

# Malformations of Cortical Development in Children with Congenital Cytomegalovirus Infection – A Study of Nine Children with Proven Congenital Cytomegalovirus Infection

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## ABSTRACT

*Congenital cytomegalovirus (CMV) infection is the most common vertically transmitted disease with the rate of the infection ranging from 0.2 to 2.4% in newborn infants. Congenital CMV infection causes multiorgan affection, but the most severe and permanent sequelae are those affecting central nervous system such as mental retardation, cerebral palsy, sensorineural hearing loss, chorioretinitis and seizures as a result of direct interference of the virus with neurogenesis. The time of acquiring infection is strongly connected to the level of child's disability. Infection in early pregnancy results in severe neurological sequelae, while later infection has less prominent signs. Radiological findings show connection between onset of infection and brain imaging, from lissencephaly, pachygyria, polymicrogyria, schizencephaly, calcification, cerebellar hypoplasia and/or hypoplasia/agenesis of corpus callosum as a result of an early infection, to white matter abnormalities including disturbed myelination as a result of a late infection. We present nine patients with proven congenital CMV infection and malformations of cortical development and their computed tomography/magnetic resonance (CT/MRI) findings along with clinical assessments. According to CT/MRI results we assume that two of our children with lissencephaly had an early onset of infection. The other seven with less severe cortical dysplasia in form of pachy/polymicrogyria were probably infected later. Cerebellar hypoplasia and/or calcifications in our patients also confirm an early onset of infection. Developmental outcome in all of our children was poor: moderate to severe psychomotor retardation has been diagnosed in all children; five of them have developed cerebral palsy (four have bilateral spastic and one dyskinetic) and one is estimated to have minor motor dysfunction. Seven out of nine developed epilepsy, chorioretinitis was found in three of them and sensorineural deafness in two of them. All of our children, except one, were presented by symptomatic infection, yet only four of them were recognized at birth. Therefore, congenital CMV infection should be considered as one of the reasons for childhood disability more often.*

**Key words:** congenital cytomegalovirus infection, cortical malformations, brain development, lissencephaly, pachygyria, polymicrogyria

## Introduction

Congenital cytomegalovirus (CMV) infection is the most common vertically transmitted disease. The rate of the infection is reported in 0.2 to 2.4% of newborn infants. Approximately 10% of those children will have symptoms at birth: neonatal sepsis, intrauterine growth

retardation, jaundice, petechiae, thrombocytopenia, hepatosplenomegaly and/or microcephaly<sup>1</sup>. Asymptomatically infected children have a 10% chance to develop neurodevelopmental sequelae later in childhood. It has been largely assumed that higher risk of fetal infection

and poorer outcome are often associated with primary and symptomatic maternal CMV infection<sup>1</sup>, although cortical maldevelopment in congenital CMV infection has been reported in infants born from mothers with preconceptional immunity<sup>2</sup>.

Congenital CMV infection causes multiorgan affection, but most severe and permanent sequelae are those affecting central nervous system (CNS) such as mental retardation, cerebral palsy, sensorineural hearing loss, chorioretinitis and seizures<sup>1,3</sup>. CMV infection has also been associated with autism, hyperactivity, behavior problems, poor impulse control and reduced pain sensation<sup>1,4</sup>. The time of acquiring infection is strongly connected to the level of child's disability. Infection in early pregnancy results in severe neurological sequelae, while later infection has less prominent signs. Neuroradiological findings of computed tomography/magnetic resonance (CT/MRI) also show connection between an onset of infection and brain abnormalities, from lissencephaly, pachygyria, polymicrogyria, schizencephaly, calcification and cerebellar hypoplasia as a result of an early infection, to white matter abnormalities including disturbed myelination as a result of a late infection<sup>5,6</sup>.

Congenital CMV infection can be nowadays more accurately and easily diagnosed by advanced laboratory tests, in particular detection of polymerase chain reaction for CMV DNA and neuroimaging findings, especially MRI of the brain which is superior in visualization of dis-

turbed neuronal migration, parenchymal defects, white matter abnormalities and/or delayed myelination as well as other brain disorders<sup>7,8</sup>.

The aim of our study was to correlate malformations of cortical development as visualized by CT/MRI with neurodevelopmental outcome in nine children with proven congenital CMV infection and try to connect onset of infection with the severity of abnormalities.

