Scientific African 17 (2022) e01356

Contents lists available at ScienceDirect

Scientific African

journal homepage: www.elsevier.com/locate/sciaf

Dengue virus among HIV-infected pregnant women attending antenatal care in Luanda, Angola: An emerging public health concern

Cruz S. Sebastião[a,b,c,d], Zoraima Neto[b], Domingos Jandondo[b], Marinela Mirandela[b], Joana Morais[b,e], Miguel Brito[a,f,*]

[a] Centro de Investigação em Saúde de Angola (CISA), Caxito, Angola
[b] Instituto Nacional de Investigação em Saúde (INIS), Luanda, Angola
[c] Instituto Superior de Ciências da Saúde (ISCISA), Universidade Agostinho Neto (UAN), Luanda, Angola
[d] Programa de Pós-Graduação Ciência para o Desenvolvimento (PGCD), IGC, Lisboa, Portugal
[e] Faculdade de Medicina (FM), Universidade Agostinho Neto (UAN), Luanda, Angola
[f] Health and Technology Research Center (H&TRC), Escola Superior de Tecnologia da Saúde de Lisboa (EST&SL), Instituto Politécnico de Lisboa (IPL), Lisboa, Portugal

ARTICLE INFO

Article history:
Received 16 February 2022
Revised 15 August 2022
Accepted 30 August 2022

Editor: DR B Gyampoh

Keywords:
Dengue virus
HIV
Seroprevalence
Pregnant women
Angola

ABSTRACT

The dissemination of the dengue virus (DENV) in endemic regions with HIV is a public health concern with greater importance when there is evidence of vertical transmission of DENV during pregnancy. Herein, we investigated DENV among HIV-infected pregnant women in Luanda, the capital city of Angola. This was part of a cross-sectional study carried out on 42 pregnant women newly diagnosed with HIV. A total of 36 plasma samples from the 42 HIV-positive pregnant women were screened for DENV using RT-PCR and ELISA. None of the specimens tested positive for DENV by RT-PCR. Regarding seroprevalence, 94.4% of the samples were positive for IgG and 11.1% for IgM. Recent infection (IgG-/IgM+ or IgG+/IgM+) was detected in 11.1% of the samples and past infection (IgG+/IgM-) in 83.3%. The risk of recent infection was higher in pregnant women over 25 years of age [OR: 13.0 (95% CI: 1.14–148), $p = 0.039$]. Our study showed laboratory evidence of a recent DENV infection among HIV-infected pregnant women attending antenatal care in Luanda. Our findings provide critical data regarding DENV infection among HIV-infected pregnant women in Luanda. Future studies involving a larger sample size of HIV-infected pregnant women are necessary to support ongoing public health programs to combat arboviruses in Angola.

* Corresponding author.
E-mail address: miguel.brito@estesl.ipl.pt (M. Brito).

https://doi.org/10.1016/j.sciaf.2022.e01356

Introduction

Dengue is a mosquito-borne viral infectious disease caused by the dengue virus (DENV) transmitted to humans through the *Aedes* mosquitoes. It is endemic in more than 100 tropical and subtropical countries in Southeast Asia, the Americas, the western Pacific, Africa, and the eastern Mediterranean regions [1]. An estimated 10,000 deaths and 390 million DENV infections occur each year [2–4]. Phylogenetic analysis indicated that the DENV lineage circulating in Angola, since 1968, has American-African origins, and strongly suggested that DENV is endemic in Angola [5]. During the years 2013 and 2018, Angola experienced a DENV outbreak that was concentrated in Luanda province, the capital city of Angola [5,6]. A large proportion of the population of Luanda lives in slums and tenement housing [7]. In addition, the Luanda province is frequently visited by international business travelers mostly because of the oil trade [7].

