

## Cytomegalovirus, *Toxoplasma gondii* and Rubella Vertical Transmission Rates According to Mid-trimester Amniocentesis: A Retrospective Study

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### ABSTRACT

**Objective:** To examine vertical transmission rates of Cytomegalovirus, *Toxoplasma Gondii* and Rubella infections according to amniotic fluid PCR analysis.

**Methods:** A retrospective analysis of mid-trimester amniocenteses performed in pregnancies with diagnosed maternal infection by Cytomegalovirus (CMV), Rubella or *Toxoplasma gondii* during 1994-2008 was performed. Vertical transmission rates were observed according to the presence of the infectious agent's DNA in the amniotic fluid. A univariate regression model was also performed to investigate possible correlations between transmission and epidemiological parameters.

**Results:** Overall, 7033 amniocenteses were performed during study's period, of which 166 (2.4%) with the indication of maternal infection by CMV, Rubella or *Toxoplasma*. Mean maternal age was  $27.4 \pm 2.5$  years and the mean gestational age at amniocentesis was  $18.7 \pm 2.5$  weeks. Vertical transmission was observed in 21 cases (12.7%). Transmission rate was 17.3% in cases with infection from CMV, 9.5% from *Toxoplasma gondii* and 7.8% from Rubella ( $P = .05$ ). Maternal age was the only parameter being significantly associated with increased risk for vertical transmission ( $P = .04$ ).


**Conclusions:** According to our results, overall vertical transmission rate marginally exceeds 10%. CMV infection is characterized by relatively higher transplacental transmission rate, while increased maternal age appears to be associated with a higher risk for vertical transmission

**Keywords:** Amniocentesis, cytomegalovirus, infection, rubella, toxoplasma, vertical transmission

## INTRODUCTION

Perinatal infection is the cause of 2–3% of overall congenital anomalies. TORCH is an acronym that

encompasses the most common perinatal infections including Toxoplasmosis, Other (syphilis, parvovirus B19, varicella-zoster), Rubella, Cytomegalovirus (CMV) and Herpes.<sup>[1]</sup> Although these infections are considered to have minor clinical impact on mother in the majority of cases, they may cause serious consequences for fetus, increasing perinatal morbidity, and even mortality. Severity of neonatal disease is mostly correlated with gestational age at which maternal infection occurs as well as with former maternal immune status. Prompt treatment has been shown to improve neonatal outcomes even if vertical transmission is confirmed.<sup>[2-5]</sup> Therefore,

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early diagnosis of such cases is critical in an effort to reduce the possibility of a seriously affected child.

Serum screening for specific IgM and IgG antibodies in the maternal blood is the first step in the diagnostic approach. Indeed, the presence of IgM antibodies is associated with acute infection. However, as IgM antibodies may persist for months or even years after the infection, this examination may lead to false-positive results. Nevertheless, anxiety that such a result causes to parents may often lead to unnecessary pregnancy termination.<sup>[2,6-8]</sup> According to reports, 1 out of 5 women proceed in abortion just after the announcement of a positive IgM test for *Toxoplasma gondii*.<sup>[9,10]</sup> IgG avidity test may have a higher specificity in the detection of acute infection; however, it still cannot identify a neonatal infection.<sup>[6,8,11]</sup>

Transplacental transmission of maternal infection to the fetus may be confirmed by amniocentesis. Polymerase chain reaction (PCR) analysis of the amniotic fluid is characterized by high sensitivity and specificity regarding the detection of an affected embryo.<sup>[5,8,12]</sup> Indeed, Bessières *et al.* demonstrated a 91% sensitivity and 99.5% specificity of PCR analysis of the amniotic fluid for the diagnosis of congenital toxoplasmosis, therefore demonstrating the diagnostic supremacy of this method in the detection of transplacental transmission.<sup>[4]</sup>

Main objective of the present study was to record transmission rates of CMV, *T. gondii* and rubella, as confirmed by second trimester amniocentesis. In addition, their potential correlation with epidemiological parameters as well as outcome of affected cases was also examined.

