Lyana Lima ORCID iD: 0000-0001-5150-7803

Vanessa de Paula ORCID iD: 0000-0002-6314-754X

Seroprevalence of human Alphaherpesvirus 1 and 2 among pregnant women infected or uninfected with Zika virus from Rio de Janeiro, Brazil.

Lyana Rodrigues Pinto Lima[1], Juliana de Sousa dos Santos Pereira[2], Nathalia Alves Araújo de Almeida[1], Marcelo Damião Ferreira de Meneses[2], Shirlei Ferreira Aguiar[3], Carlos A S Fernandes[3], Renata Campos Azevedo[2], Vanessa Salete de Paula[1].

Affiliation:

1. Laboratory of Molecular Virology Molecular, Oswaldo Cruz Institution, Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, Brazil.
2. Department of Virology. Institution of Microbiology Paulo de Góes, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil;

3. Central Laboratory of Public Heath Noel Nutels-LACEN-RJ, Rio de Janeiro, Brazil

***Corresponding author:** Vanessa Salete de Paula / address: Av. Brasil 4365, Pavilhão Helio Peggy Pereira, Manguinhos - Rio de Janeiro, Brazil. Email: vdepaula@ioc.fiocruz.br /Tel: +55 (21) 2562-1823.

Running head: Seroprevalence of HSV and ZIKV among pregnant women

Abstract

Pregnant women are an important group to be monitored for infection due to the risk of transmitting infections to their babies. Both HSV and ZIKV are neurotrophic viruses that can be transmitted congenitally. In this study, the prevalence and risk factors of HSV among Zika-positive and -negative pregnant women from Rio de Janeiro, Brazil were evaluated and compared. About 167 serum samples included in our study were from pregnant women with ZIKV infection symptoms, who were attended in different hospitals in Rio de Janeiro between November, 2015 to February, 2016. Blood samples collected from 167 pregnant women were used for this study. The presence of HSV antibodies and

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viremia were evaluated by commercial ELISA and quantitative real time PCR analyses, respectively. The data obtained from medical records were statistically analysed. The HSV-1 and HSV-2 prevalence among pregnant women was 80.2 and 12.5% for Zika-positive women and 84.5 and 5.6% for Zika-negative women, respectively. None of the pregnant women exhibited HSV viremia. Age, trimester of gestation and skin colour were associated with HSV-1 and HSV-2 prevalence among the groups studied. HSV-2 was more prevalent in Zika-positive pregnant women than in Zika-negative pregnant women, and this simultaneous infection should be better investigated in future studies.

KEYWORDS

Zika, HSV, pregnant women, Brazil

1. INTRODUCTION

Zika virus (ZIKV) is an arbovirus of the family *Flaviviridae* that is transmitted by arthropod vectors, primarily members of the *Aedes* genus (e.g., *Ae. aegypti*), and can also be transmitted through bodily fluid contact, blood transfusions, sexual transmission and vertical transmission[1]. First reported in Brazil in May 2015, ZIKV infection has subsequently affected thousands of individuals, initially in the northeast of Brazil and eventually spreading throughout the country[2,3]

In previous outbreaks, ZIKV infection was characterized by a classic clinical pattern, fever, rash, arthralgia and conjunctivitis[4]. However, in ZIKV-infected pregnant women in Brazil, a remarkable 42% of foetuses exhibited some neurological abnormality [5]. Thus, ZIKV-infected pregnant women are a high-risk group due to the ability of this virus to be transmitted via the transplacental route[6], resulting in abortion, stillbirth, or infants being born preterm and/or with congenital Zika syndrome, characterized by microcephaly, cerebral calcifications, ventriculomegaly, and arthrogryposis [7].

Due to the worrying outcome for foetuses associated with Zika maternal infection, the prevalence of this virus has been determined in some studies in Brazil. In a study in Rio de Janeiro, 37.2% of the pregnant women who visited the exanthematic disease unit had a confirmed Zika infection [8]. In a survey of HIV-infected pregnant women, Zika

infection was confirmed in 72% of the cases of recent infections in this group, which resulted in four infants having neurological abnormalities[9].

