

Brief Original Article

Evaluating vertical transmission of sexually transmitted infections to newborns

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Abstract

Introduction: Sexually transmitted infections are among the most frequent infections affecting pregnant women. We assessed the transmission of hepatitis B virus, human immunodeficiency virus type 1 and *Treponema pallidum* to newborns from infected parturients.

Methodology: An observational, cross-sectional, analytical facility-based survey was conducted among 57 newborns in Irene Neto Maternity, Lubango city, Huíla province, Angola. Hepatitis B virus DNA molecular identification was done through nested PCR. Human immunodeficiency virus type 1 proviral DNA detection was carried out by two successive nested PCRs. Real-time PCR was performed to examine the presence of *T. pallidum* DNA. Amplicons from PCR positive samples were sequenced for identity search and genotype assignment.

Results: Hepatitis B virus DNA genotype E was detected in 3/41 (7.3%) newborns from HBsAg (hepatitis B surface antigen) positive mothers. To analyse the association between mothers HBeAg (hepatitis B e antigen) positivity and hepatitis B virus vertical transmission to newborns, a Fisher's exact test was performed, showing a highly significant association ($p = 0.006$). Human immunodeficiency virus type 1 provirus or *T. pallidum* DNA was not detected in any newborn.

Conclusions: To prevent hepatitis B virus vertical transmission in Angola it is important to promote universal antenatal screening, expanding hepatitis B virus markers (viral load and/or HBeAg), risk-based infected mothers' antiviral therapy and newborn passive immunoprophylaxis.

Key words: vertical transmission; HBV; HIV-1; *Treponema pallidum*; newborns; Angola.

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Introduction

Infections remain a major cause of maternal and fetal morbidity and mortality worldwide. Antenatal screening is essential in managing infections, as well as to prevent pathogen vertical transmission (VT). Sexually transmitted infections (STI) are among the most frequent infections affecting pregnant women [1]. The risk of hepatitis B virus (HBV) VT is estimated to be higher than 90% when maternal viral load is greater than 10^5 copies/mL, 70-90% in HBeAg (hepatitis B e antigen) positive and 10-40% in HBeAg-negative mothers [2]. Human immunodeficiency virus (HIV) VT rates range from 20-45% without intervention [3]. Untreated women have a 70% chance of transmitting syphilis to their fetus during the first four years of infection [4].

In Angola, studies on STI during pregnancy and VT are scarce. We assessed the transmission of HBV, HIV-1 and *Treponema pallidum* to newborns from infected

parturients admitted to Irene Neto Maternity (INM), Lubango city, Huíla province, Angola.

Methodology

Study Design

An observational, cross-sectional, analytical facility-based survey was conducted among 500 parturients at INM, from October 2016 to September 2017 [5]. INM is the reference maternity in Huíla, the second most populous province in Angola.

Sample Characterization

Blood samples were obtained from 57 newborns of 36, 12, four and two mothers infected with HBV, HIV-1, *T. pallidum* and HBV/HIV-1, respectively [5]. There were three twin pregnancies among the former, totalizing 41 newborns from HBV-infected mothers. Maternal HBV infection was defined by HBsAg (hepatitis B surface antigen) positivity in a rapid test (Laboquick HBsAg Test, Koroglu Medical Devices

Ltd., Turkey). An algorithm with three rapid tests was used for maternal HIV-1 diagnosis: first-line assay was a fourth-generation test (Determine™ HIV-1/2 Ag/Ab Combo, Alere Ltd., UK) and the other assays were third-generation tests (Hexagon HIV, Human, Germany and Info Anti-HIV 1/2, Türklab, Turkey). Inconclusive results were confirmed by Western blot (Genscreen™ HIV-1/2 Version 2, Bio-Rad, France). Maternal *T. pallidum* antibodies were screened with a treponemal rapid test (Laboquick Anti-Syphilis Test, Koroglu Medical Devices Ltd., Turkey) and, when reactive, the result was confirmed with RPR (Syphilis RPR Test, Human, Germany) [5]. We also performed HBeAg detection in 33/38 HBsAg-positive mothers (HBe Ag&Ab, DIA.PRO, Italy).

Molecular Identification of HBV, HIV-1 and T. pallidum in Newborns

Shortly after birth, a single capillary blood sample was collected from each newborn through foot heel puncture and conserved in filter paper (Grade 2, Whatman, UK). A QIAamp DNA Mini Kit (Qiagen, Germany) was used for DNA extraction and purification, according to manufacturer's instructions.

Molecular identification of HBV DNA was conducted through nested PCR targeting a 342 bp highly conserved fragment of the genome *S/P* region, as described by Oluyinka *et al.* [6]. HIV-1 proviral DNA detection was carried out by two successive nested PCRs for amplification of protease and reverse transcriptase coding regions (460 and 650 bp, respectively) [7]. Real-time PCR was performed to examine the presence of *T. pallidum* DNA, using primers targeting DNA polymerase I (*polA*) gene and a TaqMan probe [8], both designed by TIB MOLBIOL (Germany). A commercial reaction mix (NZYSpeedy qPCR Probe Master Mix, NZYTech, Portugal) was used and amplification was carried out in a Rotor-Gene 3000 (Corbett, Australia) thermal cycler.