## Patients and Methods

Nine patients (4 boys, 5 girls) with congenital CMV infection were followed up in a period from 2000 to 2009. CMV infection was confirmed in all children by specific serology (IgG/IgM of mother and child) and/or polymerase chain reaction for CMV DNA in serum and/or isolation of CMV virus from urine. Either cranial CT or MRI was performed on all of the patients, and three of them underwent both diagnostic procedures. They all had prospective follow up with a final neurodevelopmental assessment from 5 months to 15 years of age respectively.

## Results

In Table 1 we present results of laboratory tests required for confirmation of congenital CMV infection along with results of clinical assessment of children examined

**TABLE 1**  
CLINICAL ASSESSMENT IN CHILDREN WITH CONGENITAL CMV INFECTION AND MALFORMATIONS OF CORTICAL DEVELOPMENT

Patient Sex/Age	CMV infection proven by	Clinical assessment	
		Neonatal age	Follow up
1. I.L. M/7 y	PCR for CMV DNA in serum	1 <sup>st</sup> born, Term born, Asymptomatic	Mental retardation, Bilateral spastic CP, Epilepsy, Microcephaly
2. K.I. M/11 y	Specific serology, Isolation of CMV from urine	3 <sup>rd</sup> pregnancy, Term born, Microcephaly	Mental retardation, Bilateral spastic CP, Epilepsy, Microcephaly
3. L.P.K. F/15 y	Specific serology, Isolation of CMV from urine	3 <sup>rd</sup> pregnancy-mother suffered mild infection, Term born, Hepatosplenomegaly, Jaundice, Petechiae	Mental retardation, Bilateral spastic CP, Epilepsy, Sensorineural deafness, Microcephaly
4. J.L. F/11 y	Specific serology, Isolation of CMV from urine	1 <sup>st</sup> born, Term born, IUGR, Microcephaly	Mental retardation, Dyskinetic CP, Epilepsy, Sensorineural deafness, Chorioretinitis, Microcephaly,
5. S.P. M/4 y	Specific serology, PCR for CMV DNA in serum	1 <sup>st</sup> born, Term born, IUGR	Mental retardation, Minor motor dysfunction, Microcephaly
6. H.H. F/6 mo	Specific serology in mother during pregnancy and child	1 <sup>st</sup> born, Term born, Microcephaly	Epilepsy, Severe psychomotor delay, Microcephaly
7. K.N. M/2 y	Specific serology in mother and child	5 <sup>th</sup> pregnancy, Term born, Sepsis	Mental retardation, Spastic syndrome, West Syndrome, Chorioretinitis, Microcephaly
8. R.T. F/1,5 y	Specific serology, PCR for CMV DNA in serum	1 <sup>st</sup> born, Preterm, Microcephaly, Jaundice	Mental retardation, Spastic syndrome, Epilepsy, Chorioretinitis, Microcephaly
9. M.P. F/6 y	Specific serology, PCR for CMV DNA in serum	1 <sup>st</sup> born, IUGR, Hyperbilirubinemia, Microcephaly	Mental retardation, Bilateral spastic CP, Microcephaly

CP – cerebral palsy, IUGR – intrauterine growth retardation, PCR – polymerase chain reaction

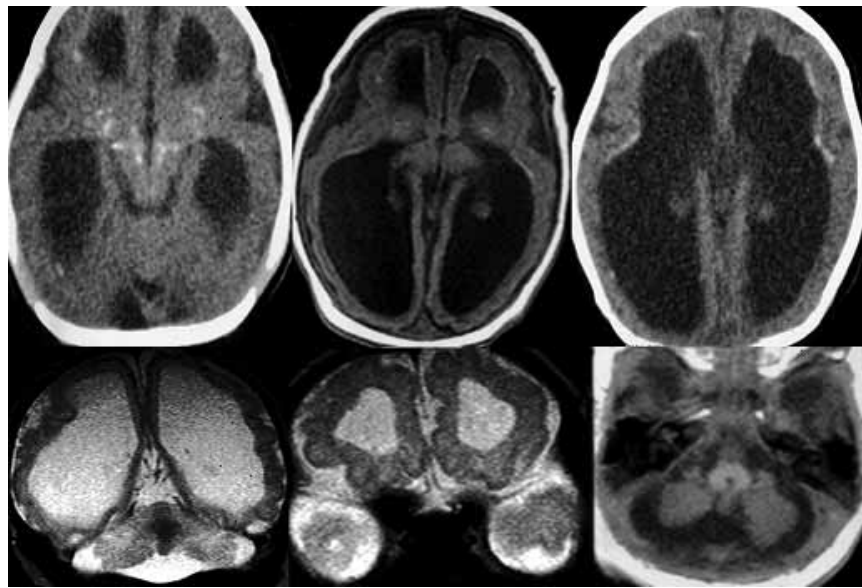


Fig. 1. Neuroimaging findings (Computed Tomography/Magnetic Resonance Imaging) in child 6 with congenital CMV infection show polymicrogyria associated with ventriculomegaly, hypoplastic corpus callosum, cerebellar hypoplasia and hypoplastic brain stem. Periventricular calcifications are better shown in images obtained by computed tomography.

