

NO EVIDENCE OF VERTICAL TRANSMISSION OF HTLV-I IN BOTTLE-FED CHILDREN

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SUMMARY

The most frequent pathway of vertical transmission of HTLV-I is breast-feeding, however bottle fed children may also become infected in a frequency varying from 4 to 14%. In these children the most probable routes of infection are transplacental or contamination in the birth canal.

Forty-one bottle-fed children of HTLV-I seropositive mothers in ages varying from three to 39 months (average age of 11 months) were submitted to nested polymerase chain reaction analysis (*pol* and *tax* genes). 81.5% of the children were born by an elective cesarean section.

No case of infection was detected. The absence of HTLV-I infection in these cases indicates that transmission by transplacental route may be very infrequent.

KEYWORDS: HTLV-I infection; HTLV-I vertical transmission; Transmission through breast-feeding; Diagnosis by PCR.

INTRODUCTION

The most frequent pathway of vertical transmission of HTLV-I is breast-feeding. Through prenatal screening for HTLV-I and the refraining from breast-feeding for the carrier mothers a reduction of ~ 80% of vertical transmission has been observed in Japan⁵. Serologic surveys of breast-fed children of carrier mothers using different methods of detection reported infection rates varying from 10% to 28% and indicate that the rate of transmission is directly related to the time of breast-feeding^{5-7,15,18,20}. However bottle-fed children may also become infected vertically in a frequency varying from 4% to 14%^{5-7,15,18,20}.

Besides breast-feeding the other possible mechanisms of vertical transmission of infections are: transplacental or hematogenous, by ascending microorganisms from the vagina (ascending via), by fetus contamination in the birth canal during delivery or by intimate contact between mother and child. The mechanism of vertical transmission of HTLV-I in bottle-fed infants remains to be established, however the most probable routes of infection may be transplacental or contamination in birth canal. Many researchers have shown that elective cesarian delivery reduces mother-to-child transmission in other blood born viral infections mainly because direct contact with blood is avoided^{14,8,11-13,19}. On the other hand, in labor uterine contractions can disrupt the placental barrier resulting in transplacental microtransfusions from mother to fetus. It has been shown that maternal microtransfusion to the fetus can be affected

by different modes of delivery, but elective cesarean presents the least microtransfusion risk¹¹.

Considering these facts, we decided to evaluate the vertical transmission of HTLV-I in breast-fed children born through elective cesarean section.

MATERIAL AND METHODS

The mothers were screened serologically for HTLV-I through ELISA and Western blot. The methods of screening were detailed in another paper². The mothers were informed about the research and those who consented were submitted to cesarean section and were advised to refrain from breast-feeding. Pediatric assistance and an alternative nutritional supply were provided for the babies. Testing for HTLV-I DNA was performed by nested polymerase chain reaction (PCR) on peripheral blood mononuclear cells of forty-one bottle-fed children. Permission was obtained from all the mothers of the children included in this study.

PCR testing: Mononuclear cells were isolated from peripheral blood of children by density centrifugation through Ficoll-Conray gradient. The peripheral blood mononuclear cells pellet was washed twice with 0.9% NaCl and re-suspended in 100 µl of lysis buffer (10 mM Tris-buffer [pH 8.3], 0.5% Nonidet P-40, 0.5% Tween 20) and digested with 40 µg of proteinase K at 65 °C for 2 hours. All samples were subjected to

