Antenatal Detection of Congenital Cytomegalovirus Infection (A Comparative Study between Serological and Molecular Identification of Cytomegalovirus Infection)

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Abstract: Background & objectives: Cytomegalovirus (CMV) is a common virus that infects most people worldwide. CMV infection is usually harmless and rarely causes illness. A healthy immune system can hold the virus in check. However, if a person's immune system is seriously weakened in any way, the virus can become active and cause CMV disease. **Methods:** The samples consisted of 120 pregnant women, prospectively screened for CMV by serology from March 2012 to November 2012. The women were presenting for routine antenatal care at a tertiary referral women's hospital Maternal and Children Hospital (M.C.H.) Buridah, Elqassem, All subjects gave written consent. CMV IgG and IgM were detected in patient serum by using a commercial microparticle enzyme immunoassay, and determination of the presence of CMV genome by PCR in Amniotic Fluid**. Results:** The age range of the pregnant women was 18 to 46 years, with no significant difference seen between the mean age of seropositive and seronegative women. The CMV seropositivity rate for the pregnant women showed that, overall, 76.8% women were CMV IgG positive at pregnancy. Pregnant women were considered in high risk due to (i) documented seroconversion to positively for CMV from 3 months before conception to the end of the first trimester of pregnancy (four patients) (ii) CMV symptoms (increase in liver enzyme levels and/or fever) and presence of CMV immunoglobulin M (IgM) (five patients) (iii) intrauterine growth retardation as detected by ultrasound (three patients). PCR was used to detect excretion of CMV from the amniotic fluid in 5 of six cases (83.3%) were sero positive, and 1 false negative(16.7%). In group without infection, PCR was positive in 1 case (1 false positive). **Conclusion:** Congenital CMV infection is a major health problem that should be approached on the basis of which women should be enrolled in prenatal diagnostic programs, which clinical specimens should be tested, and which laboratory procedures should be adopted for the diagnosis of congenital CMV transmission or infection. Determination of the presence of CMV genome by PCR in amniotic fluid can be considered as a rapid,non invasive and accurate tool for the prenatal diagnosis of congenital CMV infection but negative results cannot rule out of infection,So that diagnosis of this infection based on correlation between serological and PCR Identification.
[Hany A. Abdel-Hafeez, Gamal. F.M. Gad and Abeer A. Abdelmonem. **Antenatal Detection of Congenital Cytomegalovirus Infection (A Comparative Study between Serological and Molecular Identification of Cytomegalovirus Infection).** *Life Sci J* 2014;11(12):1070-1076]. (ISSN:1097-8135). http://www.lifesciencesite.com. 183

Keywords: Cytomegalovirus Infection; Prenatal Diagnosis; CMV enzyme immunoassay, PCR,Amniocentesis.

Introduction

Cytomegalovirus, or CMV, is a common virus that infects most people worldwide. CMV infection is usually harmless and rarely causes illness. A healthy immune system can hold the virus in check. However, if a person's immune system is seriously weakened in any way, the virus can become active and cause CMV disease.(1)

Cytomegalovirus is a member of the herpesvirus family. Other members of the herpesvirus family cause chickenpox, infectious mononucleosis, fever blisters, and genital herpes. These viruses all share the ability to remain alive, but dormant, in the body for life.(2)

A first infection with CMV usually causes no symptoms. The virus continues to live in the body silently without causing obvious damage or illness. It rarely becomes active for the first time or reactivates (causes illness again in the same person) unless the immune system weakens and is no longer able to hold the virus in check.(3)

CMV is spread from person to person. Any person with a CMV infection, even without symptoms, can pass it to others. In an infected person, the virus is present in many body fluids, including urine, blood, saliva, semen, cervical secretions, (4) breast milk.

CMV can be spread by any close contact that allows infected body fluids to pass to another person. CMV can spread in households and child-care centers through hand-to-mouth contact with infected body fluids. CMV can spread by sexual contact, blood transfusions, organ transplants, and breastfeeding. CMV can also be passed from an infected pregnant woman to her fetus or newborn.(5)

Active infection in otherwise healthy children and adults can cause prolonged high fever, chills, severe tiredness, a generally ill feeling, headache, and an enlarged spleen.