at neonatal age and the follow up. Table 2 and Figure 1 present brain CT/MRI findings in children with congenital CMV infection.

Table 1 demonstrates that only one child out of nine examined was asymptomatic at birth. He was referred to our hospital at the age of 10 months due to psychomotor delay and hypotonia. All the other children presented typical symptoms at newborn age, but in four of them they remained unrecognized. Patients 3, 5, 6 and 7 presented one or more of the following symptoms at birth: intrauterine growth retardation, microcephaly, neonatal sepsis, hepatosplenomegaly, jaundice and/or petechiae.

Four patients unrecognized at neonatal age were presented with epilepsy and/or psychomotor delay only at the age of 3–11 months respectively. One of the children was preterm and six out of nine were firstborns. At the follow up assessment microcephaly and moderate to severe psychomotor retardation has been diagnosed in all children; five of them have developed cerebral palsy (CP), four have bilateral spastic and one dyskinetic CP, and one child is estimated to have minor motor dysfunction. Seven out of nine developed epilepsy, chorioretinitis was found in three of them and sensorineural deafness in two of them. Patients 6, 7 and 8 were too young to be classified as CP but they have intractable epilepsy, psychomotor delay and microcephaly.

Table 2 presents results of CT and/or MRI scans. All of the children had malformation of cortical development of various degrees. In six out of nine CT scan revealed calcifications. CT and/or MRI showed pachygyria in four of them, lissencephaly in two and polymicrogyria in three of the examined children. Cerebellar hypoplasia was reported in four children, hypoplastic corpus callosum in three and hypoplastic brain stem in one of them. In three

children leukoencephalopathy was found. CT was proven as superior in revealing calcifications, while MR was better in showing all other features.

Two of our children, S.P. and R.T. received ganciclovir therapy. S.P. received 3 weeks of intravenous ganciclovir treatment at the age of 2 months. At the time he doesn't have hearing problems and his brainstem-evoked response (BSER) at the age of one month and 13 months were normal. R.T. received two cycles of 3 weeks of intravenous ganciclovir treatment because of recurrent CMV viraemia at the age of 7 months. At that time BSER showed bilateral hypoacusis. Nine months after beginning of therapy her BSER was normal.

## Discussion

Wide spectrum of brain structural abnormalities in patients with congenital CMV infection is a result of a period of brain development in which injury occurred. Proliferation of neurons occurs in the germinal zone in periventricular region from 8th to 16–20th week of gestational age and their migration to the cortex continues until 24–26th week<sup>5</sup>. According to Rakic population of neural stem cells transform into radial glial cells which over time produce migrating neurons and eventually undergo apoptosis or generate ependymal cells, astrocytes, glial progenitors or astrocytic stem cells that retain neurogenic potential<sup>9</sup>. The process of neural migration along radial glial fibers to cortical plate is then followed by cortical organization. At the end of neuronal production period begins the period of astrocytes proliferation. During the 26th week of gestation germinal zone is at its maximum and astrocytes are predominant proliferating cell type<sup>5</sup>. Astrocytes than also migrate along radial glial fi-

**TABLE 2**  
NEUROIMAGING FINDINGS (CT/MRI) IN CHILDREN WITH  
CONGENITAL CMV INFECTION AND MALFORMATIONS OF  
CORTICAL DEVELOPMENT