Many infectious viruses such as DENV, Zika virus (ZIKV), and Chikungunya virus (CHIKV), coexist in tropical and subtropical regions. This circulation of infectious agents has contributed to the high rates of morbidity and mortality in high-risk populations such as pregnant women. The determinants of the pathogenesis of DENV infection among human immunodeficiency virus (HIV) infected patients are not fully understood [8–11]. Previous studies showed that DENV infection during pregnancy is associated with vertical transmission, miscarriage, and maternal mortality [11–15].

Limited published studies have assessed DENV infection prevalence among HIV-infected pregnant women, as well as the consequences for their infants in Angola [6,16,17]. In the present study, we aimed to investigate the seroprevalence of DENV infection among HIV-infected pregnant women attending antenatal care in Luanda province. Our results could support public health programs for the prevention and elimination of the risk factors of adverse pregnancy outcomes in HIV-infected pregnant women from endemic areas with DENV in Angola.

Materials and methods

Study design and setting

The current study was part of a cross-sectional study carried out on 42 pregnant women newly diagnosed with HIV regardless of the gestational period. All the patients were attending antenatal care at the Lucrecia Paim Maternity hospital from April to June 2018, in Luanda province, the capital city of Angola. The Lucrecia Paim Maternity is a tertiary health unit and a reference center for research, training, and antenatal care for pregnant women from all provinces of Angola. The HIV-infected pregnant women were interviewed after signing written informed consent. A structured questionnaire was administered to obtain personal demographic characteristics such as age, local residence, level of education, occupation, and gestational period.

Sample collection and molecular testing

Plasma samples separated from a 5 mL venous blood sample obtained from each participant were stored at −80 °C until further analysis. The total viral ribonucleic acid (RNA) was manually extracted from 140μL of plasma samples using the QIAamp Viral RNA kit (QIAGEN, Germany) following the manufacturer's instructions. The HIV infection was confirmed by nested polymerase chain reaction (PCR) using the protocol described previously [18]. The presence of the DENV RNA was screened using real-time reverse-transcription polymerase chain reaction (RT-PCR) with the Applied Biosystems 7500 Fast RT-PCR System (Thermo Fisher Scientific). The Centers for Disease Control and Prevention (CDC) Trioplex real-time RT-PCR assay was used [19,20]. Briefly, the RT-PCR was carried out using 10µL of the RNA in a final reaction volume of 25µL containing primers and dual-labelled hydrolysis (TaqMan®) probes targeting *in vitro* qualitative detection of the DENV, ZIKV, and CHIKV. Cycling conditions for the RT-PCR consisted of 30 min at 50 °C followed by 45 cycles of 2 min each at 95 °C, 15 s at 95 °C, and 1 min at 60 °C. The fluorescence capture was set to detect light emissions by the DENV, ZIKV, and CHIKV through the fluorescent dye FAM, Texas Red, and VIC, respectively. The amplification curves were evaluated individually for each target virus and the threshold line was placed above the beginning of the exponential phase of the curve. Positive and negative control samples were included. The results of RT-PCR were considered valid when positive control samples showed a cycle threshold (CT) value below 31. Specimens with CT values below 31 were considered positive while specimens with CT values equal to or above 31 were considered negative.

Serological testing

Commercially available indirect enzyme-linked immunosorbent (ELISA) assay was used to screen the presence of immunoglobulin M (IgM) (EUROIMMUN, Germany) and immunoglobulin G (IgG) (EUROIMMUN, Germany) antibodies against DENV following the manufacturer's instructions. Briefly, 100μL of 1:100 diluted plasma samples were added to wells coated with anti-human IgM or IgG and incubated at 37 °C. Afterward, the wells were washed three times and incubated at room temperature (RT) with 100µL of the conjugate. Then, the wells were again washed three times and incubated at RT in the dark with 100μL of the substrate. Finally, 100μL of the stop solution was added and the plate was read ten minutes later. The absorbance value was measured at 450 and 620 nm. Positive and negative control were included in each ELISA assay. A positive ELISA result was defined as an absorbance twice that of the negative control according to the manufacturer's

instructions. The serological results of DENV infection were grouped as follows: no infection (IgG-/IgM-), past infection (IgG+/IgM-), and recent infection (IgG-/IgM+ or IgG+/IgM+). The molecular and serological testing were performed at the molecular biology laboratory of the Instituto Nacional de Investigação em Saúde (INIS), in Luanda, Angola.

