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Presence of Cytomegalovirus in urine and blood of pregnant women with primary infection might be associated with fetal infection

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ABSTRACT

Background: Cytomegalovirus (CMV) congenital infection can result from primary infection, reinfection or reactivation among pregnant women. The risk of vertical transmission is much higher in case of primary infection, and the transmission rate increases with gestational age.

However there are still many questions about maternal markers that can predict whether the virus will be transmitted to the fetus.

Objectives: To investigate the relationship between the presence and the quantity of CMV in urine and blood of women presenting a primary CMV infection during pregnancy and the presence of congenital infection in their offspring.

Study design: Detection and quantification of CMV DNA was performed on 150 urine samples and 114 blood samples from 150 pregnant women with proven CMV primary infection.

Results: Transmission rate was 36.7% (55/150). A statistically significant association was found between the presence of CMV in maternal urine and newborn infection (OR 2.03 95%CI 1.03-3.99). A clearly significant association was found between the presence of CMV in maternal blood and newborn infection (OR 3.14 95% CI 1.38-7.16). Taking into consideration those samples that are positive for CMV in maternal urine, the median value of viral load was significantly higher in those patients who transmitted to offspring ($P = 0.015$). No significant association between viral load in maternal blood and newborn infection was observed.

Conclusion: The presence of CMV in maternal urine and maternal blood correlated to the transmission of CMV to offspring in our cohort. The median viral load in urine is higher in women who transmitted. These markers may help to identify pregnant women at risk to transmit to the fetus.

1. Background

Human cytomegalovirus (CMV) is a herpesvirus which has a ubiquitous and worldwide distribution, and is the most frequent cause of congenital infection [1]. In developed countries, the prevalence is around 0.5-1% of all live births and is the leading cause of sensorineural hearing loss and mental retardation [2,3]. CMV congenital infection can result from primary infection, reinfection or reactivation among pregnant women. The mean rate of vertical transmission from mothers with primary infection during the pregnancy ranges from 35% to 45% [4–7] and the rate is going down to 1.4% in case of non-primary infection [1]. Despite numerous publications in the past decades, questions about the factors influencing the transmission to the fetus are still pending. Transmission rate increases with gestational age but the fetus is at greater risk of becoming symptomatic when maternal infection occurs during the periconceptional period or in the first trimester [4,5]. However there are still many unanswered questions about markers in the mother that can predict whether the virus will be transmitted to the fetus.

2. Objectives

The primary objective of this study was to investigate the relationship between the presence of CMV in urine and blood of women

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presenting a primary CMV infection during pregnancy and the presence of congenital infection in their offspring. As a secondary objective, we aimed to investigate the relationship between CMV viral load in urine and blood of the same patients and the presence of congenital infection in their offspring.

3. Study design

3.1. Patients and samples

Between November 2001 and November 2011, we investigated 150 pregnant women with CMV primary infection. Primary infection was diagnosed by one of the following criteria: presence of a seroconversion of IgG, presence of IgM with low IgG avidity, a significant rise of IgG in presence of IgM, clinical symptoms and/or biological alterations compatible with CMV infection in the presence of CMV-specific IgM antibodies.

Maternal age, gestational age and time between infection and sampling were collected. The time of infection was arbitrarily estimated on gestational age at detection of the first positive serology. Pregnant women negative for CMV antibodies were routinely screened approximately every month. The median interval between last CMV antibodies negative blood (when known: 141/150) and first positive serology was 5 (p25: 4, p75: 7) weeks. Maternal urine and blood were collected at first visit, after written informed consent was obtained.

3.2. Detection and quantification of CMV in urine and whole blood samples

Between November 2001 and March 2004, CMV DNA was detected using an in-house nested polymerase chain reaction (PCR) targeting the pp150 CMV gene, performed as previously described [8]. Samples collected between April 2004 and January 2011 were assayed using a real-time in-house PCR targeting the pp150 CMV gene. Extraction was done using the Qiagen DNA blood mini kit (Qiagen, Benelux). PCR was performed on a Light Cycler480 PCR system(Roche Diagnostics) with the forward primer 5′-CTG ATG AGG TTT GGG CTT TAA-3′, the reverse primer 5′-TCC GAG GAG TCG TCG TCT T-3′, and the probe 5′-FAM-CAA ACT GCA GAG TCA CCG GTC GAA-TAMRA-3′.