Most infected newborns have no symptoms at birth, but, in some cases, symptoms will appear over the next several years. These include mental and developmental problems and vision or hearing problems. In rare cases, a newborn can have a life-threatening infection at birth. Infants and children who get CMV infection after birth have few, if any, symptoms or complications. When symptoms do appear, they include lung problems, poor weight gain, swollen glands, rash, liver problems, and blood problems.(6)

People with weakened immune systems can have more serious, potentially life-threatening illnesses, with fever, pneumonia, liver infection, and anemia. Illnesses can last for weeks or months and can be fatal. In persons with HIV infection, CMV can infect the retina of the eye (CMV retinitis) and cause blindness.

Blood test for IgG antibodies (special immune system proteins) is how your doctor can determine whether you've ever been infected with CMV. By itself, this test won't show whether you were infected during your pregnancy, nor will it tell you whether your fetus has been infected with CMV. If you test negative before your pregnancy, you should take special care to prevent CMV infection while you are pregnant.(7)

New CMV infections can be found by doing testing for a type of antibodies ("IgG" antibodies) on blood samples taken at different times. If the first sample is negative and the second sample is positive, then you became infected with CMV sometime between the two samples. A new method, called IgG avidity testing, needs only one blood sample to show whether you have a recent CMV infection. However, this test is currently not commercially available in the U.S. In the past, a test for IgM antibodies (another immune system protein) has been done to try to detect recent infections. Because this test is often positive when there is no new CMV infection, it should not be used without the other tests.(11)

For these reasons, and because there are no safe and effective CMV drugs for pregnant women, we do not recommend routine screening to see if a fetus is infected with CMV. Blood tests are sometimes used to find women who have new CMV infections. These women have a high risk (about 1/3) of passing the virus to their fetus. Tests of amniotic fluid or fetal blood, along with ultrasound readings of women, often can identify which of these women passed the virus to their fetus. However, these tests are invasive and don't always correctly identify infected fetuses or fetuses who will have health problems.(9)

A newborn has congenital CMV if the virus can be found in their urine, saliva, or blood during the first 3 weeks after birth. Rather than detecting antibodies, tests must identify the virus itself. Congenital CMV cannot be diagnosed if the baby is tested more than 3 weeks after birth, since she/he could have been infected after birth. Babies infected after birth are not at risk for disabilities. If your baby has congenital CMV, you should have her hearing and vision tested regularly. Most CMV-infected babies grow up with normal health, but if your child has delayed hearing or vision problems, early detection can help his or her development.(8)

If you are planning to become pregnant, a CMV blood test (which tests for IgG antibodies - special immune system proteins) can help you know how careful you must be to prevent CMV infection. If you test positive, you will know that there is little chance that your baby will be harmed by CMV. If you test negative, carefully follow the recommendations for preventing CMV infection before and during your pregnancy. Either way, it is always a good idea to follow the prevention guidelines because they will help you avoid other infections as well. (10)

The duration of disease varies, depending on the type of infection and the age and health of the infected person. Serious CMV infections that were acquired before birth can cause developmental problems that can affect a child for a lifetime. CMV infections in transplant recipients, cancer patients, and persons with HIV infection can be life threatening and require many weeks of hospital treatment. On the other hand, infections in young adults might cause symptoms for only 2 to 3 weeks.(11)

Many parents desire antenatal diagnosis of intrauterine infection so that they are informed of the possible outcomes for their child, as opposed to antenatal testing for selective termination (12). There is therefore a need for a low-risk, noninvasive diagnostic test. Laboratory methods are required to diagnose acute CMV infections since most present nonspecific symptoms. Women are not routinely screened for CMV prior to conception so CMV seroconversion is infrequently documented, making diagnosis of primary CMV infections difficult. The presence of CMV-specific immunoglobulin M (IgM) may not be indicative of primary infection, since it is also produced during reactivation and reinfection (13). IgG antigen avidity has been used to clarify primary or non primary infections by measuring the binding affinity of IgG antibodies. IgG of low avidity are produced at the onset of infections, and subsequent maturation of the antibody increases its avidity over time(14).

