Significance of Specific Immunoglobulins Determined by the Chemiluminescence Reaction in Intrauterine Maternal-fetal Infections of TORCH Syndrome

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Because the TORCH Syndrome groups infectious agents which cross the placenta during pregnancy and due to that cause the majority of malformative fetopaties, our porpose was to determin specific IgG and IgM antibodies for Cytomegalovirus, Rubella virus and Toxoplasma gondii. We studied 95 serum samples from 93 pregnant females and 2 cilds, being analyzed by a private laboratory in Timisoara between july 2015 – june 2016. Specific antibodies IgG and IgM were found for Rubella virus in 75 of the samples, for Cytomegalovirus in 82 and for toxoplasmosis 92. In this process of determination we used Access 2 (Beckman Coulter) and Immulite 1000 (Siemens) analysers, which use the chemiluminescence reaction. For 72 cases (77.42%) specific antibodies for all tests of the TORCH complex were performed. From all the patients 11 chosed to determine antibodies just for one of the infections of the TORCH Syndrome and 10 patients asked for determining of specific antibodies for two infections of the TORCH Syndrome. 53 (64.63%) of the 82 patients tested for anti-CMV IgG specific antibodies had positive result, which signifies former presence of CMV. 64 (85.33%) of the 75 patients tested for anti-rubella specific antibodies had an IgG positive result. Interpreting of results took in counter the vaccinal status. 6 patients (8%) did not contact the Rubella virus and did were not vaccinated. The level of specific IgG anti-Toxoplasma gondii antibodies was positive for 2 (2.17%) patients of 92. IgM specific antibody test was negative. In pregnant women TORCH Syndrome diagnosis is a must, especially if the medical abortion is the correct decision. Identifyeing infectious agents implicated in TORCH Syndrome can lead to an important decrease of maternal and fetal mortality and morbidity.

Keywords: TORCH Syndrome, Cytomegalovirus, Rubella virus, Toxoplasma gondii, IgG and IgM antibodies/ immunoglobulines, the chemiluminescence reaction

Intrauterine maternal-fetal infections are intrapartum infections, from the mother to the fetus. They have a polymorphic etiology, which may be of the following: viral, bacterial, parasitic and fungal [1].

During the intrauterine life, the fetus is sterile and the placenta is impermeable to most microorganisms except some viruses (rubella, cytomegalovirus, HIV etc.), some bacteria (*Treponema pallidum*) and parasites (*Toxoplasma gondii*) [2].

Infectious agents can be transmitted from the mother to the fetus through the following pathways: haematogenic (transplacentar) - the most common, ascending - ascending amniotic infection or ascending fetal-placental infection, intrapartum - after uterine maneuvers performed for diagnosis or rarely therapeutically [1].

Due to diminishing the functional capacity of T lymphocytes, the pregnant woman is more prone to infection. During pregnancy there are immunological and hormonal changes that can aggravate or reactivate certain infections [3- 7].

The gestational age at which the mother is infected, the fetal entrance gate, virulence and pathogenic cell tropism are just a few of the factors that influence fetal and neonatal impairments. Depending on the time of contamination, maternal-fetal infections may cause pregnancy to stop, spontaneous abortion, fetal infection, fetal malformations, premature birth and abnormalities that can be highlighted sometimes late in childhood. At fetal infection in small gestational age, spontaneous abortion or even death at birth of the newborn are the most common consequences. At the same time, mother's primary infection presents the highest risk for a congenital infection during pregnancy, but intrauterine fetal damage does not occur in all cases of maternal infection [1, 8, 9].

The *TORCH complex* (T - toxoplasmosis, O - other, R - rubella, C - cytomegalovirus, H - herpes) groups infectious agents that easily cross the placenta and are responsible for most malformative fetopathies. Infections have in common: inapparent or light mother disease, similar clinical manifestations in children (hepatosplenomegaly, encephalitis, ocular lesions, low birth weight.) They can cause sequelae, spontaneous abortion or even death of the fetus [1, 4].

The incidence of congenital infections with TORCH complex microorganisms is approximately 0.25% of the live infant. Of these, the highest incidence has the cytomegalovirus infection (CMV).