Amplicons from PCR positive samples were sent to STAB VIDA (Portugal) to be sequenced (Sanger) in both directions (forward and reverse). The complementary sequences were edited in BioEdit Sequence Alignment Editor (v.7.0.9.0.) and submitted to NCBI BLASTn (Basic Local Alignment Search Tool) for identity search and genotype assignment.

Statistical Analysis

To analyse the association between mothers HBeAg positivity and HBV VT to newborns, a Fisher's exact test was performed, at the 1% level of significance,

using SPSS (Statistical Package for the Social Sciences) software v.26.

Ethical Considerations

Parturients signed an informed consent form to be included in this study, which was approved by the Angola Ethics Committee (No. 35/2017).

Results

HBV DNA genotype E was detected in 3/41 (7.3%) newborns from HBsAg-positive mothers. None of the corresponding three parturients was coinfecting with HIV-1 and only one had been aware of her HBV infection, but neither her viral load was evaluated, nor did she receive antiviral therapy (AVT).

HBeAg was detected in 2/33 HBsAg-positive mothers (6.1%). These transmitted the infection to their newborns (100%), while of the 31 HBeAg-negative, only one transmitted the infection (3.2%). There was an association between mothers HBeAg positivity and HBV VT to newborns ($p=0.006$).

HIV-1 provirus or *T. pallidum* DNA was not detected in any newborn from infected mothers. Regarding the 14 mothers infected with HIV-1, the majority (71.4%) received antiretroviral therapy (ART) before and/or during pregnancy and/or intrapartum (data on specific antiretrovirals largely incomplete), but four (28.6%) did not receive any ART. Among the four mothers infected with *T. pallidum* (RPR titers 1:2-1:8), one did not present her pregnancy card, two had a negative antenatal screening and one was treated with one dose of benzathine penicillin G at 14 weeks of pregnancy along with her partner.

Discussion

Prevention of VT is a major issue to consider in the management of STI during pregnancy. VT is related to adverse pregnancy outcomes, particularly in resource-limited countries, where access to adequate antenatal care is scarce. To our knowledge, this is one of the few reports on VT in Angola, the first on hepatitis B and syphilis.

Regarding HBV VT, the risk is higher in HBeAg-positive mothers (70-90%) [2]. In our study, despite the small numbers involved, a HBV VT rate of 100% in HBeAg-positive versus 3.2% in HBeAg-negative mothers was found, with an association between mothers' HBeAg and HBV VT to newborns ($p = 0.006$), as described before. For example, for Africa, a study previously conducted in Cameroon reported HBeAg as a serum marker associated with HBV VT [9].

Prevention of HBV VT includes universal antenatal screening, maternal AVT if high viral load is present and immunoprophylaxis in infants born to infected mothers. Evidence on the impact of HBeAg positivity must be taken into account when designing policies concerning prevention of HBV VT, particularly in low and middle-income countries. For instance, Lu *et al.* [10] suggested that vaccine alone may be enough for preventing HBV transmission in neonates of HBsAg-positive/HBeAg-negative mothers. Furthermore, Ségéral *et al.* [11] found that an algorithm of HBsAg and HBeAg rapid diagnostic tests could be a low-cost strategy to identify HBV-infected pregnant women at risk of VT when DNA quantification is not routinely available. Although universal HBV vaccination at birth is implemented in INM, other measures to prevent HBV VT in Lubango are missing. Only one of the three parturients with HBV DNA positive newborns had been aware of her HBV infection, but viral load had not been evaluated and AVT had not been administered. It is important to highlight that HBV VT remains a major source of perpetuation of chronically infected individuals' reservoir, having a huge impact in countries with a high burden of disease, as Angola, a country with a reported high HBV carrier rate of 15.1% [12].

There are several HBV genotypes, with distinct geographical distributions. They are important epidemiological markers, but they can also influence transmission patterns and clinical outcomes. In our study, the three HBV DNA positive newborns were infected with genotype E. In Angolan patients, this genotype is highly predominant [12,13], but genotypes A and D have also been reported [12].

HIV-1 provirus or *T. pallidum* DNA was not detected in any newborn from infected mothers. Regarding HIV-1, VT rate can be reduced to well below 5% with effective interventions [3]. We evaluated HIV-1 VT in 14 infected mothers with no data on viral load and absence of any preventive intervention was observed in four of them. High HIV-1 genetic diversity in Angola is also a challenge for molecular diagnosis [14]. Concerning *T. pallidum* VT among four infected mothers, there was almost no disease history available. They presented low nontreponemal titers (1:2-1:8), which could suggest the possibility of a serofast syphilis or recent treated syphilis, among others, and could explain the absence of congenital syphilis cases.

Among major limitations of this study, one should refer that it did not include women giving birth at home, which is very frequent in Angola, and maternal occult HBV infection was not evaluated.

Conclusions

Our study identified three cases of HBV genotype E VT, from mothers who were not properly managed during pregnancy regarding its prevention. In addition, a highly significant association between mothers HBeAg positivity and HBV VT to newborns was found. To prevent HBV VT in Angola it is important to promote universal antenatal HBV screening, expanding HBV markers (viral load and/or HBeAg), risk-based infected mothers' AVT and newborn passive immunoprophylaxis. A stronger integrated multisectoral commitment and further research are clearly needed in this field in Angola.

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