Patient number	Computed Tomography (CT)	Magnetic Resonance Imaging (MRI)
1.	–	Polymicrogyria, Ventriculomegaly, Cerebellar hypoplasia
2.	Pachygyria, Diffuse leukoencephalopathy, Bilateral parenchymal calcifications	–
3.	Pachygyria, Periventricular calcifications	–
4.	Pachygyria, Cerebellar hypoplasia, Leukoencephalopathy, Paraventricular occipital cyst, Calcifications	Pachygyria, Cerebellar hypoplasia, Leukoencephalopathy, Paraventricular occipital cyst
5.	Pachygyria, Calcifications	Pachygyria, Hypoplastic corpus callosum, Periventricular leucomalation
6.	Polymicrogyria, Cerebellar hypoplasia, Hypoplastic corpus callosum, Periventricular calcifications	Polymicrogyria, Cerebellar hypoplasia, Hypoplastic corpus callosum, Hypoplastic brain stem
7.	Lyssencephaly, Periventricular calcifications	–
8.	Lyssencephaly, Arachnoidal cysts	–
9.	–	Polymicrogyria, Cerebellar hypoplasia, Subependymal heterotopy, Hypoplastic corpus callosum

bers to cortical plate and accurate migration pattern seems to be necessary for appropriate cortical organization. In the first half of the third trimester oligodendrocytes are produced by differentiation of astrocytes and are involved in myelination of central nervous system<sup>5</sup>. Therefore infection in early pregnancy results in reduction of all cell types with neuroradiological findings of lissencephaly<sup>5</sup>. Infection occurring after 22–24th week results in normal number of produced neurons, but reduced number of astroglia, and appears as polymicrogyria, while infection in third trimester results in white matter abnormalities and/or disturbed myelination<sup>5</sup>. As cerebellum develops early in pregnancy cerebellar hypoplasia as well as calcifications and are also signs of early infection<sup>5</sup>. In our study we could not clinically estimate the time of infection in none of the children as most of infections were asymptomatic. Only one mother reported very mild infectious symptoms during 3–4th month of pregnancy and again at the end of pregnancy, but the in-

fection wasn't confirmed by any laboratory test. According to CT/MRI findings we assume that two of our children with lissencephaly had an early onset of infection. Other seven out of nine children with less severe cortical dysplasia in form of pachy/polymicrogyria were probably infected later, between 18th and 24th week of gestation. Coexisting cerebellar hypoplasia and/or calcifications also confirm an early onset of infection. Similar results were reported by Hayward et al. in the study of five children with lissencephaly-pachygyria associated with congenital CMV infection. All of them had pachygyria, calcifications and abnormal periventricular white-matter signal and some had ventriculomegaly<sup>10</sup>.

Pathogenesis of brain damage is still debated. Some studies suggest that the severity of outcome is associated with ischemic damage mediated by vasculitis related to the viral presence in the endothelial cells of placenta or brain<sup>7</sup>. Others assume that the main reason would be direct viral inhibition of proliferation and differentiation of the neural progenitor cells (NPC) to neuronal and glial cells in addition to induction of neuronal cell loss by apoptosis<sup>11</sup>. Immune inflammatory response to CMV in the infected brain is also considered<sup>7</sup>. Luo et al found that human neuronal progenitor cells (NPC) and their derivatives, glial and neuronal cells are fully permissive for human CMV infection<sup>12</sup>. All three cell types showed expression of viral genes and established the viral replication. While NPC and astroglia soon went to apoptosis, some neurons showed long-term survival during which they released small quantities of virus. Lytic infection of NPC causes loss of all cell types, mostly affecting astroglia thus disturbing appropriate cortical organization<sup>13</sup>. In addition, radial glial cells, as precursors of all cell types and scaffoldings of neuronal migration, were the main targets of infection in mouse embryos in some experiments<sup>14</sup>. These processes result in severe brain malformation in early pregnancy. On the other hand, persistent neuronal infection presumably causes brain dysfunctions and also serves as viral reservoir<sup>12,13</sup>.

Studies of Boppana et al. showed that abnormal CT scans (especially intracerebral calcification) was noted in 70% of symptomatic newborns and that most of them (90%) would develop at least one neurodevelopmental sequela<sup>15</sup>. Moreover, Noyola et al. defined combination of microcephaly and calcification on CT as best predictor of poor neurodevelopmental outcome<sup>1</sup>. Our results are similar: almost all children were symptomatic, all had microcephaly and six of them had calcifications. Developmental outcome in all of our children was poor with multiple and severe neurodevelopmental disorders. Instead of using CT scan, De Vries et al. recommend usage of magnetic resonance imaging since MRI is superior in diagnosis of neocortical dysplasia, temporal cysts, cerebellar hypoplasia, periventricular (pseudo)cysts and shape of lateral ventricles<sup>8</sup> what can be also seen in our study. Nevertheless we found CT more accurate in depicting parenchymal calcifications as one of the major signs in congenital CMV infection.