It is estimated that about one third of the global population is infected with *Toxoplasma gondii*, the average prevalence in Romania of *congenital toxoplasmosis* being around 1.45% ooo [10-12]. Generally, toxoplasmosis is asymptomatic in immunocompetent individuals, but in the case of immunocompromised and in congenital transmission it may develop severely.

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Congenital toxoplasmosis occurs after the mother's primary infection during pregnancy. Generally, infected pregnant women are asymptomatic, but this does not rule out the fetus's damage. The risk of transmission of infection increases with the advancement of pregnancy, but the severity of the disease in the fetus decreases (table 1) [13].

Table 1

CORRELATION BETWEEN GESTATIONAL AGE, TRANSMISSION RATE AND SEQUELAE OCCURRENCE

Gestational period	Rate of transmission	Rate of sequelae
1-st quarter	14%	41%
2-nd quarter	29%	8%
3-rd quarter	59%	0%

Although transplacental transmission is achieved in a low percentage during the first trimester of pregnancy, the consequences for the development of the fetus are severe, with congenital malformations or even abortion. In 10% of newborns, the disease can be fatal, and in 25-50% it can cause premature births [10, 14, 15].

Rubeola is an eruptive, contagious and viral disease that in most cases has a benign evolution, but has a major impact on the pregnant woman and a teratogenic effect on the fetus [9, 16].

Also, the newborn with congenital rubella is a source of infection, being contagious 6-12 months [1, 17].

Currently, rubella virus is known as the most potent teratogenic infectious agent identified so far, congenital rubella having an incidence of 3-5% [1, 18].

In pregnant women, the infection can cause spontaneous abortion, pregnancy arrest, premature birth, congenital rubella - when the rubella virus is transmitted in utero during maternal primary infection [1, 8].

Congenital evolutionary rubella causes plurivisceral lesions, which can regress or evolve with definitive scaffolds. Intrauterine growth restriction is the most common, with a mortality of 20%.

The classic triad of congenital rubella syndrome is represented by: congenital heart malformations, cataracts and deafness [1]. In addition, there are other clinical manifestations of the disease.

Specific prevention by vaccinating with MMR vaccine (mucosal, mumps, rubella vaccine) with live attenuated virus is prophylactically administered, providing immunity for up to 15 years to up to 95% of those who received it. It is administered during the first 12 months of life, and the immunization of the previously unvaccinated individuals takes place during puberty (12-18 years) [17].

Cytomegalovirus - CMV (disease of cytomegalovirus inclusions) causes infections with varying degrees of severity and a similar infectious to infectious mononucleosis.

The cytomegalovirus is ubiquitous in the human population and is transmitted by blood, body fluids, or transplacentar. Primoinfection of the teenager and adult is contracted oraly, by sexual intercourse, or blood transfusion. The consequences of infection depend on the age and immune responsiveness of the host [1]. Infections are often asymptomatic, and the virus can remain latent, so most people do not even know they were infected.

Congenital infection is contracting transplacentally in pregnant women with a primary infection or a reactivated infection [1, 8, 17]. The severity of fetal injuries is higher in the case of primary infection and those occurring in the first half of the gestation period. Maternal immunity does not prevent transplacental transmission, but fetal damage decreases.

Congenital infection with CMV occurs in 0.2-2.2% of infants born whose mothers have developed the infection. The form of clinical illness occurs primarily after a mother's primary infection in the first half of pregnancy.

Most frequently, the newborn is asymptomatic, but may develop symptoms similar to that of the primary infection mononucleosis. Of infants with congenital CMV infection, only 10% are symptomatic at birth [8].

Objectives

The purpose of this study is to determine the prevalence and incidence of congenital and perinatal infections produced by *Cytomegalovirus* (CMV), *Rubella virus* and *Toxoplasma gondii* parasite within the TORCH complex.

Experimental part

Material and method

The serum samples were collected between July 2015 and June 2016 from a total of 95 patients (93 pregnant and 2 children) who addressed a private laboratory in Timisoara. From the serum of patients, specific IgG and Ig M types for *Cytomegalovirus* (CMV), *Rubella virus* and *Toxoplasma gondii* parasite were determined.

