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Epidemiology of Human Herpes Virus 8 in Pregnant Women and their Newborns - A cross-sectional delivery survey in Central Gabon

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SUMMARY

Objectives: On the background of a high prevalence of HHV-8 infection in pre-pubertal Central African children, this study investigated the potential for *in utero* transmission of HHV-8.
Patients: Gabonese pregnant women were invited to provide peripheral and cord blood samples for serological and PCR diagnostics of HHV-8 infection at delivery for this cross-sectional survey.
Results: Out of 344 participants 120 (35%, 95% CI: 30-40%) were serologically positive for HHV-8. 31% (95% CI: 22-40%) of cord blood samples of seropositive women had detectable IgG antibodies. Among all seropositive participants HHV-8 was detected by PCR in one maternal peripheral blood sample at delivery (1%, 95% CI: 0.2-7%) and in none of cord blood samples. There was no association between demographic characteristics and infection status. Similarly, there was no difference in risk for premature delivery, low birth weight, and maternal anaemia in HHV-8 seropositive women.
Discussion: These data suggest a high seroprevalence of HHV-8 infection in pregnant women, however viraemia at delivery does not commonly occur in Central Africa. Based on these observations it may be speculated that infection of children may occur more commonly either antepartum or later on in infancy and childhood.

1. Introduction

Human herpes virus-8 (HHV-8) – also known as Kaposi's sarcoma associated herpes virus – is an infectious agent causing Kaposi's sarcoma, primary effusion lymphoma, and multicentric Castleman's disease.[1–6] HHV-8 infection is thought to be primarily transmitted by sexual contact.[2,7,8] However, the observation of high seroprevalence in pre-pubertal children residing in Africa and the Western and Eastern Mediterranean region[9–12] suggests other epidemiologically relevant routes of transmission. However, little is known about the potential for vertical transmission of HHV-8.

Active infection during pregnancy with several herpes viruses may affect the human placenta, including herpes simplex virus-1, varicella-zoster virus, and cytomegalovirus, potentially leading to placental insufficiency, premature delivery, miscarriage, or major congenital abnormalities.[13–15] To date, only limited data are available about the impact of maternal HHV-8 infection on birth outcomes and about the potential for vertical transmission of HHV-8. Previous studies report that seropositivity in newborns was mostly caused by transplacental passage of maternal antibodies.[9,16] However, HHV-8 DNA was detected in newborns in Zambia in peripheral mononuclear cells (PBMCs)[17] indicating vertical transmission during pregnancy. To further investigate the

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potential for vertical transmission and the impact of HHV-8 infection on birth outcomes this study assessed the prevalence of HHV-8 infection in pregnant women at delivery and in cord blood samples of their offspring in the Central African country Gabon.

2. Material and Methods

This study was conducted at the Centre de Recherches Médicales de Lambaréné at the Albert Schweitzer Hospital in Lambaréné, and the Ngounié Medical Research Centre in Fougamou, Gabon.[18] Pregnant women participating in a multicentre study evaluating alternative drugs for intermittent preventive treatment of malaria in pregnancy (MIPPAD study: "Evaluation of the safety and efficacy of mefloquine as intermittent preventive treatment of malaria in pregnancy (IPTp; NCT 00811421)."') were invited to participate in this cross-sectional study by providing written informed consent.[19,20] In accordance with inclusion criteria of MIPPAD only HIV-negative women were recruited. Other major inclusion criteria were gestational age ≤28 weeks, no history of allergy to sulfa drugs or mefloquine (MQ), no history of severe renal, hepatic, psychiatric, or neurological disease and no recent MQ or halofantrine treatment. No further inclusion or exclusion criteria other than specific informed consent for this study were applied.

