

# Cytomegalovirus (CMV) screening results in pregnant women admitted to a tertiary center in the Middle Anatolia

## Orta Anadolu bölgesinde tersiyer bir merkeze başvuran gebelerde Sitomegalovirüs (CMV) tarama sonuçları

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### ABSTRACT

**Objective:** Human cytomegalovirus (CMV) is a common cause of mother-to-child infection that may lead to severe long-term sequelae in affected infants and is the most common non-genetic cause of hearing loss. CMV prevalence is reported to be higher in developing countries and seroprevalence varies between countries and even regions. The aim of this study was to evaluate the seroprevalence of CMV in pregnant women who applied to regional reference hospital and to investigate the efficacy of antenatal CMV screening.

**Methods:** A total of 3362 patients admitted to a university hospital pregnant outpatient clinic between January 2016 and September 2018 were included in the study. After serological examination, avidity test was performed in cases with results suggestive of active infection. Amniocentesis was recommended to patients with low avidity and these patients were evaluated by Polymerase Chain Reaction (PCR) method.

**Results:** The frequency of CMV immunoglobulin

### ÖZET

**Amaç:** Anneden çocuğa geçen enfeksiyonların önemli bir nedeni olan insan sitomegalovirüsü (CMV), etkilenen bebeklerde uzun dönemde ciddi sekellere yol açabilir ve yenidoğanlarda genetik olmayan konjenital işitme kaybının en yaygın nedenini oluşturmaktadır. Gelişmekte olan ülkelerde CMV prevalansının gelişmiş ülkelere oranla daha yüksek olduğu bilinmektedir ve seroprevalansın ülkeler, hatta bölgeler arasında dahi ciddi farklılık gösterdiği bildirilmektedir. Bu çalışmanın amacı, bir bölge referans hastanesine başvuran gebe kadınlarda CMV seroprevalansının değerlendirilmesi ve gebelik sırasında CMV taramasının etkinliğinin araştırılmasıdır.

**Yöntem:** Ocak 2016 ile Eylül 2018 tarihleri arasında bir üniversite hastanesi gebe polikliniğine ayaktan başvuran toplam 3362 hasta çalışmaya dahil edilmiştir. Serolojik inceleme sonrasında aktif enfeksiyon olduğu düşünülen olgularda avidite testi uygulanmıştır. Bu test sonucu düşük avidite izlenen olgulara invaziv amniyosentez işlemi önerilmiştir ve alınan örnekler Polimeraz Zincir Reaksiyonu (PCR) metodu ile incelenmiştir.

**Bulgular:** CMV immunoglobulin (Ig) G ve Ig M seropozitiflik

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(Ig) G and M seropositivity rates were 96.40% and 1.75%, respectively. According to avidity test results of patients with CMV infection; low, intermediate and high avidity levels were found in 10 (20.83%), 3(6.25%) and 35 (72.91%) patients, respectively. PCR analysis results showed primary infection in 3 of the cases with low avidity. Only one infant had signs of congenital CMV infection at the time of birth.

**Conclusion:** Although routine CMV screening in pregnancy is not recommended due to lack of adequate studies on the validity of treatment and cost-effectiveness, serological examination may be beneficial especially in risky groups in developing countries. Further studies on vaccination and anti-viral therapy may provide more comprehensive information about the necessity and efficacy of screening.

**Key Words:** CMV, congenital infection, pregnancy, screening, serology

oranları sırasıyla %96,40 ve %1,75 olarak bulunmuştur. CMV enfeksiyonu düşünülen hastalardaki avidite test sonuçları incelendiğinde 10 (%20,83) olguda düşük avidite, 3 (%6,25) olguda ara düzeyde avidite ve 35 (%72,91) olguda yüksek avidite olduğu izlenmiştir. Düşük avidite saptanan 10 olguda yapılan PCR analiz sonucunda bu hastaların üçünde akut primer enfeksiyon bulguları gözlenmiştir. Doğum sonrası yapılan muayenelerde bu yeni doğanların birinde konjenital CMV enfeksiyonu bulguları saptanmıştır.

