chi^2=8.89$; $df=1$, $p=0.003$) (Table 3). Data generated from the prevention of water exposure indicated that a greater proportion of participants who consistently covered their household water containers were positive for IgG to

Table 2 Seroprevalence of dengue virus IgG in association with risk factors among patients attending UATH, Gwagwalada, FCT-Abuja, Nigeria

Risk factors	No of subject tested	No of subjects with anti-DENV IgG positive (%)	p-value
Residence in proximity to refuse dumpsite or water collection spots			
No	40	26 (65.0)	0.003*
Yes	138	53 (38.4)	
Consistent covering of household water containers			
No	154	66 (42.9)	0.300
Yes	24	13 (54.2)	
Frequent wearing of long sleeve shirts and trousers			
No	132	52 (39.4)	0.023*
Yes	46	27 (58.7)	
Indoor mosquito repellents			
No	148	62 (41.9)	0.137
Yes	30	17 (71.6)	
Frequent use of indoor insecticide sprays			
No	149	61 (40.9)	0.036*
Yes	29	18 (62.1)	

Note: *Significant for $p < 0.05$.

Abbreviations: DENV, dengue virus serotypes ; FCT, Federal Capital Territory; UATH, University of Abuja Teaching Hospital.

Table 3 Coinfection of dengue virus and malaria in the study population

Malaria status	Anti-dengue virus IgG	
	No of subjects tested	Subjects positive (%)
Negative	126	56 (44.4)
Positive	52	23 (44.2)
Total	178	79 (44.4)

dengue virus with a prevalence of 54.2%, while those who did not cover their household water containers were 42.9%. There was no statistically significant difference between the consistent covering of water containers and the presence of IgG to dengue virus ($X^2=1.08$; $df=1$, $p=0.300$). In terms of the use of protective clothing, IgG to dengue virus was relatively higher in participants who frequently wore long sleeves and trousers with a prevalence of 58.7% compared to those who less frequently used such clothing with a prevalence of 39.4%. There was a statistically significant difference between the frequent wearing of long sleeves with trousers and the presence of IgG to dengue virus ($X^2=5.15$; $df=1$, $p=0.023$). The level of IgG to dengue virus was also observed as relatively higher in participants who were exposed to indoor mosquito repellents with a prevalence of 71.6% compared to those that are not exposed with a prevalence of 41.9%. There was no statistically significant difference between exposure to mosquito repellents, water containers and the presence of IgG to

dengue virus ($X^2=2.206$; $df=1$, $p=0.138$). On the frequent use of indoor insecticides, participants who use these chemicals had indications of higher levels of IgG to dengue virus with a prevalence of 62.1% compared to their counterparts who less frequently used these insecticides with a prevalence of 40.9%. There was a statistically significant difference between the frequent use of indoor insecticides and the presence of IgG to dengue virus ($X^2=4.39$; $df=1$, $p=0.036$).

Discussion

Recently, dengue fever infection has been considered an emerging public health problem in several African countries with risk of severe infections.^{15,16} Most febrile cases are routinely diagnosed and treated for typhoid and/or malaria without proper investigation for other conditions including viral infections.

The objectives of this study were to determine the seroprevalence and coinfection rate of dengue virus among febrile and HIV patients recruited at the UATH, Gwagwalada, FCT-Abuja, Nigeria. Despite Nigeria being among the countries with the most cases of new HIV infection and dengue hyper-endemic, there have been no similar studies. This current study reported a prevalence of 44.4% for dengue-specific IgG antibody among participants, which is in accordance with the findings of Sultana et al,¹⁷ who observed 42.9% seroprevalence of anti-dengue IgG in Chittagong, Bangladesh. The seroprevalence from this study is not in conformity with dengue virus-specific IgG seroprevalence of 67.2% by Mahmood et al,¹⁸ 77% by Adeleke et al,¹⁹ 79% by Peyerl-Hoffmann et al²⁰ and 98% by Yamashiro et al.²¹ The observed discrepancies in prevalence reports could be due to the level of awareness of dengue, urbanization and environment deterioration.²² The high circulation of dengue virus in the study area could be attributed to several factors including misdiagnosis of febrile cases, the movement of migrants from endemic countries and the proliferation of breeding sites of *Aedes* mosquitoes.²³ These factors may have enhanced the high circulation of dengue virus in Gwagwalada. The mean total rainfall in this area is ~1650 mm/annum. About 60% of this rain falls between May and August during which samples were collected. The area council is an industrial zone of FCT that stands out as the second most cosmopolitan city of the FCT, after the capital city with 10 political wards, and consists of more than 26 federal organizations, which include the University of Abuja, the UATH, etc. These have brought about the inflow of people into the council. About 75% of Gwagwalada residents live in close proximity to poor drainage system, several potholes on their streets and indiscriminate environmental dumpsites.²⁴ This

often encourages the collection of artificial water containers and, thus, serves as the breeding sites for mosquito vectors of dengue virus infections.

