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Review Article

Human Cytomegalovirus: Infections and Diagnosis

M. Ziyaeyan, F. Sabahi[1]

Abstract
Human cytomegalovirus (HCMV) is a ubiquitous virus whose sole host is humans. Since HCMV can contagion from person to person through numerous ways, vast populations of humans are infected. HCMV infections can potentially have a range from asymptomatic infection in immuno-competent hosts to life-threatening diseases in organ recipients and patients with AIDS. The present article reviews the occurrence of HCMV infections and diseases in humans with different physiological and immunological status, and evaluates the existing laboratory methods for diagnosis of the disease.
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Keywords ● Human cytomegalovirus ● infections ● diagnosis

Introduction

Human cytomegalovirus (HCMV) is the vernacular name of human herpesvirus 5, a highly host-specific virus of the Herpesviridae family. It is the largest known human herpesvirus, with a genome of about 230 kbp.[1,2] The virus has double stranded linear DNA surrounded by a proteinaceous matrix (the tegument), which is enveloped by a lipid bilayer containing viral glycoproteins.[1,2] Human cytomegalovirus can be transmitted via saliva, sexual contact, placental transfer, breastfeeding, blood transfusion, or solid-organ and hematopoietic stem-cell transplantations. HCMV infections are common and lead to lifelong infections.[3] In immunocompetent individuals, primary infections are mostly subclinical or may be associated with a self-limited mononucleosis-like syndrome. However, among immunosuppressed patients, HCMV provokes various outcomes.

Neonatal and Congenital Infections

HCMV infects humans of all ages, although the peak period of viral acquisition in general population occurs early in life. Serological surveys have demonstrated maternal antibody prevalence rates of 30% to nearly 100%, reflecting wide variation in infection rates between populations.[4,5] Infants may acquire HCMV transplacentally as the result of maternal viremia, or perinatally via breast milk. Later during the childhood close physical contact facilitates the transmission.[6] The timing of infection and the serological status of the mother play an important role in defining the transmission rate and the sequelae in affected children.[7,8] Due to latency following primary infection and periodic reactivation of HCMV replication causing recurrent infections, in utero transmission of HCMV may follow either primary or recurrent infections.[9]

Primary HCMV infections are transmitted to the fetus more frequently and more likely to cause fetal damage, than

Department of Virology,
Professor Alborzi Clinical Microbiology Research Center,
Namazi Hospital,
Shiraz University of Medical Sciences,
Shiraz, Iran.
[1]Department of Virology,
Faculty of Medical Sciences,
Tarbiat Modares University,
Tehran, Iran.

Correspondence:
Mazyar Ziyaeyan PhD,
Department of Virology,
Professor Alborzi Clinical Microbiology Research Center,
Namazi Hospital,
Shiraz University of Medical Sciences,
Shiraz, Iran.
Tel: +98 711 6474296
Fax: +98 711 6474303
Email: ziyaeyanm@sums.ac.ir
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recurrent infections. It seems that primary infection occurring at an earlier gestational age leads to a worse outcome.[6] Congenital HCMV infection occurs in 0.2 to 2.2% of all live-born infants.[10,11] Ten percent of congenitally infected infants are symptomatic at birth. Neonatal symptoms include hepatosplenomegaly, thrombocytopenia, purpura, jaundice, hemolytic anemia, hepatitis, microcephaly, chorioretinitis and cerebral calcifications (1–10 per 10,000 births).[10,12] The mortality rate of symptomatic infections is high, approximately 25% in the days or weeks following birth. More than 90% of all surviving symptomatic newborns will develop long-term sequelae, mostly hearing loss and psychomotor retardation.[5] Ninety percent of all congenital HCMV children are asymptomatic at birth. Five to seventeen percent of these asymptomatic newborns will develop symptoms usually in the form of hearing deficits and subtle neurodevelopmental problems.[5,13]

Infection in Immunocompetent Hosts

Primary cytomegalovirus infection in the immunocompetent host rarely causes serious illnesses. Uncommonly, it can result in a mononucleosis syndrome, which is indistinguishable from primary Epstein-Barr virus (EBV) infection. It may present with persistent fever (generally for 2–3 weeks), myalgia, and cervical adenopathy, which is unlike EBV-associated tonsillopharyngitis or great splenomegaly. In developed countries, delayed exposure to cytomegalovirus increases the incidence of infections among middle-aged adults. Less frequent complications of primary infections include arthralgia and arthritis, ulcerative colitis, pneumonitis, hepatitis, aseptic meningitis, and myocarditis.[14]

Infection in Immunocompromised Patients

Initial infection with cytomegalovirus induces a primary immune response and subsequent establishment of long-term immunity, which restrains viral replication after reactivation from latency. Long-term immunosuppression can lead to uncontrolled replication and serious diseases.