Data description

The sociodemographic and clinical data were coded and analyzed using the Statistical Package for the Social Sciences (SPSS) version 25 for windows (IBM SPSS Statistics, USA). The descriptive analysis was presented as frequencies and percentages. Normally distributed data were presented as mean and standard deviation. The patient demographics were categorized and dichotomized as follows: age (<25 years old *vs.* ≥25 years old), local residence (urbanized area *vs.* rural area), level of education (pregnant women illiterate and with primary education were categorized as a low educational level while pregnant women with secondary or tertiary education levels were categorized as high educational level), occupation (unemployed *vs.* employed), and gestational period (first trimester *vs.* second or third trimester). A Chi-square test and logistic regression analysis were carried out with all explanatory variables to check the potential interactions between patient demographics and DENV infection in HIV-infected pregnant women. The odds ratio (OR) and their 95% confidence intervals (CIs) were calculated to determine the strength and direction of the interaction between variables. The reported p-value are two-tailed and deemed statistically significant when $p<0.05$.

Ethics approval

The study protocol was reviewed and ethical approval was obtained from the Ethics Committee of Angola (nr.13/2018) and the general directorate from Lucrecia Paim Maternity hospital (nr.083/GDG/MLP/2018). All HIV-infected pregnant women undergoing antenatal care examination were invited and consent to participate was secured from each participant or legal guardians.

Results

Between April to June 2018, a period of a DENV outbreak in Luanda province, 42 HIV-infected pregnant women were enrolled in our study. The mean age of HIV-infected pregnant women was 28±6 years old, ranging from 14 to 42 years old. The majority of pregnant women was in the age group ≥25 years (78.6%, 33/42), living in rural area (52.4%, 22/42), with low educational level (85.7%, 36/42), and unemployed (64.3%, 27/42). When the pregnant women were enrolled, 9 (21.4%) were in the first trimester of gestation, 11 (26.2%) in the second trimester of gestation, and 22 (52.4%) in the third trimester of gestation.

DENV infection among HIV-infected pregnant women

The results of nested-PCR confirmed HIV infection in all specimens collected. CT values of 30.2, 24.0, and 26.6 were obtained from RT-PCR in the positive control samples used for target DENV, ZIKV, and CHIKV, respectively. None of the 42 samples subjected to RT-PCR tested positive for DENV, ZIKV, or CHIKV. The presence of IgG and IgM antibodies against DENV infection could only be screened in 36 plasma samples out of 42 specimens collected due to inadequate plasma samples. The demographic characterization and seroprevalence of DENV infection among HIV-infected pregnant women enrolled in this study are shown in Table 1. The overall seroprevalence of DENV infection detected by ELISA among HIV-infected pregnant women was 94.4%. A total of 94.4% (34/36) and 11.1% (4/36) of plasma samples were IgG and IgM-positive, respectively. Recent infection (IgG-/IgM+ or IgG+/IgM+) was detected in 11.1% (4/36) of the plasma samples whereas non-infection (IgG-/IgM-) and past infection (IgG+/IgM-) were detected in 5.6% (2/36) and 83.3% (30/36), respectively (Table 1).