Other neurotropic viruses exhibiting high incidences in Brazil that can be transmitted via the transplacental route are Alphaherpesvirus 1 and 2 (HSV- 1 and HSV-2, respectively). HSV-1 and HSV-2 are members of the *Herpesviridae f*amily and can be transmitted during sexual practices, leading to genital herpes. Symptomatic genital herpes is characterized by the appearance of macules and papules, followed by vesicles, pustules and ulcers in the genital tract that contain large amounts of replicating viral particles [10]. HSV infection is latent and persistent, and while HSV-2 may be reactivated, leading to recurrent genital lesions or asymptomatic viral shedding, HSV-1 rarely produces a recurrent infection at the site of infection [11].

There are different factors that lead to HSV genital reactivation, one of which is pregnancy. Both HSV and ZIKV can be transmitted congenitally, although this mode of transmission is rare accounts for only 5% of cases [12]. HSV maternal transmission may result in miscarriage, growth restriction, prematurity, neurological abnormalities and neonatal herpes [13].

In Brazil, previous studies showed 94.5% pregnant women were HSV seropositive [14], while HSV-1 prevalence ranged from 16-28% [15,16] and HSV-2 ranged from 12.6-59.7% [15-18]. Neonatal herpes is rare, occurring in an estimated 10 out of every 100,000 births globally, but is a serious condition that can lead to lasting neurologic disability or death[19] (WHO, 2020). So, the higher prevalence rates among pregnant women may represent a risk for congenital transmission.

At present, the mechanism associated with the transplacental transmission of ZIKV and congenital Zika syndrome are unknown. However, one hypothesis involves the coinfection. A recent study involving cultures of human placental cells showed a significant increase in the permissiveness of ZIKV when these cells were previously infected with HSV-2, which may explain the observed teratogenic effects of Zika infection [20]. Based on these findings, the goal of this study was to investigate the presence of HSV-1 and HSV-2

infection among pregnant women who were infected or uninfected with ZIKV and to verify the risks factors associated

through the epidemiological analysis of patients' medical records.

2. MATERIALS AND METHODS

2.1 Ethics and study population

This study was previously approved by the Ethics Committee of Clementino Fraga Filho University Hospital (HUCFF/UFRJ) (number: 80709). A total of 167 samples from pregnant women with zika symptoms: fever, headache, arthragia, myalgia and exanthema were randomly selected to be evaluated in this study. Pregnant women who were attended in different hospitals in metropolitan region of Rio de Janeiro had their blood collected to confirm clinical diagnosis. The period of selection was from November 2015 to February 2016. Samples were from patients of 17 different municipalities in Rio de Janeiro State, being more prevalent in Rio de Janeiro, Duque de Caxias and São Gonçalo municipalities. The majority was resident in urban areas.

2.2 Zika laboratory diagnosis

To confirm the clinical diagnoses, 167 blood samples were sent to LACEN-RJ. These blood samples were centrifuged at 2500 rpm for 15 minutes to obtain the serum samples. Subsequently, viral RNA was extracted from the serum samples using a commercial nucleic acid extraction kit (High Pure Viral Nucleic Acid Kit, Roche Life Science) followed by ZIKV cDNA detection via qRT-PCR using the protocol described by Lanciotti et al.2008 [21]. Some negative-ZIKV samples were tested to Dengue (O Panbio® Dengue IgM Capture ELISA, Abbott and Plateia™ Dengue NS1 Ag, BioRad) and Rubeola (Anti RUBE IgG SYM, Symbiosys)

2.3 Serological diagnosis of HSV-1 and HSV-2

To evaluate the immune status with respect to HSV infection, all serum samples were tested using a commercial immunoassay to detect IgG against HSV-2 (Biokit®

Bioelisa HSV-2 IgG, Werfen Company, Barcelona, Spain) and HSV-1 (Anti HSV-1 IgG, Euroimmun, Luebeck, Germany) to evaluate the past infection. All serum samples testing positive for IgG were tested to detect the presence of IgM against HSV-2 (Ridascreen® HSV-2 IgM, R-Biopharm, Darmstadt, Germany) and HSV-1 (Anti HSV-1 IgM, Euroimmun, Luebeck, Germany) to investigate of recent infection or reactivation. The commercial kits used in this study have sensitivity and specificities greater than 84.5% for HSV-2 IgM; 96% for HSV-2 IgG; 98.6% for HSV-1 IgG and 98.2 for HSV1 IgM.