Peripheral maternal and cord blood samples were obtained at delivery. Blood samples were centrifuged at 2500 rpm for 10 minutes to obtain plasma for the detection of specific antibodies directed against latent nuclear antigen of HHV-8. Whole blood was used for the detection of virus specific DNA by PCR. Plasma samples were tested at a 1/20 dilution for the presence of HHV-8-specific IgG by an indirect immunofluorescence assay (HHV-8 IFA; ABI, Columbia, MD) following instructions by the manufacturer. No quantification of antibody titres was performed. In this assay specific HHV-8 antibodies binding to the antigen of infected cells were visualized after incubation with Fluorescein Isothiocyanate under a fluorescence microscope.[21] Based on the high sensitivity of the serologic test, only seropositive samples were further analysed by PCR.

DNA was extracted from maternal and cord blood using QIAamp tissue kit (Qiagen, Bechman Instruments Inc, US). DNA extracts were further assayed by RT-PCR using the LightCycler® carousel-based system. Amplification was carried out in a 15 μl PCR master mix containing 10 pmol/μl of each primer,[22,23] 3 mM of MgCl2, 1x Lightcycler® LightCycler® FastStart DNA Master SYBR Green I (Roche Diagnostic GmbH, Mannheim, Germany) and 1.5 μl of DNA. To detect HHV-8, two sets of primers were used. The first set of primers amplified a 233-bp region of open-reading frame 26 (ORF26);[23] the second set of primers was specific for the ORF25 region.[22]

Birth weight was measured within 24 h after birth in all newborns. For estimation of gestational age, the Ballard score was used.

Data were recorded on paper record forms, transcribed into an electronic database, and further analysed (JMP 7.0, SAS Institute Inc., NC). Ethics clearance of this study was obtained by the Comité d'Ethique du Centre de Rercherches Médicales de Lambaréné, at the Albert Schweitzer Hospital in Gabon.

3. Results

Between May 2010 and October 2011, 344 pregnant women delivering at the two health care centres were recruited for his study. Mean age at recruitment was 24 years ranging from 14 to 49 years, and mean birth weight was 2,954 g (SD: 581 g).

A total of 120 pregnant women (35%, 95% CI: 30-40%) tested positive for HHV-8 specific antibodies in the serological assay.

Table 1
Analysis of baseline characteristics as potential factors associated with HHV-8 seropositivity in pregnant women in Gabon

Parameters	Total number of participants	HHV-8 seropositive n(%)	p-Value[b]
Age group			
14-17	45	16 (36%)	0.944
18-20	92	34 (37%)	
21-30	139	48 (35%)	
31-49	68	22 (32%)	
Literacy			
Literate	283	102 (36)	0.331
Illiterate	61	18 (30%)	
Gravidity			
Primigravid	86	28 (33%)	0.070
Secundigravid	71	33 (46%)	
Multigravid	187	59 (32%)	
Syphilis[a]			
Positive	5	2 (40%)	0.825
Negative	332	117 (35%)	

[a] Syphilis infection was assessed by Rapid Plasma Reagin testing.
[b] Chi-squared test.

Proportions of seropositivity in primigravidae, secundigravidae and multigravidae were 33%, 47%, and 32%, respectively. No difference in the distribution of baseline and demographic characteristics including age and parity were observed in HHV-8 positive versus negative individuals (Table 1). HHV-8 prevalence was comparable in syphilis positive (40%, 95% CI: 11.8-76.9%) and negative individuals (35%, 95% CI: 30.3-40.5%; Table 1), respectively. No association between HHV-8 seropositivity and adverse birth outcomes was found (Table 2).

Ninety-eight cord blood samples from seropositive women were collected for further serological analysis. Among those, 30 cord blood samples (31%, 95% CI: 22-40%) tested positive for the presence of HHV-8 specific IgG. 76 paired samples from maternal and cord blood were available for further PCR analysis (inadequate DNA extraction in 22 samples). One maternal blood sample (1%, 95% CI: 0-7%) yielded a positive result for HHV-8 DNA. All other maternal samples and all cord blood samples were negative in PCR analysis.