**Sonuç:** CMV enfeksiyonunun tedavisinde kullanılan ajanların gerek maliyet, gerek terapötik etkinliği ile ilgili yeterli çalışma bulunmaması nedeniyle gebelikte rutin CMV taraması önerilmemektedir. Ancak, özellikle gelişmekte olan ülkelerdeki riskli gruplarda serolojik değerlendirme faydalı olabilir. Aşı ve anti-viral tedavi hakkında yapılacak sonraki geniş kapsamlı çalışmalar ile taramanın gerekliliği ve etkinliği hakkında daha fazla bilgi sahibi olunabilir.

**Anahtar Kelimeler:** CMV, konjenital enfeksiyon, gebelik, tarama, seroloji

## INTRODUCTION

Human cytomegalovirus (CMV) is an enveloped, double-stranded DNA virus within the family of  $\beta$ -herpesviruses. It is affecting approximately 40,000 infants each year in the United States and is the leading non-genetic cause of congenital hearing loss (1, 2). The prevalence of congenital infection varies significantly between regions and countries, and as is known, higher rates are observed in developing countries. A systematic review of birth prevalence of congenital CMV in developing countries included 11 studies with sample sizes ranging from 317 to 12,195 and reported rates from 0.6% to 6.1% (3). In a study conducted in Turkey that included almost 1000 patients, the seroprevalence has been reported to be 1.9% (4).

Maternal acquisition of infection might resemble from multiple ways including sexual and non-sexual contact, blood products and organ transplant (5). Although the infection is often asymptomatic or vague, it may present with flu-like symptoms including fever, myalgia, lymphadenopathy and fatigue. After causing primary infection, viral components can be found in many body fluids such as urine, saliva, vaginal secretions and breast milk for months (6).

Congenital infections are the result of transplacental transmission of CMV and infection might occur due to primary CMV infection, re-infection with a new strain of CMV or re-activation of latent infection (7, 8). While most of the infected neonates have no signs of infection, sensorineural

type hearing loss, visual disorders and impaired psychomotor development will occur in 5 to 10% percent of this newborns (9, 10). Symptoms such as intrauterine growth restriction (IUGR), microcephaly, thrombocytopenia, anemia and jaundice develop in %10-15 in severely affected cases. The most important causes of mortality in these neonates are disseminated intravascular coagulation, hepatic insufficiency and concomitant infections (11, 12).

Diagnosis of congenital infection can be made by isolation of virus with culture techniques, detection of CMV antibodies in serological tests and identification of CMV-DNA by PCR from various samples (13, 14). Serological tests, which can also be used for screening, are frequently used among these methods. Primary infection diagnosis is based on detection of immunoglobulin (Ig) G in the serum of the patient who was previously seronegative or detection of Ig M antibodies with low Ig G avidity (15).

At present, antenatal CMV screening necessity remains controversial due to lack of suitable vaccines and treatment. In addition, differences between seropositivity rates among regions and countries deepen this uncertainty. However, recent studies demonstrated benefits of early diagnosis and prospective success of antiviral therapy in congenital CMV cases. The aim of this study was to evaluate the seroprevalence of CMV in pregnant women who applied to regional reference hospital and to investigate the possible profits and handicaps of antenatal CMV screening.

## MATERIAL and METHOD

We retrospectively reviewed patients who applied for pregnancy follow-up in a university obstetrics outpatient clinic between January 2016 and September 2018. After obtaining approval from the hospital institutional review board (reference number: 2019-146), demographic data and clinical characteristics of the patients were collected from patient charts and hospital records. Written informed

consent for all of the gynecological interventions was obtained for future use. The study was conducted according to the principles of the World Medical Association Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects, amended in October 2013).

A total of 3362 patients with intrauterine pregnancy at first trimester were included in this study. Intrauterine pregnancy diagnose was made by ultrasound and recorded in hospital database. Demographical features including patients' age, obstetrical history and comorbidities at the first prenatal visit were reviewed from patient files.

CMV IgG and IgM antibodies were tested according to clinical standard application and pregnancy follow-up protocol on the first prenatal visit after fetal heart beat was observed. CMV electrochemiluminescence immunoassay (ECLIA) (Elecsys, Roche Diagnostics, Mannheim, Germany) was utilized to measure antibody titers. The tests were performed on the Cobas 6000 analyzer. According to manufacturer's instructions, values of IgG and IgM levels greater than 6 IU/ml and 1 IU/ml were regarded as positive, respectively.