Immunoassay automated Access 2 (Beckman Coulter) and Immulite 1000 (Siemens) immunology analyzers based on the chemiluminescence reaction (property of substances to emit photons when oxidized) were used to perform immunological determinations in the serum.

Results and disscusions

Of the 95 samples collected IgG and Ig M specific Ig rubella antibodies were determined in 75 of the samples, for *Cytomegalovirus* (CMV) at 82 samples and for toxoplasmosis in 92 samples (table 2).

Table 2

NUMBER OF SAMPLES TESTED FOR EACH TYPE OF INFECTION

Rubella	Cytomegalovirus	Toxoplasmosis
75	82	92

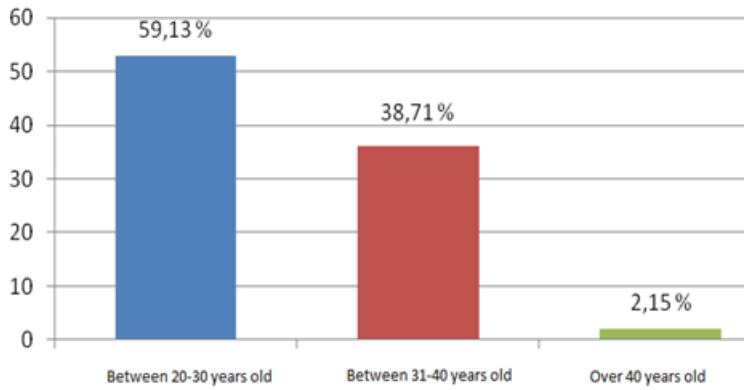

Fig. 1. Distribution of patients by age

Table 3

NUMBER OF PATIENTS DEPENDING ON SPECIFIC ANTIBODIES THAT WERE DETERMINED

Disease	Nr.	%
Toxoplasmosis	10	10.75 %
Rubella	1	1.07 %
CMV	-	-
Toxoplasmosis + Rubella	1	1.07 %
Toxoplasmosis + CMV	9	9.67 %
Rubella + CMV	-	-
TORCH complex	72	77.42 %
TOTAL	**93**	**100 %**

Depending on the age of the patients, it was observed that the highest incidence was between 20 and 30 years - 55 patients (59.13%). Over 40 years, the incidence was the lowest with only 2 patients (2.15%). 36 patients were between 31 and 40 years of age (38.71%) (fig. 1).

Most of the patients who addressed the laboratory performed the TORCH test for the three infectious agents: 72 (77.42%) of the 93 patients.

10 patients only required the determination of toxoplasmosis-specific IgG and IgM antibody titers, 1 patient only required rubella-specific antibodies, and no patient wanted to detect only specific CMV antibodies.

10 of the patients wanted the determination of the antipodes specific for two of the TORCH complex infections: specific toxoplasmosis and CMV antibodies were determined in 9 of the patients, and for the toxoplasmosis and rubella in a single patient (table 3).

Cytomegalovirus infection (CMV)

Of the 82 sera studied in which IgG anti-CMV antibodies were detected, the result at 53 was positive with a level above 1.1 indicating the presence of a *Cytomegalovirus* virus but did not confirm the presence of an infection. Therefore, for a certain diagnosis, detection of IgM-specific antibodies is recommended.

The negative result occurs if the exposure to the *Cytomegalovirus* is absent. Only 24 (29.27%) of patients were negative for IgG antibodies, but considering that most patients are pregnant, they should repeat the test in the IV month of the task for a certain diagnosis (table 4).

Table 4

THE LEVEL OF ANTI-CMV IgG ANTIBODIES (BIOLOGICAL REFERENCE RANGES)

Ig G		
Negative	Inconclusive	Positive
0<IgG<0.9	0.9<IgG<1.1	IgG>1.1
24	5	53
82		

In the case of the two children, the presence of anti-CMV IgG specific antibodies indicates either they have come into contact with the virus in the past or the antibodies have been transmitted intrauterine, but in this case confirmation of contamination is required in the first 2 weeks of life.

The prevalence of IgG antibodies present in women was 64.63%, while in 29.27% they were not detected. The repetition of the test was only necesary in 6.10% of patients (fig. 2).