Determinants of DENV infection among HIV-infected pregnant women

Significant differences were observed between recent infection and age groups, being more frequent in the under 25 years old age class (Table 1). Non-infection and past infection were significantly associated with the gestational period ($p<0.05$). On the other hand, there were no significant differences between IgG or IgM seropositive by place of residence, educational level, and occupation (Table 1). The chances of recent infection were higher in HIV-infected pregnant women younger than 25 years old [OR: 13.0 (95% CI: 1.14–148), $p = 0.039$] (Table 2). On the other hand, despite not being statistical significance all recent DENV infections were observed in pregnant women with low educational levels and unemployed (Table 1). Moreover, a putative chance related to DENV infection has been observed in low educational levels and the unemployed pregnant women, and pregnant women living in rural areas (Table 2).

Discussion

Although DENV and HIV infections are endemic and major public health problems in Angola, there is a lack of published studies assessing the seroprevalence of DENV fever in HIV-infected patients in Angola [6,16,17]. To the best of our knowledge, this seroprevalence study is the first description of DENV infection among HIV-infected pregnant women in Luanda

Table 1
Seroprevalence and determinants of DENV infection among HIV-infected pregnant women attending antenatal care in Luanda, Angola, 2018.

Patient demographics	No. of pregnant women (%)	Non-infection (IgG-/IgM-)			Past infection (IgG+/IgM-)			Recent infection (IgG-/IgM+ or IgG+/IgM+)		
		No (%)	Yes (%)	p-value	No (%)	Yes (%)	p-value	No (%)	Yes (%)	p-value
Overall	36 (100)	34 (94.4)	2 (5.6)		6 (16.7)	30 (83.3)		32 (88.9)	4 (11.1)	
Age groups										
<25 years	9 (25.0)	9 (100)	0 (0.0)	0.401	3 (33.3)	6 (66.7)	0.121	6 (66.7)	3 (33.3)	0.014*
≥25 years	27 (75.0)	25 (92.6)	2 (7.4)		3 (11.1)	24 (88.9)		26 (96.3)	1 (3.7)	
Place of residence										
Urban area	18 (50.0)	18 (100)	0 (0.0)	0.146	1 (5.6)	17 (94.4)	0.074	17 (94.4)	1 (5.6)	0.289
Rural area	18 (50.0)	16 (88.9)	2 (11.1)		5 (27.8)	13 (72.2)		15 (83.3)	3 (16.7)	
Educational level										
Low	33 (91.7)	31 (93.9)	2 (6.1)	0.661	6 (18.2)	27 (81.8)	0.418	29 (87.9)	4 (12.1)	0.522
High	3 (8.3)	3 (100)	0 (0.0)		0 (0.0)	3 (100)		3 (100)	0 (0.0)	
Occupation										
Unemployed	25 (69.4)	24 (96.0)	1 (4.0)	0.539	5 (20.0)	20 (80.0)	0.418	21 (84.0)	4 (16.0)	0.159
Employed	11 (30.6)	10 (90.9)	1 (9.1)		1 (9.1)	10 (90.9)		11 (100)	0 (0.0)	
Gestational period										
First trimestrer	8 (22.2)	6 (75.0)	2 (25.0)	0.006*	4 (50.0)	4 (50.0)	0.004*	6 (75.0)	2 (25.0)	0.156
Second or third trimestrer	28 (77.8)	28 (100)	0 (0.0)		2 (7.1)	26 (92.9)		26 (92.9)	2 (7.1)	

* The variables were statistically significant for the Chi-square test ($p<0.05$).

Table 2
Determinants of recent DENV infection among HIV-infected pregnant women attending antenatal care in Luanda, Angola, 2018.

Patient demographics	Univariate analysis		Multivariate analysis†	
	OR (95% CI)	p-value	AOR (95% CI)	p-value
Age groups				
<25 years old	13.0 (1.14–148)	**0.039**	10.0 (0.56–178)	0.117
≥25 years old	1.00	–	1.00	–
Place of residence				
Urban area	0.29 (0.03–3.14)	0.311	0.27 (0.02–4.79)	0.374
Rural area	1.00	–	1.00	–
Educational level				
Low	1.00	–	1.00	–
High	0 (0.0–0.0)	0.999	0 (0.0–0.0)	0.999
Occupation				
Unemployed	1.00	–	1.00	–
Employed	0 (0.0–0.0)	0.999	0 (0.0–0.0)	0.999
Gestational period				
First trimester	4.3 (0.50–37.3)	0.182	7.38 (0.43–126)	0.167
Second or third trimester	1.00	–	1.00	–