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PCR amplification for HTLV *tax* gene. Due to technical problems, we could only perform PCR for the *pol* gene on 28 of the 41 samples. For the *pol* gene, 1 µg of DNA was amplified in one round with primers SK110 (4384-4405 5'CCCTACAATCAACCAGCTCAG) and SK111 (4569-4547 5'GTGGTGAAGCTGCCATCGGGTTTT) in a reaction mixture containing 3.0 mM MgCl₂ and 0.2 mM each primer. The PCR product was detected by liquid hybridization with a radio labeled probe SK112 (4452-4573 5'GTACTTTACTGACAAACCCGACCTAC) for HTLV-I. To detect the *tax* gene, DNA was amplified by a nested PCR with SK43 (6985-7003 5'CGGATACCCAGTCTACGT) and SK44 (7143-7123 5'GAGCCGATAACGCGTCCATCG) in the first round and TAX1 (7001-7020 5'-GTGTTTGGCGATTGTGTACA-3') and TAX2 (7128-7112 5'-CCATCGATGGGGTCCCA-3') in the second round in a reaction mixture containing 2.5 mM MgCl₂ and 0.2 mM each primer. The PCR product was analyzed upon electrophoresis on a 1% agarose gel¹⁶. A T-cell line (MT-2) infected with HTLV-1 was used as positive control. PBMC from seronegative individuals were used as negative controls. Positive and negative controls were included in every run. All samples were also submitted to PCR for the beta globin gene to detect the presence of PCR inhibitors.

RESULTS

From 52 HTLV-I+ mothers included in a previous study² only forty-one non breast-fed children could be contacted for PCR studies in ages varying from three months to 39 months (average age of 11 months). 81.5% of the mothers have had an elective cesarean section. Sixty per cent of these mothers were previously submitted to PCR studies with positive results². Four children were born prematurely and one, born at term, was small for gestational age. Twenty-one were male and 20 were female. None of the children received blood transfusions. PCR amplification for the *tax* gene was done in all the cases and for the *pol* gene in 28 cases, and the results were negative for both amplifications.

DISCUSSION

In South America there has been no evaluation of vertical transmission of the HTLV-I until now. It is known that in Salvador, Bahia, 0.84% of pregnant women of low socioeconomic class are HTLV-I carriers². Furthermore, the occurrence of many cases of adult T-cell leukemia/lymphoma¹ and infective dermatitis in Salvador, Bahia³, diseases associated with the vertical transmission of HTLV-I^{10,17}, indicates the importance of this route of infection in this city.

In this study the children were tested by nested PCR because this method is more sensitive for the detection of HTLV-I infection than conventional serology and is very useful for diagnosis of this infection early in life, before seroconversion, that occurs between one and three years of age^{5,9}. In the laboratory of Molecular Biology, Fundação Pró-Sangue/Hemocentro, USP, Brazil the sensitivity of PCR was 90% when seropositive blood donors were used as positive controls¹⁶. Using conventional serology MONPLAISIR *et al.* (1993)¹⁴ found 7% of infection among 27 children (ages varying from 2 to 12 years) born to HTLV-I seropositive mothers, but when they used PCR the frequency of transmission increased to 41%.

As previously stated, bottle-fed children can be infected vertically in a frequency varying from 4% to 14%. However, in the present study

no case of vertical transmission was observed in 41 bottle-fed children. The absence of infection determined by a sensitive method indicates that transmission by the transplacental route may be very infrequent. It is possible that the artificial delivery that occurred in 81.5% of the cases may have contributed to the absence of transmission.

RESUMO

Ausência de transmissão vertical do HTLV-I em crianças não amamentadas

A amamentação é o meio mais freqüente de transmissão vertical do HTLV-I. No entanto, crianças não amamentadas mostram-se infectadas em freqüências que variam de 4 a 14%. Nestes casos, os meios mais prováveis de infecção devem ser através da placenta ou por contaminação no canal de parto. Quarenta e um filhos de portadoras do HTLV-I aleitados artificialmente foram submetidos a pesquisa do vírus pela reação em cadeia da polimerase. 81,5% destas crianças nasceram através de cesária eletiva. Nenhum caso de infecção pelo HTLV-I foi detectado. Este fato indica que a infecção por via transplacentária é pouco freqüente e que é provável que o parto artificial tenha contribuído para a ausência de transmissão do vírus.

ACKNOWLEDGMENTS

The authors are grateful to Dr Aldely Rocha Dias for providing the nutritional support for the babies and for Drs James Cadidé and Magnolia Santos for the obstetrical assistance. This work was supported by Superintendência de Apoio ao Desenvolvimento Científico e Tecnológico (CADCT), and CNPq.

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Received: 28 October 2001

Accepted: 11 April 2002