2.4 HSV-1 and HSV-2 DNA detection

To evaluate the presence HSV-1 and HSV-2 DNA, nucleic extraction was performed on the serum samples using a commercial kit (High Pure Viral Nucleic Acid Kit, Roche®). Subsequently, HSV-1 and HSV-2 DNA was detected via real time PCR using the protocol described by Lima et al. 2017 [22]

2.5 Epidemiological analysis

The data obtained from the medical records of the pregnant women were used for epidemiological analysis (age, skin color, area of residence and trimester of gestation). The adjusted frequency distributions of the variables were calculated with a 95% confidence interval (CI). The difference between the prevalence among the two groups (with and without Zika) was statistically analysed by a chi-squared test. Logistic regression models were used to predict the odds ratio (OR) with a 95% confidence interval. The analysis was performed using the statistical package R studio Desktop, with significance set at $p > 0.05$.

3. RESULTS

3.1 Population characteristics and ZIKV diagnosis

In this study, 167 pregnant women with suspected of Zika infection were recruited. After a qRT-PCR analysis to test for ZIKV, 96 pregnant women were classified as being Zika-positive and 71 were Zika-negative. Thus, the prevalence of ZIKV infection was 57.5% (96/167) among the pregnant women admitted in Rio de Janeiro hospitals.

Regarding the demographic characteristics of the overall population, the median age of the pregnant women was 25.3 years old, 9.6% (6/62) were black, 45.2% (28/62) were white and 45.2% (28/62) were brown. The majority of the population (96.5%) lived in an urban area. With respect to the gestational age, 16% (19/119) were in the 1st trimester, 55.4% (66/119) were in the 2nd trimester and 28.6% (34/119) were in the 3rd trimester (TABLE 1).

3.2 HSV detection

All Zika-positive and -negative pregnant women were assessed for their immune status with respect to HSV. The overall seroprevalence of HSV-1 IgG and HSV-2 IgG was 82% (137/167) and 9.5% (16/167), where 80.2% (77/96) of Zika-positive pregnant women and 84.5% (60/71) of Zika-negative pregnant women tested positive for HSV-1 and 12.5% (12/96) of Zika-positive pregnant women and 5.6% (4/71) of Zika-negative pregnant women tested positive for HSV-2 (TABLE 2).

The presence of IgM was also evaluated in samples that tested positive for HSV-1 and HSV-2 IgG. Only one Zika-positive pregnant woman (1/12) and one Zika-negative pregnant woman (1/4) tested positive for HSV-2 IgM and IgG. None of the pregnant women who tested positive for HSV-1 IgG did so for IgM. All of the pregnant women were tested for the presence of HSV viremia by qPCR, but HSV DNA was not detected in the blood of any individual.

3.3 Statistical analysis

Among the variables analysed, the age of 21-30 years was associated as a risk factor for HSV-1 (p=0.01; OR=1.3 [1.3-10.9] and p<0.001; OR 8.5 [2.4-56.6]) and as a protective factor for HSV-2 (p=0,001; OR= 0.09 [0.01-0.3] and p=0.04; OR=0.05[0.03-0.2]) among both ZIV-positive and -negative pregnant women. White pregnant women (Zika-positive and -negative) were more likely to be seropositive for HSV-1 than black pregnant women (p= 0.02 OR= 3.2 [1.2-9.7] and p=0.05; OR = 1.9 [1.3-4.9]), and HSV-1 was a risk factor associated with Zika-negative pregnant women in the 2nd trimester of gestation (p= 0.02; OR= 9.9 [1.9-18.3]) (TABLE 3 AND TABLE 4). The OR was also used to assess

whether there were associations between HSV-1 and HSV-2 seroprevalence with ZIKV infection, the results of which showed that pregnant women with ZIKV were 2.3-fold (p=0.22; OR =2.3 [0.71-7.48]) more likely to be seropositive for HSV-2 than those without ZIKV, although this result did not show significance.

4. DISCUSSION

In Brazil, HSV-1 and HSV-2 infections are endemic and primarily affect young individuals [23]. Within this population, pregnant women stand out as an important group due to the possibility vertically transmitting an infection to their babies through the congenital route, via peripartum or postpartum transmission. As a result of the Zika epidemic and the consequent increase in cases of microcephaly and neurological alterations in babies born to mothers infected with Zika during pregnancy, different factors have been investigated to identify the mechanisms that lead to the congenital transmission of ZIKV.