Abbreviations: OR, odds ratio; CI, confidence interval; AOR, adjusted odds ratio.
† Adjusted for all the explanatory variables listed.

province. In this analysis of DENV infection in HIV-infected pregnant women during the recent DENV outbreak in the year 2018, the overall seropositivity was 94.4%. Almost all HIV-infected pregnant women (83.3%, 30/36) had laboratory evidence of past DENV infection whereas 4/36 (11.1%, 95%CI 3.1–26.1)had recent DENV infection (Table 1). The recent DENV outbreak in Luanda could have contributed to the high seropositivity found in this studied population [6].

The negative RT-PCR viral DENV results observed in IgM positive samples (4/36) in our study, could be ascribed to the fact that IgM antibodies could still be detectable 7 days after the of onset of symptoms as well as two to three months after viral exposure. On the other hand, the DENV viremia period is short (about five days) indicating that the investigation of recent DENV infection through serological markers (IgM/IgG) may not be sensitive enough in endemic regions with a large number of asymptomatic patients [21].

The high level of DENV IgG positive (94.4%, 34/36) observed in our study, suggests the previous infection by DENV in almost all HIV-infected pregnant women. Moreover, our results also indicated that Luanda province is an endemic region with DENV in Angola with a high risk of spread of infectious diseases transmitted by an arthropod. Similar results of a high prevalence of DENV IgG positivity were also observed in 92% of the HIV-infected pregnant women screened for DENV infection in Brazil [22], whereas different results including a low prevalence of DENV IgG positivity were found in pregnant women from the Democratic Republic of Sao Tome and Principe [23] and China [24]. The unprecedented population growth, uncontrolled urbanization, house-to-house movements of people living in precarious conditions of basic sanitation, international trade, travel of people from countries with active DENV transmission, climate change, breach of public health

infrastructure, and breach of vector control programs, contribute disproportionately to spread of local and imported cases of DENV in Angola [1,25].

The prevalence of recent DENV infection (IgM positive – 11.1%) identified in our study was higher compared to a previous report on pregnant women (2.8 – 10.6%) from Brazil [21,26]. Although adult pregnant women are the population most affected by HIV infection (75.0%, 27/36) (Table 1), a statistically significant highr risk of DENV infection was observed in younger HIV-infected pregnant women [OR: 13.0 (95% CI: 1.14–148), $p = 0.039$] (Table 2). One of the possible explanations for the high likelihood of recent DENV infection among younger pregnant women in Luanda could be attributed to the fact that these young women work or have more outdoor activities, therefore increasing exposure to the *Aedes* mosquito bite that causes DENV infection. These results are not surprising since 91.7% and 69.4% of the screened HIV-infected pregnant women had a lower academic level and were unemployed, respectively (Table 1).

A previous study showed an increase in incidence, hospitalization and severity of DENV infection among younger patients over the past ten years indicating a possible change in the population at risk of infection, from adults to younger people [27]. This epidemiological change and the high risk of recent DENV infection in younger patients observed in our study could be explained by the fact that adults develop immune responses to all dengue serotypes over time making younger people more susceptible to DENV infection. However, these changes should serve as a warning to Angolan health authorities to provide timely health care for younger patients. We also cannot exclude the possibility that DENV infection leads to increased risk of miscarriage or even maternal mortality, and in older age groups these events could be more frequent, leading to a reduction in successful pregnancies. Indeed, other studies have shown a higher risk of miscarriage [14] or higher mortality [15] with DENV infection during pregnancy.