We reported 44.2% co-occurrence of dengue virus IgG antibodies and malaria parasitemia. This value is closely similar to that reported by Adeleke et al¹⁹ who reported a dengue IgG and malaria prevalence of 33% in Osogbo, Southwestern Nigeria; however, this was higher than the seroprevalence of 1.3% by Idoko et al²⁵ in Kaduna; 2.2% by Dawurung et al²⁸ in Jos, Plateau State; and 10.1% by Idris et al²³ in Maiduguri, Northern Nigeria. The difference in dengue virus serology as reported by earlier studies with lower prevalence is due to the IgM to dengue virus that was investigated, unlike the present study that investigated only dengue virus IgG. IgM accounts only for current exposure to dengue infections which dissipate, but not completely, with time. However, IgG accounts for both previous and present exposure to dengue virus and appears from 5 to 7 days of infection. Reinfections with the virus could result to pathological IgG titer and severe symptoms including DHF.²⁷ The high prevalence of malaria and dengue coinfection in this present study is an indication of poor vector control measures against dengue vector in the community.

Regarding the gender-related prevalence of DENV IgG from this study, more females were being infected than their male counterparts. This observation is in consonance with Bello et al³⁰ and Adeleke et al,¹⁹ studies but not with others' studies^{18,20,31,32,33} which reported higher prevalence for dengue infection in males than in females. The disparity in the prevalence for gender could be the relatively large number of females recruited for the study compared to fewer males. The females were exposed more to the risk factors such as being engaged in most domestic activities. Since the dengue vector is predominantly indoor biting mosquitoes,²⁹ women may be more likely to have a relatively higher prevalence of dengue virus-specific IgG.

There was no association between age and dengue infection, which was in conformity with the report of previous studies.^{4,30} This observation is, however, in contrast to literature by Peyerl-Hoffmann et al²⁰ and Reiskind et al,³⁴ who reported an age-dependent increase of anti-dengue antibodies in participants with risk of infections. The prevalence of dengue virus IgG from this study has a significant association with the occupation of participants. Farmers appeared to the group with the highest cases of DENV IgG seropositivity. This agrees with the findings of Bello et al²⁸ and Oladipo et al,³⁵ which indicates that farming activities are predisposed to dengue virus infection as a result of sylvatic-to-urban spillover.

Findings from this study indicated a higher prevalence of dengue virus IgG seropositivity in study participants who adopted various protective measures against the vector bites compared to those who did not adhere to such protective measures. In contrast to these observations on the use of protective measures such as using indoor insecticide sprays in preventing the spread of dengue virus, Mahmood et al¹⁸ reported a higher dengue burden in participants who never used indoor anti-mosquito sprays compared to those who frequently used such sprays, and their findings indicated no significant association between anti-dengue seropositivity and frequency in the use of these insecticides. Unlike the previous study that recruited apparently healthy adult participants, our study involved the study of immunocompromised participants. Viral infections, generally, suppress the natural immunity of the affected individual, which often predisposes the individual to opportunistic infections.²⁶ Therefore, coinfection with dengue virus, HIV, and malaria as observed in this study could be very devastating to the affected participants who are immunosuppressed, thus lacking the ability to mount immune response by producing sufficient serum levels of anti-dengue IgG in the presence of increasing pathogenic burden.

Of the 178 subjects tested, 23 (44.2%) were malaria patients with anti-dengue IgG positivity. This finding was higher than those of Idoko et al²⁵ and Baba et al²⁶ who reported 1.3% and 5.8% malaria/dengue coinfection in Kaduna and Maiduguri cities of Nigeria, respectively. The higher rates of malaria/dengue coinfection from this study could be due to differences in sample size and design, type of laboratory test used, difference in climatic conditions and in malaria and dengue endemicity of these cities. This finding is not surprising, because *Anopheles* and *Aedes* mosquitoes have severally been reported to be in existence in Abuja;²⁷ therefore, the presence of dengue and malaria in this study is expected. This result is very important because Nigeria is one of the few African countries that limit the investigation of febrile illnesses to malaria and typhoid with complete neglect to viral infections. In general, viral infections suppress the natural immunity of the host, and this often allows opportunistic infections.²⁸ Therefore, a coinfection of dengue and malaria as observed in this study could be very devastating to HIV-infected persons. Sustainable vector control should be the paramount technical strategy for malaria and dengue prevention and control. Considering the hyperendemicity of both infections in Nigeria, prevention of mosquito breeding sites and/or bites should be emphasized at all community levels.

Conclusion

There exist statistical associations between dengue IgG seropositivity and proximity to waste dump sites, wearing protective clothing and frequent use of indoor insecticide sprays. Our findings provide the baseline data of the level of dengue virus circulating in the study population. These observations seek a prompt and adequate response from the individuals, community and government policy makers to adopt preventive and control measures that can mitigate the resultant effects of morbidity and mortality resulting from dengue burden especially in immunosuppressed individuals.

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Disclosure

The authors report no conflicts of interest in this work.

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