In this study, we investigated potential risk factors and the occurrence of HSV infection among Zika-negative and -positive pregnant women admitted in hospitals of Rio de Janeiro. Statistical analysis revealed that pregnant women (Zika-positive and -negative) aged 21-30 years old were more likely to be seropositive for HSV-1 than younger pregnant women, a result that is comparable with those of recent studies describing that increases age is associated with an increased risk of being HSV-1 seropositive [24, 25].The age group of 21-30 years old was also to associated with being HSV-2 positive, but as a protection factor, suggesting that HSV-2 infection is happening early in the sex life of this group.

Epidemiological analysis showed Zika-negative pregnant women in the 2nd trimester were more likely to be seropositive for HSV-1 than pregnant women in the 1st trimester, although these results require further investigation to verify the implications of this factor on the congenital transmission of HSV. Although a white skin colour was identified as a risk factor for HSV-1 prevalence among Zika-positive and -negative pregnant women, we believe that this association is not accurate due to a lack of information in the medical records of a large number of patients.

In this study, HSV-1 seroprevalence was higher for both the Zika-positive (80.2%) and Zika negative (84.5%) pregnant women evaluated compared with that observed in general population of Brazil (67.2%) [23] and for pregnant women from Brazil [15,16] (16 and 28%, respectively), EUA (59.3%) [26] and Finland (45 and 69.5%) [27]. The HSV-2 prevalence among Zika-positive pregnant women was similar (12.5%) to that of the general population of Brazil (11.3%) [23] and was similar to Germany populations (13.3 and 9.6%, respectively)[25] but was lower that detected among pregnant women from Rio de Janeiro, Brazil (20.6-59.7%) [17,18] and from USA (21.1%) [26].

In addition to the HSV-2 prevalence reported in this study being lower than those previously described in Rio de Janeiro, when we compared the HSV-2 prevalence between the two groups studied, it was possible to verify that the prevalence of HSV-2 among pregnant women with ZIKV (12.5%) was twice as high as that observed among pregnant women without ZIKV (5.6%). HSV-1 was also to investigated in the two groups, but there was no significant difference between the prevalence of HSV-1 among Zika-positive (80.2%) and Zika-negative pregnant women (84.5%).

Both ZIKV and HSV-2 can be transplacentally transmitted during pregnancy, and an acute ZIKV infection in a pregnant woman with a latent HSV-2 infection represents a risk of HSV-2 reactivation and as a consequence an active coinfection by HSV-2/ZIKV. Data from a previous study showed that approximately 75% of women with a history of genital herpes caused by HSV-2 and acquired prior to pregnancy have at least one case of recurrence during pregnancy, and 14% develop specific symptoms or lesions prior to delivery [28].

Thus, HSV-2 seropositive samples were assessed for viremia to verify possible episodes of HSV-2 reactivation during acute ZIKV infection among the Zika-positive pregnant women. However, none of the assayed individuals had detectable levels of HSV-2 DNA in the serum at the time of collection, suggesting that there were no reactivation episodes of HSV-2 among the evaluated women. Since this study was cross-sectional and reactivation of HSV-2 can occur at any time, it is important that there is continuous monitoring of HSV-2 infection to assess active coinfection in patients with ZIKV.

In a study performed by Aldo et al. 2016 [20] active ZIKV/HSV-2 coinfection in human trophoblast cells and in an animal, model led to increased placental permissiveness to Zika, allowing transmission of the virus to the foetus. In this study, the prevalence of HSV-1 and HSV-2, viruses with oral and genital transmission routes, were estimated in women with and without Zika infection. Although HSV reactivation was not detected in this study, the prevalence of HSV-2 was higher in women infected with ZIKV than in those who were uninfected, whereas the prevalence of HSV-1 was similar in both groups. Epidemiological investigation revealed that about 45% of pregnant women who experienced zika asymptomatic or symptomatic infection present birth defects [29], suggesting the importance of co-factors to allow virus transborder through the placenta. Pregnancies' outcomes were not accessed in this study, therefore, ZIKV/HSV-2 active coinfection should be further investigated, and pregnant women who are seropositive and have active HSV-2 infections should be monitored during pregnancy to avoid transplacental transmission of both HSV-2 and ZIKA.

Abbreviations

HSV= Herpes Simples Virus

ZIKV – ZIKA virus

OR= Odds ratio

USA= United State of America

DECLARATIONS

Experimental Ethics.

This study was previously approved by Ethics Committee of Clementino Fraga Filho University Hospital (HUCFF/UFRJ) - number 80709. The informed consent was signed for all study's participants and when the participant was under 16 years old, the parent signed the informed consent for him.