Women who had high levels of CMV IgG and IgM levels were additionally tested with avidity test to determine whether acute or chronic infection. While, avidity index greater than 0.65 for CMV Ig G was regarded as high avidity, less than 0.40 was regarded as low avidity and used as a potential marker for acute infection. All positive test results were reevaluated and controlled. Values between 0.40 and 0.65 were considered as intermediate values and necessary further investigations were made.

All data analyses were performed using SPSS (Statistical Packages for The Social Sciences) software, version 22.0 (SPSS Inc., Chicago, USA). Numbers and percentages were used as descriptive statistical methods in evaluating the data.

## RESULTS

The mean age of the patients who applied to a tertiary care hospital between January 2016 and December 2018 was 25.4 years (range 15-44). The frequency of CMV IgG and IgM seropositivity was found to be 96.40% and 1.75%, respectively (Table 1). Only one patient had IgM seropositivity with negative IgG result. In this case, repeated IgM test result was reported as negative. When avidity test was performed in both IgG and IgM positive 48 patients; low, intermediate and high avidity levels were found in 10, three and 35 patients, respectively. Nine patients were lost to follow-up and data about

these patients could not be reached. All intermediate avidity antibody test results (n=3) were repeated and later confirmed as high avidity.

Ten patients with low avidity were recommended for perinatology referral and PCR DNA analysis with amniocentesis and eight patients were accepted for invasive testing. Three were found to have positive test results for PCR. In one of these patients, IUGR developed and in another case, sonographic examination performed at 24th week showed megacysterna magna and periventricular echogenicity and this neonate diagnosed as congenital CMV infection (Figure 1).

Table 1. CMV IgG and M seropositivity rates by years

Years	Total Test n	CMV IgG (+) n (%)	CMV IgM (+) n (%)
2016	1587	1504 (94.77%)	26 (1.63%)
2017	1044	1016 (97.31%)	20 (1.91%)
2018	731	721 (98.63%)	13 (1.77%)
Total	3362	3241 (96.40%)	59 (1.75%)

Abbreviations: CMV; cytomegalovirus, IG; immunoglobulin

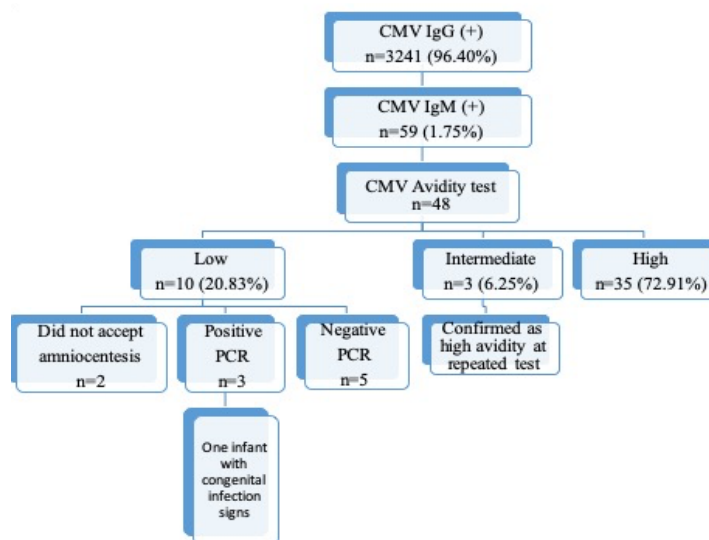


Figure 1. Flowchart for CMV screening

## DISCUSSION

Our data demonstrated the seroprevalence and epidemiology of CMV infection in the mid-northern region of Turkey. We examined retrospectively data of more than 3,000 pregnant women and found that the seroprevalence of CMV was 96.40% in our cohort, which is similar to previous reports from Turkey (16-18). It is well known that seropositivity rates are higher in developing countries. As a result of high seroprevalence, a large supply of CMV continuously exists in the population. Among the reasons behind the higher rates of CMV seropositivity compared to developed countries, crowded family life, inadequate infrastructure and low socioeconomic status are major subjects (7).

CMV IgM positivity rates also vary in population based studies. Similar to our results, Aynioğlu et al. reported that IgM positivity in their study population was 2% (19). In another study from eastern region of Turkey, authors found 1.7% positivity rates for IgM (20). In explaining these high rates, the authors also highlighted the impact of low socioeconomic status and especially the impact of family life with more children.