4. Discussion

This study demonstrates a high prevalence of HHV-8 seropositive HIV negative pregnant women indicating that HHV-8 infection

Table 2
Birth outcome and HHV-8 infection in pregnant women in Gabon

Parameters	n	HHV-8 seropositive n(%)	p-Value
Delivery			
Livebirth	336	119 (35%)	0.179
Stillbirth	8	1 (13%)	
Delivery characteristics*			
Vaginal	325	113 (35%)	0.965
Caesarean	17	6 (35%)	
Delivery anaemia*			
No anaemia	192	66 (34%)	0.818
Anaemia[b]	149	53 (36%)	
Term delivery*			
Normal	314	114 (36%)	0.197
Preterm[c]	15	3 (20%)	
Birth weight*			
Normal	276	97 (35%)	0.724
Low[d]	64	21 (33%)	

[a] HHV-8 infection classified based on serology.
[b] anaemia: haemoglobin < 11.0 g/dl.
[c] preterm delivery: < 37 weeks of gestation.
[d] low birth weight: defined as < 2500 g at delivery.
* Data not available for all 344 individuals.

is highly prevalent in this rural Central African population. Interestingly, the proportion of illiterate and very young pregnant women – an important surrogate marker for socioeconomic status – was not different between infected and uninfected individuals.[24] This finding indicates that HHV-8 is rather uniformly distributed in this African population without evidence for particular risk factors for HHV-8 infection. Importantly, there was no difference in prevalence of HHV-8 seroprevalence depending on the age and parity of pregnant women. Interestingly, prevalence of syphilis was low in both groups. The low rate of syphilis in our cohort might be explained by the fact that HIV was defined as an exclusion criterion and thus women with high risk sexual behaviour were excluded. In Europe and North America unprotected sexual contacts are considered to be the major route of transmission for HHV-8.[2,25] However, in Central Africa there is epidemiological evidence for early increase of HHV-8 seroprevalence during childhood. A sero-epidemiological survey in a highly endemic region in Uganda showed that HHV-8 transmission occurs during childhood increasing progressively up to 50% in adults. Similarly, a Cameroonian study reported a gradual increase of seropositivity from 13% in 7-24 month olds to 39% in 12-14 year old children, reaching 45% in adult women.[9,10] These data from the Central African region demonstrate that routes of transmission other than by sexual contact play the most important role for transmission of HHV-8 in this pre-pubertal population. Importantly, other modes of transmission including exposure to saliva are increasingly incriminated for the transmission of HHV-8 and may potentially explain the epidemiological pattern of HHV-8 transmission in Central Africa.[26]

In our study, seropositivity was detected in 31% of cord blood samples of seropositive women. This finding may be explained by passive transfer of maternal IgG antibodies and is per se no conclusive proof for vertical transmission. Importantly, all cord blood samples were tested negative for the presence of HHV-8 DNA in PCR analysis. This finding indicates that infection and subsequent viraemia in the newborn does not occur commonly. However, this study did not investigate the potential for intrapartum transmission of HHV-8. In addition longitudinal sampling of pregnant women was not performed during pregnancy due to the cross section study design, which constitutes a limitation of our study. Further follow up and blood sampling during the first year of life would have been necessary to address this research question but was beyond the scope of this work.

Whereas active infections with other herpes viruses during pregnancy are reportedly associated with adverse pregnancy outcome including spontaneous abortion, premature delivery, and miscarriage,[13–15] this survey did not show such an association for HHV-8 seropositive pregnant women. However, viraemia was not detected in all but one woman and therefore the sample size of this study was not large enough to reliably detect more subtle risk differences between viraemic and non-infected individuals.

In summary these data confirm a uniformly high prevalence of HHV-8 seropositivity in Gabon. No evidence for *in utero* transmission of HHV-8 and viraemia in cord blood was found in this study. Future investigations should further characterize transmission characteristics in infancy and childhood in Central Africa potentially focussing on oro-oral transmission patterns.

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Ethical approval: Ethics clearance of this study was obtained by the Comité d'Ethique du Centre de Rercherches Médicales de Lambaréné, at the Albert Schweitzer Hospital in Gabon.

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Conflicts of Interest: MR is serving as Corresponding Editor in the International Journal of Infectious Diseases. The authors declare no other potential conflicts of interest.

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