Infection in Solid-Organ and Hematopoietic Stem-Cell Transplantation

Recent studies indicate that 50% to 75% of solid-organ transplant recipients develop HCMV infection and approximately one third of the infected patients develop HCMV diseases.[15,16] In the era before introduction of ganciclovir, cytomegalovirus infection and pneumonia were developed in 38% and 17% of recipients of allogeneic stem cell transplants, respectively, while mortality due to HCMV pneumonia was 85%.[17] HCMV infection and disease occur in close temporal association with maximal host immunosuppression and, thus, are frequent during the first months after transplantation with a peak incidence between 2 and 4 months, although the disease can occur years after transplantation.[18,19]

Three types of HCMV infections can occur in transplant recipients: primary, reactivation, and super infection. Primary infection invariably occurs after blood transfusion or organ transmission of HCMV from a seropositive donor (D+) to a seronegative recipient (R-). This type of infection causes HCMV disease with greater frequency than reactivation (secondary) infection. Primary infection is more frequently associated with severe clinical disease manifestations, and recurs more commonly even after initial successful treatment.

Secondary infection is caused by reactivation of the latent virus subsequent to the suppression of host defenses. Super infection occurs when a new strain of HCMV infects a previously seropositive patient. Secondary infection and super infection can lead to clinical manifestations of HCMV disease in approximately 10% to 20% of solid-organ transplants,[20] and in 30% of stem cell transplants recipients. Such patients generally show milder symptoms than those caused by primary infection.[21-23] It is noteworthy that nearly twice as many D+/R+ patients develop HCMV disease, compared with D-/R+ patients. This may indicate that concurrent reactivation infection and super infection are not uncommon events after transplantation.[24,25] The incidence of symptoms associated with HCMV infection varies among different types of allograft recipients. In general, liver, pancreas, lung, intestinal, and heart transplant recipients show greater incidence of HCMV disease, than do kidney transplant recipients. Symptomatic infections occur in approximately 39% to 41% of heart-lung, 9% to 35% of heart, 22% to 29% of liver and pancreas, and 8% to 32% of renal transplant recipients not receiving antiviral prophylaxis.[20,24,26,27]

HIV Infection

HCMV infection is one of the latent human infections that, although controlled by the cellular immune response, it is activated after HIV attaches to CD4 lymphocytes. The incidence of HCMV infection among patients with advanced HIV disease is high. Epidemiological studies have shown that nearly half of HIV-infected patients eventually develop HCMV as an end-organ disease, with its most prominent manifestations being chorioretinitis, esophagitis, colitis, pneumonitis, and central nervous system (CNS) diseases. Despite the high prevalence of

HCMV antibody in HIV infection, the clinical manifestations of HCMV disease are not generally present until the CD4 count drops below 100 cells/mm^3. [28,29]

Diagnosis

Serology

Humoral response to HCMV infection is manifested by the production of HCMV-specific antibodies. Immunoglobulin M (IgM) antibody against HCMV occurs early (e.g., within 2 to 4 weeks following primary infection), and IgG antibody production occurs soon thereafter; both can be detected by a variety of methods. Detection of IgM in a single serum sample from a newborn is diagnostic of congenital HCMV infection because IgM does not cross the placenta.[30,31] In the field of transplantation, HCMV serology does not indicate diagnosed HCMV disease, because HCMV infection is widely prevalent and most adults are, thus, seropositive (IgG) for HCMV. Furthermore, the time lag between primary infection and IgM production, the persistence of IgM antibody in some healthy individuals, and the incapability of some transplant recipients (e.g., hematopoietic stem cell recipients) to produce IgM antibody significantly decrease the clinical utility of serology in diagnosing HCMV disease.[32,33]