## METHODS

In Greece, all pregnant women are offered screening for CMV, rubella and toxoplasmosis infection in the first trimester of pregnancy. In cases where a recent infection is suspected, a mid-trimester amniocentesis is offered followed by real-time PCR analysis for infectious agent's DNA. This was a retrospective study of all amniocenteses conducted in a single institution, following the diagnosis of a first trimester maternal infection by CMV, rubella and toxoplasmosis. All cases with the former infections were diagnosed by positive maternal serum specific IgM antibodies during the first trimester of pregnancy. Patients were advised not to perform amniocentesis before 20<sup>th</sup> gestational week regarding CMV and rubella and 18<sup>th</sup> gestational week regarding *T. gondii* because of the increased possibility of false-negative results. However, in case of maternal request and given the relative small interval until the limit of 24<sup>th</sup> gestational week for pregnancy termination according to Greek

legislation, amniocentesis could also be performed earlier than suggested. Data were collected from the medical records of all the patients during the period 1994–2008. Institutional Review Board approved the present study.

Epidemiological characteristics available for analysis included maternal age, parity, and ethnicity. Specifically, we estimated mean values of maternal age and parity, as well as rates of women aged  $\geq 35$  years old, nulliparous and natives. Placenta location and gestational age at the amniocentesis procedure were also recorded. All the aforementioned parameters were studied for the total of patients as well as for each specific infection independently.

The main objective of our analysis was to define the rate of vertical transplacental transmission in cases where specific IgM antibodies were detected in the maternal serum during the first trimester of the present pregnancy. Transmission was confirmed by real-time PCR analysis of the amniotic fluid aspirated by amniocentesis. Vertical transmission rate was estimated for the total of cases while a sub-analysis was also made for the three different studied maternal infections. In addition, a univariate regression analysis was used in order to correlate vertical transmission confirmed by second trimester amniocentesis with epidemiological characteristics. The amniocentesis procedure was conducted as described thoroughly in a previous report.<sup>[13]</sup>

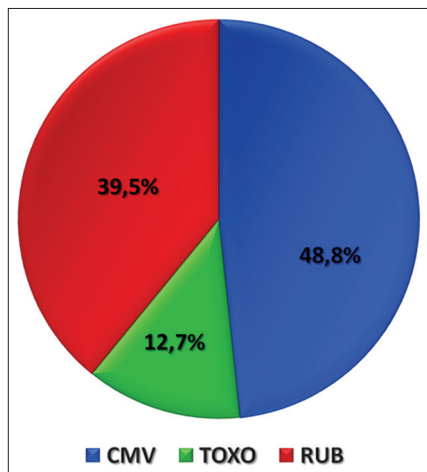
## Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Science version 17.0 (SPSS Inc., Chicago, IL, USA). Independent samples *t*-test was used for continuous data to assess mean values and standard deviations. Fisher's exact test analysis was used to compare cross-tabulated variables. Univariate logistic regression model (relative risk; 95% confidence interval) was used to correlate the vertical transmission rate of the different infections of the study with epidemiological parameters. Differences between groups were considered statistically significant at  $P < 0.05$ . All *P* values were two-sided.

## RESULTS

There were overall 7033 amniocenteses performed during the period of the study, of which 166 (2.4%) were performed with the indication of maternal infection by CMV, rubella, and toxoplasmosis. Mean maternal age of the women was  $27.4 \pm 4.9$  years and 13 (7.8%) were older than 35 years old. Mean parity was 1.5, while 110 (66.3%) women were nulliparous. Mean gestational week at the time of the amniocentesis was  $18.7 \pm 2.5$  weeks. Placenta location was mainly anterior (54.2%). There were 2 of 166 cases

that involved twin pregnancies (1.2%). No significant difference was observed in terms of maternal age, parity,



**Figure 1: Overview of amniocenteses performed with the indication of maternal infection during 1994–2008**

**Table 1: Epidemiological characteristics of women performing amniocentesis according to the different types of maternal infection**