In addition to serological tests, imaging examinations are frequently used as an important tool for screening congenital CMV infection. Studies have shown that sonographic abnormalities, such as intracranial calcification, IUGR, periventricular echogenities, ventriculomegaly and microcephaly are essential signs for congenital CMV prediction (21, 22). In our study, the fetus with periventricular echogenicity and mega cisterna magna during antenatal sonography was diagnosed as congenital CMV after birth. Although IUGR was observed in another case, the neonate was not significantly affected by CMV infection in postnatal examination. Since there are many factors affecting intrauterine growth and enlargement, IUGR related signs have lower sensitivity for congenital CMV diagnosis

antenatally. Nevertheless, it should be noted that in case of monitoring these abnormalities in sonography, intrauterine infections should be considered and evaluated regardless of seropositivity.

An important feature of this study is that it analyzes the results of congenital CMV screening of more than 3000 pregnant patients. As mentioned earlier, CMV affects 0.2-2.2% of all neonates and only 5-10% of that newborns are symptomatic at birth (23, 24). In our study, it was observed that seroprevalence of congenital CMV rates was in support of these classical data. Although high seroprevalence in society is thought to be protective for symptomatic infection, its efficacy for predicting long-term effects is limited (25). In the same study, the authors reported a positive correlation between high seroprevalence and congenital infection and explained this paradoxical condition with increased risk of infection due to viral load in the host.

Although congenital CMV infection is the most common non-genetic cause of deafness and one of the main causes of neurological sequelae in neonates, opinions about screening are contradictory in the literature. The underlying reason for this condition is that the serological evaluation is insufficient in special circumstances, screening is not cost effective and the treatment options are limited in a possible diagnosis. As known, gold standard method for primary CMV infection diagnosis is the detection of IgG seroconversion. Since it is not known how long this IgG positivity occurs before pregnancy, IgM test is applied to secure the diagnosis. Therefore, tests for maternal serum CMV IgM are commonly used to identify primary CMV infection. However, due to long persistence in the host and detection during latent CMV reactivation, sensitivity of CMV IgM for detecting primary infection is only 20-25% (26). Therefore, IgG avidity test is applied especially in discrimination between active and reactivation of latent infection. The avidity test is based on measuring the binding

affinity of IgG antibodies to IgG antigens. Its index increases over time and while low levels of avidity indicate recent infection, high levels point out previous CMV infections (27). Studies have demonstrated that CMV IgG avidity index is a notable tool to predict congenital infection (21, 28). Although the applicability of screening programs is difficult, some groups have reported that nearly 80% of the cases can be caught by screening (29). As Sert et al. indicated in their comprehensive analysis, it may be recommended to screen particular groups, including patients with flu-like symptoms, with abnormalities in sonographic examination and patients contact and work with children (30). Society for Maternal-Fetal Medicine (SMFM) also do not recommend routine screening of all pregnant women for evidence of primary CMV infection at this time (31). In addition to the lack of sufficient studies and lack of evidence to demonstrate the effectiveness of the screening tests mentioned earlier, SMFM underlined the possible harmful side effects and unnecessary interventions due to routine screening.

As mentioned earlier, studies on antenatal CMV treatment efficacy and side effect profile are limited. The use of CMV hyperimmunglobulin (HIG) was evaluated in the CHIP study and did not show a significant reduction in congenital infection (2).

The study also showed a non-significant increase in adverse effects including preeclampsia and IUGR in HIG arm of the study population. Antiviral therapy of infected fetuses has been studied in small series and case reports. In a observational study, the authors reported that administration of valacyclovir in pregnant women with fetal CMV might decrease viral loads and provide therapeutic effect (32). In another study, supportive of the previous data, the use of oral ganciclovir showed that the viral load in the amniotic fluid was reduced and the newborn was born without congenital infection (33). In light of these data, any antenatal therapy, either with anti-virals or HIG, should only be offered as part of a research protocol (31).

In conclusion, although universal guidelines do not recommend routine screening for CMV during pregnancy, identification of risky groups especially in countries with high seroprevalence can provide improved antenatal results. Maternal flu-like symptoms and essential findings in sonographic examination during antenatal follow-up may be useful in determining risk groups. With the increasing number of studies showing the effectiveness of anti-viral treatment and analyzing the side effects more precise, the necessity of screening programs will be revealed more clearly.

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