Viral Cultures

Recovery of replicating HCMV by conventional tube or shell vial assay has traditionally been the standard method for the diagnosis of HCMV infection. HCMV can be isolated from a wide variety of specimens; however, urine, throat washings, saliva, and anticoagulated whole blood and buffy coat are the specimens most often received for diagnostic purposes in the clinical virology laboratories.[34] The recent improvement in cell culture techniques for HCMV is the shell vial assay. The assay uses 1-dram shell vials with a monolayer of cells on a round coverslip and is a centrifugation-amplified culture that uses commercially available monoclonal antibodies directed against HCMV immediate early antigen. The shell vial assay is more rapid than conventional tube cell cultures, requiring an average of 16 hours to positivity.[35] Culture methods alone may not be useful for diagnosis of active HCMV disease in most cases because shedding of HCMV (especially in urine or respiratory tract secretions) may occur in immunosuppressed patients without development of disease. However, isolation of HCMV from urine or saliva of neonates is still useful for the identification of congenital HCMV infection.[30] The quantitative detection of HCMV in cell cultures has a high correlation with HCMV disease; nevertheless, the low sensitivity of this technology limits its value in guiding pre-emptive prevention protocols, which require the detection of lower levels of HCMV replication.

Antigenemia Assay

The antigenemia assay is a rapid quantitative method that detects HCMV antigens by directly immunostaining polymorphonuclear leukocytes (PMN) from blood specimens with monoclonal antibodies directed against the HCMV lower-matrix protein pp65 (UL83). Quantitative results are expressed as the number of HCMV-infected PMN per number of cells evaluated.[36,37] The clinically relevant threshold of the number of infected PMN differs among the different patients populations. Thresholds of more than 10 positive cells per 2×10^5 cells and of 1 to 2 positive cells per 2×10^5 cells have been suggested to guide pre-emptive treatment in solid-organ and hematopoietic stem cell transplant recipients, respectively.[38,39] Currently, many virology laboratories use the pp65 antigenemia assay as the gold standard method to evaluate or validate in-house molecular methodologies. The antigenemia assay detects viremia 7 to 14 days before the onset of disease.[38,40,41] Quantification of antigenemia can be used to predict HCMV disease. Although the significant threshold for predicting disease differs among patient settings, a higher level of antigenemia has a higher predictive value for disease in all patients groups.[19,36]

Polymerase Chain Reaction (PCR)

PCR has revolutionized diagnostic virology by providing a powerful tool to detect and quantify viral DNA and RNA in various clinical specimens. Nucleic acid amplification by PCR is considered as one of the major tools used to detect HCMV infections. PCR techniques are capable of detecting viral DNA or RNA in various clinical specimens including peripheral blood leukocytes, whole blood, serum and plasma.[42] Carrying out qualitative PCR on infected leukocytes can provide rapid diagnosis of HCMV infections.[43] Since PCR techniques are very sensitive and specific and are able to detect trace of DNA elements, in some cases positive PCR results cannot differentiate between active viral replication and latent viruses. However, in seronegative patients the positive PCR results are definitely indicative of primary HCMV infection. On the other hand, a negative PCR result indicates the absence of HCMV infection.[33] Considering that HCMV replicates in infected cells and released into the plasma, the detection of viral nucleic acid in plasma or

serum reveals active infection. This point is particularly true in solid organ transplant recipients. The diagnostic value of PCR is well established when examined specimens include bronchoalveolar lavage fluid, cerebrospinal fluid and tissue biopsy samples.[44] Comparative studies carried out about PCR techniques on different leukocyte subpopulations and plasma have shown the significance of PCR in detecting HCMV DNA in PMN cells. However, since the qualitative PCR tests are not well standardized, the results are not sufficiently compatible with patients' clinical symptoms.[21,45] When compared with quantitative PCR, the qualitative counterpart is of lower diagnostic value.[22] The measurement of viral load by quantitative PCR appears to be a promising development that might be important for the diagnosis and prediction of HCMV disease, differentiation of latent from active infection, and monitoring the treatment.[22,46,47]

Methods developed for DNA quantification by PCR may be classified into three categories: semiquantitative, competitive, and noncompetitive quantitative. Quantification of HCMV DNA in blood leukocytes may have practical implications for the diagnosis of visceral organ disease during viremia. The median quantity of DNA in the leukocytes of patients with visceral organ disease is significantly greater than that in patients with viremia alone. Compared with serum samples, peripheral blood leukocyte specimens from patients with HCMV disease have generally higher HCMV DNA titers. Quantitative PCR is being touted as one of the best diagnostic methods for diagnosis of HCMV.[47]

Conclusion

It seems that the most practical and reliable method for diagnosis of HCMV active infections and follow-up the treatment in immunocompromised patients are using Real-Time quantitative PCR and antigenemia assay on PMN cells of patients. Analysis of the results obtained from the above-mentioned methods can help the physicians make proper decisions at the onset of pre-emptive therapy and monitoring the treatment.

Conflict of Interest: None declared

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