Disclosure of Conflicts of Interest.

The authors declare that they have no competing interests

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Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Authors' contributions

LRPL and JSSP performed the laboratory test. NAAA performed the statistical analysis. MDFM, SFA, CASF e RCA collected sérum samples. VSP and RCA projected this study. LRPL wrotten this article

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TABLES

Table 1: Distribution of characteristics among the pregnant women (n=167).

Variable	***N(%)***
Age	
12 – 20 years old	42 (25.1)
21 - 30 years old	86 (51.5)
>31 years old	39 (23.4)
Trimester of gestation	
1	19 (16.0)
2	66 (55.0)
3	34 (28.6)
Skin color	
White	28 (45.2)

Black	6 (9.7)
Brown	28 (45.2)
Area	
Urban	4 (3.5)
Countryside	110 (96.5)
Zika	
Negative	71 (42.5)
Positive	96 (57.5)

Table 2 – Immune status for HSV-1 and HSV-2 IgG into the two groups.

	Prevalence for HSV-1 IgG	**Prevalence for HSV-2 IgG**
Zika positive	80.2% (77/96)	12.5% (12/96)
Zika Negative	84.5% (60/71)	5.6% (4/71)

Table 3: Association of prevalence of HSV-1 and HSV-2 among ZIKV-positive pregnant women (n=96)

Variable	*N(%)*	*No information available*	*HSV1 IgG prevalence(%)*	*X^2(p-valor)*	*OR (IC 95%)*	*HSV2 IgG prevalence(%)*	*X^2(p-valor)*	*OR (IC 95%)*
Age								
12 – 20 ears old	23 (24)		18 (18,8)		1	2 (2,1)		1
21 - 30 years old	50 (52)		40 (41,7)	**0.01**	**1,3 (1,3-10,9)**	6 (6,3)	**0,001**	**0,09 (0,01-0,3)**
>31 years old	23 (24)		19 (19.8)	0,7	0,2 (0,7-0,3)	4 (4,2)		
Trimester of gestation		25						
1	8 (8,5)		6 (6,3)			1 (1)		
2	41 (43,6)		32 (34)	0,8		7 (7,4)	0,06	
3	20 (21,2)		17 (18)	0,5		1 (1)	0,5	
Skin color		56						
Black	2 (2,1)		1 (1)		1	3 (3,1)		
White	21 (22,3)		16 (17)	**0,02**	**3,2 (1,2-9,7)**	1 (1)	0,2	
Brown	15 (15,9		13 (13,8)	0,4	2,0 (0,3-	1 (1)	0,4	

	)				15,8)			
Area		29						
Urban	4 (4,2)		3 (3,1)			0		
Countryside	57 (60)		47 (50)	0.7		5 (5,3)	0,9	

Table 4: Association of prevalence of HSV-1 and HSV-2 among ZIKV-negative pregnant women (n=71)

Variable	*N(%)*	*No information available*	*HSV1 IgG prevalence(%)*	*X^2(p-valor)*	*OR (IC 95%)*	*HSV2 IgG prevalence(%)*	*X^2(p-valor)*	*OR (IC 95%)*
Age								
12 – 20 years old	19 (26)		17 (23,9)		1	1 (1,4)		1
21 - 30 years old	36 (52)		31 (43,6)	**<0,001**	**8,5 (2,4-56,6)**	2 (2,8)	**0,04**	**0,05 (0,03-0,2)**
>31 years old	16 (22)		12 (16,9)	0,2	0,3 (0,4-2,1)	1 (1,4)		
Trimester of gestation		23						
1	11 (15)		10 (13,6)		1	0		
2	25 (34,2		20 (27,3)	**0,02**	**9,9 (1,9-**	3 (4,1)	0,9	

	)				**18,3)**		
3	14 (19,1)		11 (15)	0,4	0,3 (0,1-3,4)	2 (2,7)	0,9
Skin color		49					
Black	4 (5,4)		4 (5,4)		1	0	
White	7 (9,5)		7 (9,5)	**0,05**	**1,9 (1,3-4,9)**	0	
Brown	13 (17,8)		3 (4,1)	0,9	(0,1-1,3)	2 (2,7)	1
Area		20					
Countryside	0		0				
Urban	53 (72,6)		45 (61,6)	1		1 (1,3)	1