Furthermore, since the risk of CMV intrauterine transmission increases with advancing gestation, there is a need for diagnostic tests that can be used at all

stages of gestation. In developing an appropriate diagnostic algorithm, test sensitivity, specificity, PPV, and NPV need to be estimated, utilizing known population prevalence. We outline here a noninvasive, diagnostic algorithm for congenital CMV detection at all stages during pregnancy based on initial serological screening with CMV IgG, IgM, and IgG avidity. The diagnosis of asymptomatic neonates is emphasized, since half of the children who suffer from CMV sequelae are asymptomatic at birth (18). Also, knowledge of the risk of conditions such as sensorineural hearing loss in asymptomatic neonates, can allow close monitoring, early diagnosis, and early intervention, whereas failure to detect and follow up asymptomatic neonates may have serious consequences for the development of the child.(15)

2.Material and Methods

Patients.

The samples consisted of 120 pregnant women, prospectively screened for CMV by serology from *March 2012 to November 2012*. The women were presenting for routine antenatal care at a tertiary referral women's hospital Maternal, Children Hospital (M.C.H.) Buridah, Elqassem, All subjects gave written consent.

Specimens.

Whole blood samples were collected from all women. Whole blood samples were centrifuged at 2,000 × *g* for 20 min, and the buffy coat was removed and stored at −20°C.

Serology.

CMV IgG and IgM were detected in patient serum by using a commercial microparticle enzyme immunoassay (Abbott AxSYM; Abbott Laboratories). An IgG avidity assay (CMV IgG avidity EIA; Radim, Rome, Italy) was used to distinguish between primary and recurrent CMV infections (16). A second commercially available CMV-IgM enzyme immunoassay (Eti-Cytok IgM; Sorin Biomedica, Vercelli, Italy) was also used on all sera. The procedures and interpretation of results were performed according to the manufacturer's instructions except that specimens with an avidity index of ≥35% were considered hi gh avidity.

Amniocentesis, Twenty milliliters of AF was collected by transabdominal amniocentesis under continuous ultrasound guidance. (17).

Amniocentesis was performed at 14 to 30 weeks of gestation, with informed consent obtained from all women.

(iii) Determination of the presence of CMV genome by PCR. CMV DNA was individually extracted from two to three aliquots of AF (100 μl each) with an IsoQuick Nucleic Acid Extraction Kit (Orca Research, Bothell, Wash.) and was resuspended in 50 μl of RNase-free water. Aliquots of 5 × 10^5 PMNLs were resuspended in 100 μl of PCR buffer (KCl, 50 mM; Tris-HCl, 10 mM [pH 8.3]; $MgCl_2$, 2 mM; gelatin, 0.01%), with nonionic detergents and proteinase K. Samples were incubated at 60°C for 1 h and then at 95°C for 20 min to inactivate the proteinase. A total of 30 μl of each sample of PMNLs and DNA extracted from each aliquot of AF were added to 20 μl of a reaction buffer containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2 mM $MgCl_2$, the four deoxynucleotide triphosphates at a concentration of 0.2 mM each, 50 pmol of each primer, and 1.25 U of Amplitaq DNA polymerase (Perkin-Elmer Cetus, Norwalk, Conn.). The primer pair of. (6) targeting a 435-bp sequence within the major immediate-early antigen of the CMV Towne strain was used (DIGENE Diagnostics, Beltsville, Md.), with one primer 5′ labelled with biotin. Samples were denatured at 94°C for 5 min and subjected to 40 amplification cycles. PCR products were denatured and hybridized with a CMV-specific RNA probe. RNA-DNA hybrids were transferred to streptavidin-coated capture plates. Alkaline phosphatase-labelled RNA-DNA hybrid antibody was added, and bound antibody was detected by using *p*-nitrophenylphenol. The optical density at 405 nm was read with a microplate reader at 1, 18, and 24 h after substrate addition. The color intensity was proportional to the amount of captured RNA-DNA hybrids, with maximum assay sensitivity achieved 24 h after substrate addition. The cutoff was calculated as twice the mean for the negative controls (a minimum of four per assay) plus 0.08 (Digene SHARP Signal System; DIGENE Diagnostics). AF was considered positive if at least one of the aliquots was positive.

Statistical analysis

Cross-tabulation and chi-square (with Yates' continuity correction) were used to examine the relationship between variables using a 95% confidence interval as a measure of association.