All positive samples were retrospectively quantified using the CMV R-gene kit (BioMérieux, France). The PCR was carried out on a LightCycler 480 PCR system (Roche Diagnostics, Belgium). The kit is a 5′nuclease real-time assay, targeting the gene coding for ppUL83 protein.

3.3. Diagnosis of congenital infection

A congenital infection in a newborn was determined by the detection of CMV by PCR in either urine or blood or saliva samples obtained during the two first weeks of life. In cases of termination of pregnancy, the detection of CMV was done by PCR on amniotic fluid, fetal blood and fetal tissues when available.

3.4. Statistical analysis

We performed all statistical analysis using STAT IC 12. Categorical variables were described giving absolute number of cases and percentage, and were analyzed using a chi square test. As a measure of association odds ratios (OR) were calculated. Confidence interval (CI) was calculated using the Cornfield formula. Quantitative variables were described using mean and standard deviation when normally distributed, median and first and third quartile when not normally distributed and were analyzed using Student *t*-test when prerequisites for the test were met and with a Wilcoxon Manning test if they were not. Statistical significance was considered when the p value was <0.05.

4. Results

In our total cohort, the rate of intrauterine transmission was 36.7% (55/150). For these 150 patients the presence of CMV in maternal urine was analyzed.

In Table 1 baseline characteristics and presence of CMV in maternal urine are summarized in transmission and non-transmission groups. The OR between the presence of CMV in maternal urine and transmission of the virus to offspring was found to be significant at 2.03 (95% CI 1.03–3.99, p = 0.041). There was no statistically significant difference between maternal age, gestational age at seroconversion and time elapsed between infection and sampling between those patients whom transmitted the virus to their offspring and those who did not.

In a sample subgroup when blood sample was available (n = 114), we also analyzed the presence of CMV in maternal blood. In Table 2 baseline characteristics and presence of CMV in maternal blood are summarized in transmission and non-transmission groups. Presence of CMV in maternal urine was also analyzed for this subgroup. There was no statistically significant difference between maternal age, gestational age at seroconversion, time elapsed between infection and sampling between those patients who transmitted the virus to their offspring and those who did not.

In this subgroup the OR between the presence of CMV in maternal urine and transmission of the virus to offspring was 1.9 (95% CI 0.87–4.13, p = 0.1). The OR between the presence of CMV in maternal blood and transmission of the virus to offspring was found to be clearly statistically significant at 3.14 (95% CI 1.38–7.16, p = 0.006).

The analysis of the difference of virus transmission to offspring in function of viral load in the positive urine and blood samples is summarized in Table 3. Taking into consideration those samples that are positive for CMV in maternal urine (n = 79), there was a significant higher value of viral load in the samples of patients who transmitted the virus to the offspring compared to those who did not (p = 0.015). When only the subgroup of 114 pregnancies was considered (59 positive urine samples), the viral load in those

Table 1
The relationship between the presence of CMV in maternal urine and transmission to the fetus. Descriptive statistics of baseline characteristics and univariate analysis.

	n	Transmission n = 55 (36.7%)		Non transmission n = 95		OR	95%CI	p-value
Maternal age (years)	150	29.4[a]	4.6[b]	30.3[a]	4.3[b]			0.2
Gestational age at infection (completed weeks)	150	18[c]	12–24[d]	16[c]	12–21[d]			0.26
Time between infection and sampling (completed weeks)	150	6[c]	3–9[d]	5[c]	3–8[d]			0.29
Presence of CMV in maternal urine sample (n)	150	35	63.6%	44	46.3%	2.03	1,03–3,99	0.041

[a] Mean.
[b] Standard deviation.
[c] Median.
[d] p25–p75.