Epidemiologic characteristics	CMV (n=81)	Rubella (n=21)	Toxoplasma (n=64)	P
Maternal age, (mean±SD)	27.6±4.6	27.6±5.2	25.8±6.4	NS
≥35 years old, n (%)	6 (7.4)	1 (4.8)	6 (9.4)	NS
<35 years old, n (%)	75 (92.6)	20 (95.2)	58 (90.6)	
Parity, (mean±SD)	1.5±0.8	1.5±0.9	1.5±0.7	NS
Nulliparous, n (%)	28 (34.6)	6 (28.6)	22 (34.4)	NS
Multiparous, n (%)	53 (65.4)	15 (71.4)	42 (65.6)	
Gestational age* (mean±SD)	18.9±2.4	18.2±2.7	18.7±2.6	NS
Placenta location, n (%)				
Anterior	44 (54.3)	12 (57.1)	34 (53.1)	NS
Posterior	29 (35.8)	8 (38.1)	25 (39.1)	
Fundal	2 (2.5)	0 (0)	3 (4.7)	
Lateral	6 (7.4)	1 (4.8)	2 (3.1)	
Ethnicity, n (%)				
Greek	78 (96.3)	16 (76.2)	46 (71.9)	0.001
Immigrants	3 (3.7)	5 (23.8)	18 (28.1)	

\*Gestational week at the time of amniocentesis. CMV=Cytomegalovirus, SD=Standard deviation, NS=Nonsignificant

**Table 2: Univariate logistic regression model correlating vertical transmission with the epidemiological characteristics**

Epidemiologic characteristics	Vertical transmission RR (95% CI)	P
Maternal age*	1.07 (0.97-1.17)	0.17
Maternal age (≥35 years old)	3.28 (1.03-6.27)	0.04
Parity*	0.89 (0.48-1.65)	0.71
Parity (nulliparous vs. multiparous)	0.98 (0.37-2.59)	0.27
Placenta location	1.3 (0.50-3.50)	0.15
Ethnicity (natives vs. immigrants)	0.44 (0.10-1.98)	0.41

\*Numeric variables. RR=Relative risk, CI=Confidence interval

placenta location and gestational age at amniocentesis between the different groups. However, a significantly higher percentage of native Greek women were infected by CMV in comparison to the other infections. No abnormal ultrasound markers were detected in any case with vertical transmission while no signs of intrauterine growth restriction were also detected. Comparison of women’s epidemiological characteristics according to the different types of maternal infection is presented in Table 1. Overview of amniocenteses performed because of maternal infection is presented in Figure 1.

Vertical transmission was confirmed in 21 cases out of 166 cases (12.7%), all of which were singleton pregnancies. According to the type of infection, transmission rate was 17.3% for cases affected by CMV (n = 14/81), 9.5% for *T. gondii* (n = 2/21) and 7.8% for rubella (n = 5/64), the difference being marginally significantly different (P = 0.05). When correlating the possibility of vertical transmission with epidemiological aspects, maternal age ≥35 years old was the only parameter found to significantly increase risk for transmission. Thus, vertical transmission was 30.4% in women ≥35 years old while 11.9% for women <35 years old (P = 0.04). Univariate regression model between vertical transmission and epidemiological aspects is presented in Table 2.

## DISCUSSION

The present study showed that transplacental transmission of CMV, rubella and toxoplasmosis was confirmed in 12.7% of all cases examined. More specifically, the vertical transmission rate was 17.3% for CMV, 4.7% for *T. gondii* and 9.5% for rubella.

Cytomegalovirus remains the virus most commonly responsible for congenital infection in the developed world. Transplacental transmission following primary viral infection approaches the rate of 40% while in case of recurrent infection this may not exceed 1%.<sup>[2,14]</sup> Overall transmission rate in the present study was estimated to be 17.3%. Rahav *et al.*, demonstrated a vertical transmission of CMV in about 30% of mothers infected by CMV during pregnancy.<sup>[15]</sup>