3. Results

The women were divided into two groups based upon gestation. Group A consisted of 80 patients who consented through the outpatient clinic at 20 weeks or less than 20 weeks of gestation (mean, 15 weeks). Group B consisted of 40 patients who consented at the time of glucose tolerance testing (GTT) for gestational diabetes at over 20 weeks gestation (mean, 28 weeks). All women were screened serologically for CMV IgG and IgM. Women with an equivocal serology result or an IgG-negative and IgM-positive result were screened 3 weeks later to confirm the serostatus of the patient. CMV IgG avidity was determined for all patient specimens that were CMV IgM and IgG positive. First-trimester bleeds from women in group B who were IgM positive were screened retrospectively. No

statistical differences ($P > 0.05$) were seen in the average age, parity, or CMV serostatus of the two groups.

Table 1. Show the gestation age of studied mothers:

≤20 weeks gestation(mean, 15 weeks)	80 Patients
>20 weeks gestation (mean, 28 weeks)	40 Patients
Total no. of women examined	120 Patients

The CMV seropositivity rate for the pregnant women showed that, overall, 76.8% women were CMV IgG positive at pregnancy. The age range of the pregnant women was 18 to 46 years, with no significant difference seen between the mean age of seropositive and seronegative women.

Pregnant women were considered in high risk due to (i) documented seroconversion to positively for CMV from 3 months before conception to the end of the first trimester of pregnancy (four patients) (ii) CMV symptoms (increase in liver enzyme levels and/or fever) and presence of CMV immunoglobulin M (IgM) (five patients) (iii) intrauterine growth retardation as detected by ultrasound (three patients).

Table 2. Screening data from six women diagnosed as being at high risk for CMV intrauterine transmission:

Case	Gestation at testing (wk)	CMV IgG seroconversion	CMV IgM	PCR
1	5	–	+	+
2	12	+	+	–
3	14	+	–	+
4	18	+	+	+
5	20	–	+	+
6	22	+	+	+

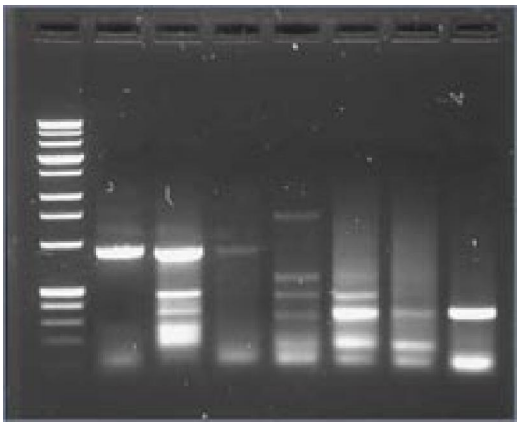

Figure 1

PCR results of CMV samples;: PCR positive control; lanes 1 : 6

PCR was used to detect excretion of CMV from the amniotic fluid in 5 of six cases(83.3%) were sero positive, and 1 false negative (16.7%), In group without infection, PCR was positive in 1 case (1 false positive) in Table 3. Accordingly,PCR has a sensitivity = 83%, specificity =97.4%, positive predictive value = 79.5%, and negative predictive value =98.3% (Figure 1)

Table 3. Results of PCR of amniotic fluid in cases with CMV infection and cases without infection:

PCR	Cases+veCMV By EIA (No.= 6)	Cases -veCMV By EIA (No.=114)	Total
Positive	5 (83.3%)	1 (0.87%)	6
Negative	1 (16.6%)	113(99.1%)	114
Total	6 (100%)	114(100%)	120

Table 4. Patient's characteristics of studied mothers in cases with CMV infection and cases without infection:

Patient's characteristics	Cases +ve CMV (No. =6)	Cases-ve CMV (No. =114)	*P* value
Maternal age (years) Mean +SD	20.15±3.2	21.85±2.3	0.812
Gravidity Mean ±SD	2.4±1.1	4.45±1.28	0.315
Parity Mean ±SD	4.2±1.7	5.11±.91	0.175
History Of abortion Mean ±SD	1.9±1.4	0.12±0.32	0.028
Gestational age (weeks) Mean ±SD	24.05±1.8	34.65±3.35	0.675

4.Discussion

In our study, the samples consisted of 120 pregnant women, prospectively screened for CMV, the women were divided into two groups based upon gestation. Group A consisted of 80 patients who consented through the outpatient clinic at 20 weeks or less than 20 weeks of gestation (mean, 15 weeks). Group B consisted of 40 patients who consented at the time of glucose tolerance testing (GTT) for gestational diabetes at over 20 weeks gestation (mean, 28 weeks). All women were screened serologically for CMV IgG and IgM. Women with an equivocal serology result or an IgG-negative and IgM-positive result were screened 3 weeks later to confirm the serostatus of the patient. CMV IgG avidity was determined for all patient specimens that were CMV IgM and IgG positive. The CMV seropositivity rate for the pregnant women showed that, overall, 76.8% women were CMV IgG positive at pregnancy.