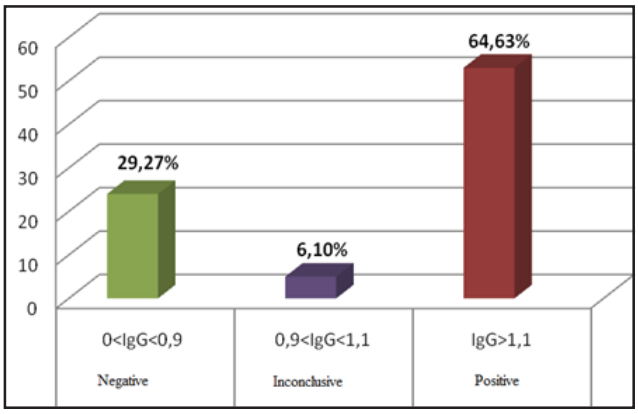

Fig. 2. Percentages on the presence or absence of IgG anti-CMV antibodies

In Romania, CMV prevalence varies between 70-80%, age being one of the factors that affects it. Studies in literature have shown an increase in seropositivity with age, so that in the general feminine population the risk is 25.80%. IgG antibody levels were higher in rural women than in urban areas due to poor living conditions and poorer hygiene that contribute to increased likelihood of contracting the infection [3, 4].

In developed countries, a low prevalence of IgG specific antibodies of 40-69% compared to 90-100% was observed in developing countries, but this depends on the virus circulation and the receptivity of the population. At the same time, there was an increase in seropositivity by parity, so that in nulliparous women it occurred in 66.7% compared to 96% in women with more than 3 births [3, 4].

In the determination of IgM specific anti-CMV antibodies, all results were negative, which means that women did not have acute CMV infection (table 5).

Table 5

THE LEVEL OF ANTI-CMV IgM ANTIBODIES (BIOLOGICAL REFERENCE RANGES)

Ig M		
Negative	Inconclusive	Positive
0<IgM<0.9	0.9<IgM<1.1	IgM>1.1
82	0	0
82		

False-negative results may occur if elevated levels of specific IgG antibodies competitive block IgM binding in CMV antigen [3, 7].

Rubella virus infection

Serological tests for the detection of IgG and IgM specific antibodies are an important tool in the monitoring and diagnosis of acute infection. The first humoral response of the infection is caused by the occurrence of specific IgM antibodies that can be detected within a few days from the onset of the symptomatology and have a maximum of 2-3 weeks after onset. After 180 days, IgM levels are rarely detectable. Approximately one week after the occurrence

of IgM-specific antibodies, specific IgG-like antibodies typically occur, and their level increases rapidly in the first 6-10 weeks. After this period, they progressively decrease to a titer of over 10 IU/mL, which is maintained throughout life. Reinfection is asymptomatic but accompanied by moderate increases in IgG antibodies [16, 18, 19].

Anti-rubella IgG maternal antibodies are transmitted to the fetus and persist for up to 6 months after birth. In congenital rubella syndrome, newborns have a trasitory increase in IgM antibodies and then IgG growth that persists long after the normal maternal antibody presence period.

Children with congenital rubella syndrome should be monitored and isolated as soon as the infection has been diagnosed. They are considered contagious in the first year of life, except for those with negative cultures [8,19].

Of the 75 patients who addressed at the laboratory, 64 (85.33%) had an IgG-type antibody above 10 IU/mL, which is a positive result and indicates either a history of infection or a recent infection. In such cases, detection of IgM-specific antibodies from the same serum is recommended, and if a negative result is obtained, the presence of immune status and the history of infection are confirmed.

For patients with inconclusive results, it is recommended to repeat the determination after approximately 7 days. For 6 (8%) of patients, the result was negative, indicating an absence of immunity (table 6). In the case of pregnant women, even in the absence of known exposure, it is recommended to repeat the determination from the fourth month of pregnancy.

Table 6

THE LEVEL OF IgG ANTI-RUBELLA ANTIBODIES (BIOLOGICAL REFERENCE RANGES)

Ig G		
Negative	Inconclusive	Positive
0<IgG<5 UI/ml	5<IgG<10 UI/ml	IgG>10 UI/ml
6	5	64
75		

The presence of IgG-specific antibodies in patients presenting at the laboratory was 85.33%. This result can also be influenced by recent vaccination, since in this case IgG-specific antibodies persist for the rest of their lives. Only 6 (8%) of the patients had no contact with the virus and were not vaccinated (fig. 3).