It has been documented that injury of brain parenchyma can progress after birth<sup>5</sup> so adequate treatment could improve the outcome of congenital infection. Though previous studies suggest that ganciclovir administered intravenously for 6 weeks in neonatal period in symptomatically infected infants prevents hearing deterioration at 6 months and may prevent hearing deterioration at the age of one year or more<sup>16</sup>, it is not recommended in treatment of asymptomatic infants because of risk of neutropenia<sup>7</sup> and nephrotoxicity<sup>17</sup>. Moreover, ganciclovir is mutagenic, teratogenic and carcinogenic in animal models and because of that exposed children should have

long-term follow up<sup>17</sup>. Two children in our study treated with ganciclovir were closely monitored and they showed no side-effects. One of those children had bilateral hypacusis before treatment and nine months after treatment her BSER was normal. The other one didn't have hearing disorder before and after treatment.

We find important to stress out that almost all of our children had symptomatic infection and yet only four of them were recognized at birth. Therefore, congenital CMV infection should be considered more often at neonatal age, because of its significant role in a wide variety of neurological disabilities.

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## MALFORMACIJE KORTIKALNOG RAZVOJA U DJECE S KONGENITALNOM CITOMEGALOVIRUSNOM INFEKCIJOM

### SAŽETAK

Kongenitalna citomegalovirusna infekcija (CMV) je najčešća bolest koja se prenosi s majke na dijete i zahvaća 0,2–2,4% novorođene djece. Infekcija uzrokuje oštećenja više organskih sustava, ali najteže i trajne posljedice su rezultat oštećenja središnjeg živčanog sustava kao rezultat izravne interferencije virusa s neurogenezom uzrokujući široki spektar neurorazvojnih odstupanja. Među njima svojom težinom se ističu razvoj mentalne retardacije, cerebralne paralize, epileptičkih napadaja, gubitak zamjedbenog sluha te pojava korioretinitisa. Razina neurorazvojnog odstupanja djeteta usko je povezana s vremenom u kojem je infekcija nastupila. Infekcije rano u trudnoći rezultiraju teškim posljedicama po razvoj mozga, dok su posljedice infekcije koja je nastupila kasnije znatno blaže. Neuroradiološki nalazi kompjutorizirane tomografije i magnetske rezonancije (CT/MR) također ukazuju na povezanost između vremena stjecanja infekcije i razvojnog poremećaja mozga. Nalazi se kreću od lizencefalije, pahigirije, polimikrogirije, shizencefalije, kalcifikacija, hipoplazije malog mozga i/ili hipoplazije/ageneze korpusa kalozuma kao rezultata rane infekcije, do abnormalnosti vezanih za razvoj bijele tvari mozga, uključujući poremećaje u mijelinizaciji koje nastupaju nakon kasnije infekcije. Prikazujemo devet bolesnika s dokazanom CMV infekcijom i malformacijama razvoja korteksa te njihove radiološke nalaze kompjutorizirane tomografije/magnetske rezonancije, uz nalaze kontinuirano provedenih kliničkih pregleda. Vodeći se neuroradiološkom i kliničkom slikom pretpostavili smo da je dvoje djece sa znakovima lizencefalije bilo zaraženo rano u trudnoći, dok je ostalih sedmero s manje teškim oblicima kortikalne displazije u smislu pahi/polimikrogirije vjerojatno zaraženo nešto kasnije. U prilog našoj pretpostavci o ranom nastupu infekcije idu i uočene hipoplazija malog mozga i/ili pojava kalcifikacija u naših pacijenata. Neurorazvojni ishod u sve naše djece je bio loš: umjerena do teška psihomotorna retardacija je utvrđena kod sve djece; petero je razvilo cerebralnu paralizu (četvero bilateralnu spastičnu

i jedno diskinetsku), dok je kod jednog djeteta utvrđena minimalna motorička disfunkcija. Sedmero od devet pacijenata ima epilepsiju, kod troje je dijagnosticiran korioretinitis, a kod dvoje zamjedbena gluhoća. Važno je istaći da su gotovo sva djeca rođena sa znakovima simptomatske infekcije, ali samo ih je četvero prepoznato neposredno nakon poroda iz čega se može zaključiti da je broj zaražene djece veći nego što se procjenjuje te bi se kongenitalna CMV infekcija morala češće uzeti u obzir kao etiološki faktor neurorazvojnih odstupanja u djetinjstvu.