Cytomegalovirus infection during pregnancy does not necessarily prove infant damage, even when vertical transmission is confirmed. According to literature, only 10–15% of newborns affected by the virus at the intrauterine period will develop mild to serious complications. The rest of embryos will be delivered free of symptoms, and only 10% of them are expected to suffer neurodevelopmental defects later in life, such as deafness and earing problems necessitating early earing survey.<sup>[16,17]</sup> However, abortion rate is relatively high in pregnancies complicated by CMV infection.<sup>[15]</sup> Finally, regarding therapy, there is no vaccine or another

prophylactic measure available in order to prevent such an infection. Nevertheless, Nigro *et al.* report that fetal damage may be limited in case anti-CMV monoclonal antibodies are infused into an infected pregnant woman.<sup>[18]</sup>

Infection with the protozoon *T. gondii* is a widely-spread disease, affecting 1 out of 3 persons worldwide. The gestational week of maternal infection is proportional to the transplacental transmission but inversely proportional to the severity of fetal disease.<sup>[3]</sup> In our analysis, vertical transmission of the protozoon was confirmed in 9.5% of the infected mothers after second trimester amniocentesis. Relatively, Yamada *et al.* detected *T. gondii* DNA in the amniotic fluid in 17.6% of infected pregnant women.<sup>[8,19,20]</sup>

There is no agreement in the literature concerning prevention of *T. gondii* transmission. However, it is suggested that infected pregnant women should receive treatment (1<sup>st</sup> trimester infection: Spiramycin, 2<sup>nd</sup> trimester virus-PCR positive on amniotic fluid: Stop spiramycin then treatment by pyrimethamin and sulfadoxin or pyrimethamin and sulfadiazine.<sup>[4,5]</sup> Although congenital infection may lead to severe lesions, as already mentioned, the vast majority of infected fetuses are born free of symptoms. Characteristically, Villena *et al.*, analyzing a population of live-born infants that were characterized by intrauterine infection, reported that 87% of them were asymptomatic. In addition, of the remaining 13% in the same study, only 3% suffered from severe disease.<sup>[5]</sup> Therefore, also in case of toxoplasmosis, proof of vertical transmission may not necessarily confirm fetal damage.

Risk for congenital rubella syndrome increases when infection occurs in early stages of pregnancy. Specifically, the percentage of infants with congenital malformation exceeds 50% in cases of infection during the first trimester of pregnancy while the relative percentage is significantly reduced after the 20<sup>th</sup> gestational week.<sup>[20-24]</sup> However, maternal viremia is not a proof of vertical transmission to the embryo, and fetal infection does not necessarily correspond to fetal damage. Namaei *et al.* reported that none of the infants of gravidas that received a measles-rubella vaccine at the interval between 3 months before and 3 months after conception appeared to experience viral consequences. This may be the reason why authors support detailed information provided to the mother in order to prevent unnecessary voluntary interruption of pregnancies.<sup>[21,25]</sup>

Prenatal diagnostic exploration should be recommended in case of positive PCR in order to detect the affected embryos, having more precise information concerning the fetal health status before the decision of pregnancy's termination.<sup>[2,6,12,26]</sup> Studies have demonstrated an association between viral load in amniotic fluid and the

risk of a symptomatic infant, whereas further investigation with a noninvasive diagnostic procedure might postulate a method to evaluate potential fetal affection.<sup>[26-29]</sup>

The main limitation of the present study is the nonavailability of follow-up of cases in which vertical transmission was diagnosed. However, our center is a private diagnostic center where pregnancies are referred for prenatal diagnosis and are afterward treated by private physicians or hospitals. In addition, amniocenteses were also in certain cases performed earlier than recommended, potentially increasing the possibility of false-negative results. However, the merit of the study was to elucidate on the vertical transmission rates in cases of maternal infection by CMV, rubella, and *T. gondii*. To the best of our knowledge, this study is one amongst only a few presenting a relatively large series of amniocenteses performed with the indication of maternal infection. Besides, our observation that maternal age may be associated with vertical transmission possibility is an interesting outcome necessitating further research.

## CONCLUSIONS

Cytomegavirus infection is characterized by significantly increased transmission rates than rubella and *T. gondii*. However, vertical transmission rate was observed to be lower than 20% for cytomegavirus and then 10% for rubella and *T. gondii*. Pregnancy termination should not be the initial approach before confirming vertical transmission and reporting to parents the evidence-based possibility of severe congenital disease so that they may make a justified decision regarding their affected fetus.

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