In anther study, It was found that,the base rate of CMV IgG seroprevalence in blood donors was shown to increase with age, from 34.9% at less than 20 years of age to 72.4% after the age of 50 years. These are the first published data of age-related CMV serostatus in an Australian population, and the results are similar to those from populations in Europe and the United States (21). Within the blood donor population significantly more women were infected with CMV than men, a finding in agreement with risk factors for other

sexually transmitted viruses, such as human papillomavirus, and herpes simplex viruses. This increased risk is consistent with a demonstrated higher efficiency of male-to-female transmission (40).

Unlike the blood donor population in other study, the rate of CMV infection in the pregnant-women did not increase with age but instead was consistently high in women of less than 30 years of age (60 to 66%). Risk factors for CMV infection have been correlated with the socioeconomic status within a community (17). The pregnant women was from the southeastern area of Sydney, which has the second highest socioeconomic status within the state of New South Wales. The Australian data presented therefore differ from published data from the United States and Western Europe, in which women of childbearing age, of middle to upper class socioeconomic status, have a lower seroprevalence rate of CMV (21).

In this study,pregnant women were considered in high risk due to (i) documented seroconversion to positively for CMV from 3 months before conception to the end of the first trimester of pregnancy (four patients) (ii) CMV symptoms (increase in liver enzyme levels and/or fever) and presence of CMV immunoglobulin M (IgM) (five patients) (iii) intrauterine growth retardation as detected by ultrasound (three patients).

Serological screening was incorporated into already-established procedures, including first-trimester screening.After the initial onset of infection the rise in IgM titer may occur prior to the rise in IgG titer, making CMV IgG avidity testing reliant on the sensitivity of the CMV IgM test.

In our study we obtained from pregnant women AF,with which we performed PCR, PCR was used to detect excretion of CMV from the amniotic fluid in 5 of six cases(83.3%) were sero positive,1 false negative (16.7),In group without infection, PCR was positive in 1 case (1 false positive). Accordingly,PCR has a sensitivity = 83%, specificity =97.4%, positive predictive value = 79.5%, and negative predictive value =98.3%.

Our results are in contrast to those of previous studies of PCR of plasma and serum (15). However, the different characteristics of CMV infection and disease in these populations (patients with AIDS and bone marrow and renal transplant recipients) in comparison with those in liver transplant recipients or differences in PCR methodology or priming efficiency may account for some of these different results(30).

CMV shedding in urine can occur in both primary and nonprimary infections, and increasing numbers of women shed with advancing gestational age (35). Only a small percentage (1.2%) of CMV seropositive women in the present study were found to be shedding CMV compared to published data. Urinary shedding has a poor correlation with intrauterine infection, although it has been identified as a risk factor (15).

Furthermore, the proportion of congenital infections detected in pregnant women in anther study, who had CMV-specific IgM, as detected by ELISA, during the first trimester of pregnancy is 9.8%. When the IgM positivity by ELISA is confirmed by PCR, the correlation with transmission is not significantly different. On the contrary, if the PCR profile is taken into consideration, the rate of transmission for women with an at-risk profile is the same as that for women who seroconverted during the first trimester of pregnancy (rates of transmission, 29 and 25%, respectively). An at-risk profile means that IgM is preferentially directed to rp52 and/or vp65 or that serum IgM reacts with more than four bands (20, 23).

However, a major drawback is that at present there is not enough evidence to predict the outcome for CMV-infected fetuses. The natural history of intrauterine CMV infection is not well understood, but it is clear that some fetuses are irreversibly damaged by the virus before delivery. Those infants would not benefit from postnatal therapy, but if infected fetuses could be detected before this irreversible stage has been reached, treatment in utero might have a significant effect on the course of the disease. Recent developments with effective antiviral agents may make such therapy possible (30).