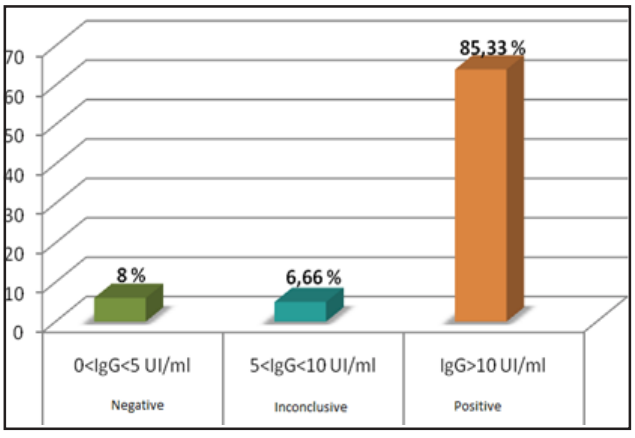

Fig. 3. Percentages on the presence or absence of IgG anti-rubella antibodies

The fact that for all patients a negative result of IgM antibodies was obtained confirms the absence of recent infection (table 7). Specific IgM antibodies appear in 80% of cases within the first 3 weeks after vaccination.

Table 7

THE LEVEL OF IgM SPECIFIC ANTI-RUBELLA ANTIBODIES (BIOLOGICAL REFERENCE INTERVALS)

Ig M		
Negative	Inconclusive	Positive
0<IgM<0.9 COI	0.9<IgM<1.1 COI	IgM>1.1 COI
75	0	0
75		

For children, diagnosis of rubella is determined by highlighting specific IgM-like antibodies. In most cases, IgM antibodies are detected in the first 3-5 months of life, becoming rarely detectable after 18 months.

A positive result for specific IgM antibodies may indicate a recent infection, but repetition of the assay is necessary as false positive results can be obtained by interfering with IgM antibodies specific to other infections: infectious mononucleosis or possible herpes virus infections [12, 18, 19].

Despite the introduction of effective vaccines and global vaccination programs, it is estimated that around 110,000 cases per year with congenital rubella syndrome exist [17].

Toxoplasmosis

The frequency of infection ranges between 20% and 80% depending on age and increasing with it. The immune response of the body to infection is represented by the appearance of IgM antibodies in the onset of the disease. They reach a maximum level in the first month and gradually decrease in 3-6 months, but there is the possibility to persist in low titres up to 2 years [20, 21].

IgG antibodies reach a maximum level within 1-2 months, persisting for life. They are useful for screening the immune status and diagnosing primary infection and reactivations [15].

Due to the fact that most people are seropositive, interpreting the titer should be based on clinical symptoms and patient history. A significant increase of more than 4 times the specific antibody titer may indicate an acute infection, but in immunocompromised patients the titers may be unsafe as these do not have the ability to produce enough antibodies to cause significant titration [15, 22].

To avoid the complications and consequences of transmission of the infection during pregnancy, it is advisable to assess the immune status prior to conception as it is believed that seropositive women before pregnancy do not transmit the infection to the future fetus. On the other hand, seronegative women have a high risk of contracting the infection during pregnancy, which is why prophylactic measures are required.

In an immunocompetent person, the previous infection leads to a negative IgM test, and resistance to infestation is indicated by the IgG positive test. Also, IgM antibodies are not present during reactivation, and IgG antibodies to *Toxoplasma gondii* do not differentiate between latent and reactivated infection [15, 22].

According to studies, the global prevalence of toxoplasmosis is about 10-80% of the population. The lowest seroprevalence, 10-30%, was observed in North Europe, North America and Asia, and the opposite is in Latin America and African countries. In Central and South Europe, which includes Romania, the prevalence is moderate, 30-50% [10, 11, 22].

Of the total of 92 patients who were tested for IgG toxoplasmosis-specific antibodies, 56 (60.86%) of these had a value of less than 6.5 IU/mL, which means they do not have a recent infection or in their history, being unimmunized (table 8). In 2 (2.17%) of patients, the IgG specific antibody titre was greater than 8 IU/mL, requiring the repeating of analysis and detection of IgM specific antibodies.