Although no statistical significance was observed, the majority of recent DENV infections $n = 3$ (75.0%, 95%CI 10.4–99.4), as well as the risk of recent infection, were identified among HIV-infected pregnant women living in rural areas of Luanda. On the other hand, the multivariate logistic regression analysis showed that although not statiscally significant, risk of recent DENV infection was 7.38 times (95% CI: 0.43 – 126) higher among HIV-infected pregnant women in their first trimester of gestation compared to pregnant women in their second or third trimester of gestation (Table 2). Our findings and the recent evidence of vertical transmission, miscarriage, and maternal mortality of DENV infection during pregnancy [12–15], indicate that continuous surveillance which includes differential screening for the acute febrile syndrome among pregnant women to prevent adverse effects and vertical transmission of DENV infection in the non-urbanized regions should be considered mainly during periods of increased viral circulation in endemic areas from Angola.

Our study has some potential limitations. The limited representativeness of the studied population diminishes the generalization of our results and might not be sufficient to support public health programs on the risk factors of vertical transmission and adverse pregnancy outcomes of DENV infection among HIV-infected pregnant women in Luanda. The prevalence of DENV infection in non-pregnant populations has not been investigated, and we suggest further studies in this approach. We are therefore unsure whether the observed prevalence in pregnant women varies significantly from that observed in the general population. The specific non-structural proteins (*e.g.*, NS1) that indicate recent DENV infection, as well as the possibility of cross-reactivity between assays based on the detection of antibodies against different flaviviruses such as ZIKV, a flavivirus closely related to DENV, were not evaluated [28]. Despite these limitations, our results highlight the concern about the risk of asymptomatic DENV infection during pregnancy suggesting that the Angolan Ministry of Health should consider the possibility of implementing screening and monitoring programs for DENV infections in HIV-infected pregnant women in the future, especially in endemic areas for DENV in Angola (*e.g.*, provinces of Luanda and Cuanza Norte). Future large-scale prospective studies with extensive laboratory testing of DENV infection in HIV-infected pregnant women are necessary, to obtain epidemiological and clinical data that could elucidate the effect of asymptomatic or severe DENV infection on the population of HIV-infected pregnant women in endemic areas of Angola.

Conclusion

The present study showed a high seroprevalence of DENV infection with laboratory evidence of a recent DENV infection among HIV-infected pregnant women attending antenatal care in Luanda province. This study provides critical data regarding the seroprevalence of DENV infection among HIV-infected pregnant women in Luanda. Future studies involving a larger sample size of HIV-infected pregnant women are necessary to support ongoing public health programs to combat arboviruses in endemic areas of Angola.

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' contributions

Study design and conceptualization of study: MB, JM, and CSS. Sample collection: CSS. Laboratory procedure: CSS, ZN, DJ, and MM. Data analysis: MB and CSS. Writing—original draft preparation: CSS. Writing—review and editing: MB, JM, and CSS. All authors approved the final version of the published manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We are grateful to the HIV-infected pregnant women for their participation, as well as the institutional support of INIS, CISA, ISCISA/Agostinho Neto University, and Lucrecia Paim Maternity. A Ph.D. scholarship from the Programa de Pós-Graduação Ciência para o Desenvolvimento (PGCD) and the Fundação para a Ciência e a Tecnologia (FCT), Portugal, was awarded to CSS (SFRH/BD/135296/2017).