With the advances in antiviral chemotherapy, prenatal diagnosis of fetal infection could also lead to treatment of the mother with an anti-CMV agent known to cross the placenta. Ganciclovir is being administered to newborn infants congenitally infected with CMV, and encouraging results have been obtained (32). However, treatment of the affected neonate is probably too late because much of the virus-induced damage to the infants may already have occurred before delivery.

In conclusion, congenital CMV infection is a major health problem that should be approached on the basis of which women should be enrolled in prenatal diagnostic programs, which clinical specimens should be tested, and which laboratory procedures should be adopted for the diagnosis of congenital CMV transmission or infection. Determination of the presence of CMV genome by PCR in amniotic fluid can be considered as a rapid, non invasive and accurate tool for the prenatal diagnosis of congenital CMV infection but negative results cannot rule out of infection, So that diagnosis of this infection based on correlation between serological and PCR Identification.

References

1. Fernando, S., J. M. Pearce, and J. C. Booth. 1993. Lymphocyte responses and virus excretion as risk

factors for intrauterine infection with cytomegalovirus. J. Med. Virol. 41:108-113. [PubMed].
2. Alfirevic, Z., K. Sundberg, and S. Brigham. Jan 20;2003, posting date. Amniocentesis and chorionic villus sampling for prenatal diagnosis. Cochrane Database Syst. Rev. [Online.]
3. Donner, C., C. Liesnard, F. Brancart, and F. Rodesch. 1994. Accuracy of amniotic fluid testing before 21 weeks gestation in prenatal diagnosis of congenital cytomegalovirus infection. Prenatal Diagn. 14:1055-1059.
4. Fox, J. C., I. M. Kidd, P. D. Griffiths, P. Sweny, and V. C. Emery. 1995. Longitudinal analysis of cytomegalovirus load in renal transplant recipients using a quantitative polymerase chain reaction: correlation with disease. J. Gen. Virol. 76:309-319. [PubMed].
5. Australian Bureau of Statistics. 1997. Population profile incorporating demographic and social indicators from the ABS 1996 census of population and housing. Australian Bureau of Statistics, Canberra, Australia.
6. Bodeus, M., S. Feyder, and P. Goubau. 1998. Avidity of IgG antibodies distinguishes primary from non-primary cytomegalovirus infection in pregnant women. Clin. Diagn. Virol. 9:9-16. [PubMed].
7. Faedo, M., C. E. Ford, R. Mehta, K. Blazek, and W. D. Rawlinson. 2004. Mouse mammary tumor-like virus is associated with p53 nuclear accumulation and progesterone receptor positivity but not estrogen positivity in human female breast cancer. Clin. Cancer Res. 10:4417-4419. [PubMed].
8. Bodeus, M., C. Hubinont, P. Bernard, A. Bouckaert, K. Thomas, and P. Goubau. 1999. Prenatal diagnosis of human cytomegalovirus by culture and polymerase chain reaction: 98 pregnancies leading to congenital infection. Prenatal Diagn. 19:314-317.
9. Kreimer, A. R., A. J. Alberg, R. Viscidi, and M. L. Gillison. 2004. Gender differences in sexual biomarkers and behaviors associated with human papillomavirus-16, -18, and -33 seroprevalence. Sex. Transm. Dis. 31:247-256. [PubMed]. 9.Boppana, S. B., L. B. Rivera, K. B. Fowler, M. Mach, and W. J. Britt. 2001. Intrauterine transmission of cytomegalovirus to infants of women with preconceptional immunity. N. Engl. J. Med. 344:1366-1371. [PubMed].
10. Breborowicz, G. H. 2001. Limits of fetal viability and its enhancement. Early Pregnancy 5:49-50. [Online.] http://www.earlypregnancy.org. [PubMed].