Table 8

THE LEVEL OF ANTI-ANTITOXOPLASMOSIS IgG ANTIBODIES (BIOLOGICAL REFERENCE INTERVALS)

Ig G		
Nonimmunized	Inconclusive	Immunized
0<IgG<6.5 UI/ml	6.5<IgG<8 UI/ml	IgG>8 UI/ml
56	34	2
92		

In our case, it can be seen that the prevalence of IgG-specific antibodies was only 2.17%, while the percentage of non-immunized individuals was 60.86%. For 36.95% of the patients the result was inconclusive and the test should be repeated (fig. 4).

The seroconversion of IgG-specific antibodies or their increase within 2-3 weeks confirms the recent infection.

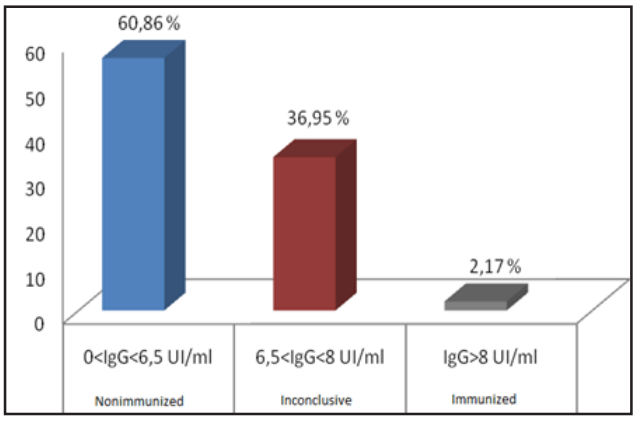

Fig. 4. Percentages representing the presence or absence of anti-antitoxoplasmosis IgG antibodies

For all patients, the negative result indicates the absence of a recent primary infection, although specific IgM antibodies may be found in those with reactivated infections. In the newborn in which specific IgM antibodies are detected and persists in several determinations, the diagnosis of congenital infection is suspected.

IgM-specific antibodies occur within the first 2 weeks of acute disease, and ultimately become undetectable, although they may be present at 18 months after acute infection, unlike IgG antibodies that grow more slowly reaching the peak within 1-2 months, and may remain elevated months or years. IgM antibodies with low IgG are specific in recent infections in immunocompetent patients.

Table 9

THE LEVEL OF IgM SPECIFIC ANTI-TOXOPLASMOSIS ANTIBODIES (BIOLOGICAL REFERENCE INTERVALS)

Ig M		
Negative	Inconclusive	Positive
0<IgM<0.9 COI	0.9<IgM 1.1 COI	IgM>1.1 COI
92	0	0
92		

Conclusions

The study aimed at determining the prevalence and incidence of congenital and perinatal infections caused by infectious agents which are grouped under the name TORCH complex.

The TORCH complex contains infectious agents that easily cross the placenta and are responsible for most malformative fetopathies.

In these infections, transplacental transmission occurs in a low percentage in the first trimester of pregnancy, but the consequences for the development of the fetus are severe, with congenital malformations or even abortion. The risk of transmission of infection increases with the advancement of pregnancy, but the severity of the disease in the fetus decreases.

In pregnant women, TORCH's laboratory diagnosis is mandatory, especially for situations where the correct decision on abortion is needed. Diagnosis involves the determination and dynamic titration of IgG and IgM specific antibodies for *Cytomegalovirus* (CMV), *Rubella virus* and *Toxoplasma gondii* parasite.

From the 95 patients (93 pregnant and 2 children) who were sent to a private laboratory in Timiºoara between July 2015 and June 2016, serum samples were collected for the IgG and Ig M antibodies to: *Cytomegalovirus* (CMV) - 82 samples, *Rubella virus* - 75 samples and *Toxoplasma gondii* - 92 samples.

Identification of infectious agents involved in TORCH syndrome may lead to a significant decrease in mortality and maternal-fetal morbidity.

It is important that pregnant women who have a risk factors for infection with TORCH syndrome agents are properly informed and tested to avoid serious complications in the baby.

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