References

[1] M.G. Guzman, Harris E. Dengue, Lancet 385 (2015) 453–465, doi:10.1016/S0140-6736(14)60572-9.
[2] S. Bhatt, P.W. Gething, O.J. Brady, J.P. Messina, A.W. Farlow, C.L. Moyes, et al., The global distribution and burden of dengue, Nature 496 (2013) 504–507, doi:10.1038/nature12060.
[3] J.P. Messina, O.J. Brady, N. Golding, M.U.G. Kraemer, G.R.W. Wint, S.E. Ray, et al., The current and future global distribution and population at risk of dengue, Nat. Microbiol. 4 (2019) 1508–1515, doi:10.1038/s41564-019-0476-8.
[4] S.T. Ogunlade, M.T. Meehan, A.I. Adekunle, D.P. Rojas, O.A. Adegboye, E.S. McBryde, A review: aedes-borne arboviral infections, controls and wolbachia-based strategies, Vaccines 9 (2021) 1–23, doi:10.3390/VACCINES9010032.
[5] Ministry of Health of Angola, Ongoing dengue epidemic — Angola, 2013, MMWR 62 (2013) 504–507, doi:10.1016/j.annemergmed.2013.10.002.
[6] de S.C. Hill, J.N. Vasconcelos, B.G. Granja, J. Thézé, D. Jandondo, Z. Neto, et al., Early genomic detection of cosmopolitan genotype of dengue virus serotype 2, Angola, 2018, Emerg. Infect. Dis. 25 (2019) 2017–2020.
[7] National Institute of StatisticsResultados Definitivos Do Recenseamento Geral Da População e Da Habitação De Angola - 2014, INE, Luanda, Angola, 2016.
[8] G. Watt, P. Kantipong, K. Jongsakul, Decrease in human immunodeficiency virus type 1 load during acute dengue fever, Clin. Infect. Dis. 36 (2003) 1067–1069, doi:10.1086/374600.
[9] M. Montoya, L. Gresh, J.C. Mercado, K.L. Williams, M.J. Vargas, G. Gutierrez, et al., Symptomatic versus inapparent outcome in repeat dengue virus infections is influenced by the time interval between infections and study year, PLoS Negl. Trop. Dis. 7 (2013) 1–10, doi:10.1371/journal.pntd.0002357.
[10] T.P. Endy, K.B. Anderson, A. Nisalak, I.K. Yoon, S. Green, A.L. Rothman, et al., Determinants of inapparent and symptomatic dengue infection in a prospective study of primary school children in Kamphaeng Phet, Thailand, PLoS Negl. Trop. Dis. 5 (2011), doi:10.1371/journal.pntd.0000975.
[11] A. Torrentes-Carvalho, E.D. Hottz, C.F. Marinho, J.B.C. da Silva, L.M. de Oliveira Pinto, L.G. Fialho, et al., Characterization of clinical and immunological features in patients coinfected with dengue virus and HIV, Clin. Immunol. 164 (2016) 95–105, doi:10.1016/j.clim.2016.01.005.
[12] X. Yin, X. Zhong, s. Pan, Case report vertical transmission of dengue infection: the first putative case reported in China, Rev. Inst. Med. Trop. Sao Paulo (2016) 14–17.
[13] S.L.C. Maroun, R.C.C. Marliere, R.C. Barcellus, C.N. Barbosa, J.R.M. Ramos, M.E.L. Moreira, Case report: vertical dengue infection, J. Pediatr. 84 (2008) 556–559, doi:10.2223/JPED.1826.
[14] P.C. Tan, M.Z. Soe, K. Lay, S.M. Wang, S. de Sekaran, S.Z. Omar, Dengue infection and miscarriage: a prospective case control study, PLoS Negl Trop Dis 6 (2012), doi:10.1371/journal.pntd.0001637.
[15] K.A. Sondo, A. Ouattara, E.A. Diendéré, I. Diallo, J. Zoungrana, G. Zémané, et al., Dengue infection during pregnancy in Burkina Faso: a cross-sectional study, BMC Infect. Dis. (2019) 997, doi:10.1186/s12879-019-4587-x.