11. Dahle, A. J., K. B. Fowler, J. D. Wright, S. B. Boppana, W. J. Britt, and R. F. Pass. 2000. Longitudinal investigation of hearing disorders in children with congenital cytomegalovirus. J. Am. Acad. Audiol. 11:283-290. [PubMed].
12. Daiminger, A., U. Bader, M. Eggers, T. Lazzarotto, and G. Enders. 1999. Evaluation of two novel enzyme immunoassays using recombinant antigens to detect cytomegalovirus-specific immunoglobulin M in sera from pregnant women. J. Clin. Virol. 13:161-171. [PubMed].
13. Ahlfors, K., S. A. Ivarsson, and S. Harris. 1999. Report on a long-term study of maternal and congenital cytomegalovirus infection in Sweden: review of prospective studies available in the literature. Scand. J. Infect. Dis. 31:443-457. [PubMed].
14. Faedo, M., C. E. Ford, R. Mehta, K. Blazek, and W. D. Rawlinson. 2004. Mouse mammary tumor-like virus is associated with p53 nuclear accumulation and progesterone receptor positivity but not estrogen positivity in human female breast cancer. Clin. Cancer Res. 10:4417-4419. [PubMed].
15. Bodeus, M., and P. Goubau. 1999. Predictive value of maternal-IgG avidity for congenital human cytomegalovirus infection. J. Clin. Virol. 12:3-8. [PubMed].
16. Fowler, K. B., S. Stagno, and R. F. Pass. 1993. Maternal age and congenital cytomegalovirus infection: screening of two diverse newborn populations, 1980-1990. J. Infect. Dis. 168:552-556. [PubMed].
17. Yoshinaga-Itano, C. 2003. Early intervention after universal neonatal hearing screening: impact on outcomes. Mental Retardation and Developmental Disabilities Res. Rev. 9:252-266.
18. Fowler, K. B. D., F. P. E. McCollister, A. J. P. Dahle, S. M. D. Boppana, W. J. M. D. Britt, and R. F. M. D. Pass. 1997. Progressive and fluctuating sensorineural hearing loss in children with asymptomatic congenital cytomegalovirus infection. J. Pediatr. 130:624-630. [PubMed].
19. Australian Bureau of Statistics. 1997. Population profile incorporating demographic and social indicators from the ABS 1996 census of population and housing. Australian Bureau of Statistics, Canberra, Australia.
20. Gaytant, M. A., G. I. Rours, E. A. Steegers, J. M. Galama, and B. A. Semmekrot. 2003. Congenital cytomegalovirus infection after recurrent infection: case reports and review of the literature. Eur. J. Pediatr. 162:248-253. [PubMed].
21. Fowler, K. B., S. Stagno, and R. F. Pass. 2003. Maternal immunity and prevention of congenital cytomegalovirus infection. JAMA 289:1008-1011. [PubMed].
22. Fowler, K. B. D., F. P. E. McCollister, A. J. P. Dahle, S. M. D. Boppana, W. J. M. D. Britt, and R. F. M. D. Pass. 1997. Progressive and fluctuating sensorineural hearing loss in children with asymptomatic congenital cytomegalovirus infection. J. Pediatr. 130:624-630. [PubMed].
23. Liesnard, C., C. Donner, F. Brancart, F. Gosselin, M. L. Delforge, and F. Rodesch. 2000. Prenatal diagnosis of congenital cytomegalovirus infection: prospective study of 237 pregnancies at risk. Obstet. Gynecol. 95:881-888. [PubMed].
24. Gaytant, M. A., G. I. Rours, E. A. Steegers, J. M. Galama, and B. A. Semmekrot. 2003. Congenital cytomegalovirus infection after recurrent infection: case reports and review of the literature. Eur. J. Pediatr. 162:248-253. [PubMed].
25. Lazzarotto, T., P. Spezzacatena, P. Pradelli, D. A. Abate, S. Varani, and M. P. Landini. 1997. Avidity of immunoglobulin G directed against human cytomegalovirus during primary and secondary infections in immunocompetent and immunocompromised subjects. Clin. Diagn. Lab. Immunol. 4:469-473. [PubMed].

