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.
Lt., 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 (HBeAg&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 coinfected 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 nonreponemal 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.

**Acknowledgements**

The authors are grateful to the parturients who participated in the study. We also want to thank the staff of the INM, particularly the nurses’ team. The study was supported by the Calouste Gulbenkian Foundation (Portugal) through a scholarship assigned to the corresponding author (grant number 135499).

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Conflict of interests: No conflict of interests is declared.