[16] Z. Neto, P.A. Martinez, S.C. Hill, D. Jandondo, J. Thézé, M. Mirandela, et al., Molecular and genomic investigation of an urban outbreak of dengue virus serotype 2 in Angola, 2017–2019. Beasley DWC, editor, PLoS Negl Trop Dis 16 (2022) e0010255, doi:10.1371/journal.pntd.0010255.
[17] C.S. Sebastião, C. Gaston, J.P. Paixão, E.N.M. Sacomboio, Z. Neto, J.N. de Vasconcelos, et al., Coinfection between SARS-CoV-2 and vector-borne diseases in Luanda, Angola, J. Med. Virol. (2021), doi:10.1002/jmv.27354.
[18] C.S. Sebastião, Z. Neto, Jesus CS De, M. Mirandela, D. Jandondo, J.C. Couto-Fernandez, et al., Genetic diversity and drug resistance of HIV-1 among infected pregnant women newly diagnosed in Luanda, Angola. PLoS One. (2019) 1–10, doi:10.1371/journal.pone.0225251.
[19] G.A. Santiago, J. Vázquez, S. Courtney, K.Y. Matías, L.E. Andersen, C. Colón, et al., Performance of the Trioplex real-time RT-PCR assay for detection of Zika, dengue, and chikungunya viruses, Nat. Commun. 9 (2018), doi:10.1038/s41467-018-03772-1.
[20] Centers for Disease Control and Prevention. Trioplex Real-time RT-PCR Assay. CDC. 2017; 57.
[21] A.F.L.T. Argolo, V.C.R. Féres, L.A. Silveira, A.C.M. Oliveira, L.A. Pereira, J.B.S. Júnior, et al., Prevalence and incidence of dengue virus and antibody placental transfer during late pregnancy in central Brazil, BMC Infect. Dis. 13 (2013), doi:10.1186/1471-2334-13-254.
[22] E.C. João, C. Ferreira O da, M.I. Gouvêa, L.B. Teixeira M de, A. Tanuri, L.M. Higa, et al., Pregnant women co-infected with HIV and zika: outcomes and birth defects in infants according to maternal symptomatology, PLoS One 13 (2018) 1–12, doi:10.1371/journal.pone.0200168.
[23] T.Y. Yen, M. Trovoada dos Santos, J. de, L.F. Tseng, S.F. Chang, C.F. Cheng, A.V. Carvalho, A. de, et al., Seroprevalence of antibodies against dengue virus among pregnant women in the Democratic Republic of Sao Tome and Principe, Acta Trop. 155 (2016) 58–62, doi:10.1016/j.actatropica.2015.12.012.
[24] L. Liu, J. Huang, M. Zhong, K. Yuan, Y. Chen, Seroprevalence of Dengue Virus among Pregnant Women in Guangdong, China. Viral Immunol. 33 (2020) 48–53, doi:10.1089/vim.2019.0046.
[25] S.T. Stoddard, B.M. Forshey, A.C. Morrison, V.A. Paz-Soldan, G.M. Vazquez-Prokopec, H. Astete, et al., House-to-house human movement drives dengue virus transmission, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 994–999, doi:10.1073/pnas.1213349110.
[26] R.C. Leite, A.I. Souza, P.M.S. Castanha, M.T. Cordeiro, C.T. Martelli, A.L.G. Ferreira, et al., Dengue infection in pregnancy and transplacental transfer of anti-dengue antibodies in Northeast, Brazil, J. Clin. Virol. 60 (2014) 16–21, doi:10.1016/j.jcv.2014.02.009.
[27] M.G. Teixeira, M.C.N. Costa, G. Coelho, M.L. Barreto, Recent shift in age pattern of dengue hemorrhagic fever, Brazil. Emerg. Infect. Dis. 14 (2008) 1663, doi:10.3201/eid1410.071164.
[28] B. Rönnberg, Å. Gustafsson, O. Vapalahti, P. Emmerich, Å. Lundkvist, J. Schmidt-Chanasit, et al., Compensating for cross-reactions using avidity and computation in a suspension multiplex immunoassay for serotyping of Zika *versus* other flavivirus infections, Med. Microbiol. Immunol. 206 (2017) 383–401, doi:10.1007/s00430-017-0517-y.