26. Gottlieb, S. L., J. M. Douglas, Jr., D. S. Schmid, G. Bolan, M. Iatesta, C. K. Malotte, J. Zenilman, M. Foster, A. E. Baron, J. F. Steiner, T. A. Peterman, M. L. Kamb, and R. S. G. Project. 2002. Seroprevalence and correlates of herpes simplex virus type 2 infection in five sexually transmitted-disease clinics. J. Infect. Dis. 186:1381-1389. [PubMed].
27. Maine, G. T., T. Lazzarotto, and M. P. Landini. 2001. New developments in the diagnosis of maternal and congenital CMV infection. Expert Rev. Mol. Diagn. 1:19-29. [PubMed].
28. Griffiths, P. D. 2002. Strategies to prevent CMV infection in the neonate. Semin. Neonatol. 7:293-299. [PubMed].
29. Guerra, B., T. Lazzarotto, S. Quarta, M. Lanari, L. Bovicelli, A. Nicolosi, and M. P. Landini. 2000. Prenatal diagnosis of symptomatic congenital cytomegalovirus infection. Am. J. Obstet. Gynecol. 183:476-482. [PubMed].
30. Hagay, Z. J., G. Biran, A. Ornoy, and E. A. Reece. 1996. Congenital cytomegalovirus infection: a long-standing problem still seeking a solution. Am. J. Obstet. Gynecol. 174:241-245. [PubMed].
31. Revello, M. G., and G. Gerna. 2002. Diagnosis and management of human cytomegalovirus infection in the mother, fetus, and newborn infant. Clin. Microbiol. Rev. 15:680-715. [PubMed].
32. Boppana, S. B., K. B. Fowler, W. J. Britt, S. Stagno, and R. F. Pass. 1999. Symptomatic congenital cytomegalovirus infection in infants born to mothers with preexisting immunity to cytomegalovirus. Pediatrics 104:55-60. [PubMed].
33. Lazzarotto, T., C. Galli, R. Pulvirenti, R. Rescaldani, R. Vezzo, A. La Gioia, C. Martinelli, S. La Rocca, G. Agresti, L. Grillner, M. Nordin, M. van Ranst, B. Combs, G. T. Maine, and M. P. Landini. 2001. Evaluation of the Abbott AxSYM cytomegalovirus (CMV) immunoglobulin M (IgM) assay in conjunction with other CMV IgM tests and a CMV IgG avidity assay. Clin. Diagn. Lab. Immunol. 8:196-198. [PubMed].
34. Gaytant, M. A., E. A. Steegers, B. A. Semmekrot, H. M. Merkus, and J. M. Galama. 2002. Congenital cytomegalovirus infection: review of the epidemiology and outcome. Obstet. Gynecol. Surv. 57:245-256. [PubMed].
35. Lazzarotto, T., S. Varani, L. Gabrielli, P. Spezzacatena, and M. P. Landini. 1999. New advances in the diagnosis of congenital cytomegalovirus infection. Intervirology 42:390-397. [PubMed].
36. Fox, J. C., I. M. Kidd, P. D. Griffiths, P. Sweny, and V. C. Emery. 1995. Longitudinal analysis of cytomegalovirus load in renal transplant recipients using a quantitative polymerase chain reaction: correlation with disease. J. Gen. Virol. 76:309-319. [PubMed].
37. Grangeot-Keros, L., M. J. Mayaux, P. Lebon, F. Freymuth, G. Eugene, R. Stricker, and E. Dussaix. 1997. Value of cytomegalovirus (CMV) IgG avidity index for the diagnosis of primary CMV infection in pregnant women. J. Infect. Dis. 175:944-946. [PubMed].
38. Maine, G. T., R. Stricker, M. Schuler, J. Spesard, S. Brojanac, B. Iriarte, K. Herwig, T. Gramins, B. Combs, J. Wise, H. Simmons, T. Gram, J. Lonze, D. Ruzicki, B. Byrne, J. D. Clifton, L. E. Chovan, D. Wachta, C. Holas, D. Wang, T. Wilson,. 2000. Development and clinical evaluation of a recombinant-antigen-based cytomegalovirus immunoglobulin M automated immunoassay using the Abbott AxSYM analyzer. J. Clin. Microbiol. 38:1476-1481. [PubMed].
39. Halwachs-Baumann, G., M. Wilders-Truschnig, G. Enzinger, M. Eibl, W. Linkesch, H. J. Dornbusch, B. I. Santner, E. Marth, and H. H. Kessler. 2001. Cytomegalovirus diagnosis in renal and bone marrow transplant recipients: the impact of molecular assays. J. Clin. Virol. 20:49-57. [PubMed].
40. Revello, M. G., M. Zavattoni, A. Sarasini, E. Percivalle, L. Simoncini, and G. Gerna. 1998. Human cytomegalovirus in blood of immunocompetent persons during primary infection: prognostic implications for pregnancy. J. Infect. Dis. 177:1170-1175. [PubMed].

12/19/2014