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CYTOMEGALOVIRUS IN IMMUNOSUPPRESSED PATIENTS: A SILENT AND POTENTIAL KILLER.

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SUMMARY

Cytomegalovirus (CMV) is a recognized cause of morbidity and mortality among immunocompromised individuals. This review will concentrate on understanding the pathogenesis, clinical manifestations and laboratory diagnostic options for CMV infection.

KEY WORDS- REVIEW, CYTOMEGALOVIRUS, IMMUNOSUPPRESSED

INTRODUCTION

Human Cytomegalovirus (CMV) is an enveloped DNA virus, a potential killer or a lifelong silent killer (1). CMV infections are common and are usually asymptomatic however clinically significant infections are encountered frequently in pregnant women, neonates and immunocompromised patients (2). CMV is an important cause of morbidity and mortality among immunosuppressed patients. After a primary infection, which usually occurs during childhood, CMV reactivation occurs when the host immune system is compromised, either through infection such as, HIV immaturity (neonate) or through other iatrogenic means following organ transplantation, the virus is able to exert its full pathogenic potential (3).

CMV is one of the commonest viral opportunistic infection in persons with Acquired Immune Deficiency Syndrome (AIDS). Clinical disease due to CMV has been recognized in up to 5-25% of patients with AIDS (4). Prior to the advent of Highly Active Antiretroviral Therapy (HAART) for HIV infection, CMV retinitis was the most common cause of blindness in adults with AIDS (5). CMV is the most common cause of congenital malformation resulting from viral intrauterine infection in developed countries (6).

Virus associated leucopenia is common in solid organ transplant recipients, also seen are obliterative bronchiolitis in lung transplant, graft atherosclerosis after heart transplantation and Cytomegalovirus-related rejection of renal allografts². Clinical trials have shown that preventive approaches utilizing antiviral agents have lead to a reduction in the rates of CMV infection and disease and indirect sequelae associated with CMV. Currently, prophylaxis or periodic monitoring and antiviral therapy targeted

towards patients with viral replication are routinely employed at many transplant centers.

The present article is meant to highlight the important features of the Cytomegaloviruses and also to increase the level of awareness on their potential to cause life threatening infections among the immunocompromised patients.

STRUCTURE OF CMV

Cytomegalovirus is a DNA virus belonging to the family Herpesviridae and the subfamily, Beta Herpesviridae which also includes the Roseolovirus and the Muromegalovirus. CMV is morphologically indistinguishable from other human Herpes viruses (7). Surface projections of envelope are distinct and spikes are dispersed evenly over the entire surface. The nucleocapsid is isometric, and surrounded by the tegument that consists of globular material which is frequently asymmetrically distributed and may be variable in amount (8). The nucleocapsid is sometimes penetrated by stain (although an intact envelope impermeable is to stain). Viral nucleocapsid is angular and has an icosahedral symmetry. There are 162 capsomeres per nucleocapsid, and capsomeres are hexagonal in cross section with a hole running half way down the long axis. The viral core consists of a fibrillar spool on which DNA is wrapped while the ends of the fibres are anchored to the underside of the capsid shell. Incomplete virus particles often present, and they represent capsids lacking the envelope.

CMV is inactivated by a number of physical and chemical treatments including; heat (56.5°C for 30 minutes), low PH, ether, ultraviolet light and cycles of freezing and thawing⁸. CMV virions contain one molecule of linear double stranded DNA. Total genome length is 120,000-220,000. Molecular virological techniques have been used to study variation among CMV strains. DNA strains have been shown to have similar but distinctive fragment

migration patterns. Antigenic heterogeneity among CMV strains has been detected in cross neutralization and other serologic assays; however evidence for distinct serotypes is limited (9-11).

EPIDEMIOLOGY OF CMV

CMV is ubiquitous, being found universally throughout all geographic locations and socio economic groups, and infects between 50% and 85% of adults in the United States by 40 years of age (12). CMV infection is more widespread in developing countries and in areas of low socio economic condition (13). In the developing countries, very young children are often virtually universally CMV antibody positive (14). The early acquisition and high prevalence of CMV antibody has been ascribed to low socio economic conditions, poor hygiene, and overcrowding (14). However reports from studies carried out on the people of the New Guinea highland villages with primitive standards of hygiene and who often lived apart from childhood until marriage as well as studies carried out on people of the New Hebrides and Solomon island who lived in crowded population but are scrupulously clean, the prevalence of CMV infection was higher in the latter than the former group. It was therefore concluded that close personal contact, rather than poor hygiene was more important for the acquisition and spread of CMV. The vehicle of transmission of CMV has been shown to include; saliva, breast milk, intrauterine infection, urine, droplet infection, blood transfusion, organ transplantation, sexual intercourse and fomites among others (6).

PATHOGENESIS OF CMV

An understanding of the process of CMV viral replication will provide insights into its molecular mechanisms of infection and pathogenesis (15). Following infection, or contact with the virus, the CMV DNA enters the nucleus of the host cells and begins the process of replication and shedding, leading to the release of new virions (viral particles) into the blood and other body fluids. This replication cycle, takes approximately 24 hours, and consist of 3 phases; an immediate early phase (4 hours)-during which regulator proteins are made followed by an early phase (8 hours) - during which viral DNA polymers are made and a late phase (12hrs) - during which structural proteins and new progeny viral particles are assembled.

In an immunocompetent host, most of the virus is destroyed by CMV-specific cytotoxic T-cells, and the infectious process becomes asymptomatic. The presence of asymptomatic CMV infection is based on the detection of CMV in body fluids or on seroconversion from a negative to a positive status. The latter is defined as the appearance of IgM or a fourfold rise in the level of CMV specific IgG antibodies.

In immunocompromised patients who are not

receiving prophylactic treatment against CMV, symptomatic CMV infection; CMV disease and organ involvement may result (16). There are various mechanisms by which CMV disease may occur and these includes; direct cellular loss through viral infection, both of individual cells, and of larger foci because of propensity to spread, leading to coalescing cells. This close cell-cell interaction protects virus from antibody inactivation. Systemic hypoxic- Ischemic insult due to systemic hypotension and immunologic injury, involving antigen antibody complex deposition may also be involved in organs such as the kidney

Eventually, CMV attains a latent state and persistent infection within T- cells, endothelial cells and monocyte-derived macrophages ensues (16). Cell mediated immunity which is required for resolution of symptoms, also contributes to symptoms while the role of antibodies are limited. Suppression of cell mediated immunity as seen in HIV infection allows recurrence of symptoms and can result in exacerbation of disease. The virus also has ability to induce immunosuppression in the body (9). The degree of morbidity induced by CMV is influenced by the degree of immunosuppression. In transplant recipients, there are two factors that influence the degree of morbidity due to CMV infection; the type and extent of immunosuppression, and the type of transplant. Morbidity is highest among bone marrow transplant (BMT) patients, and lowest among kidney transplant patients.

Multiple mechanisms of immune evasion for CMV could relate to the pathogenic role of the virus. Recently, the expression of immune evasion genes US3, US6, and US11 of CMV in the blood of solid organ transplant recipients has been investigated¹¹.

PATHOLOGY OF CMV

CMV produces characteristic cytopathic changes on infected cells which appear large, rounded and contains ground glass appearing inclusion bodies in the cytoplasm. These infected cells are the hallmark of CMV infection and indicates the presence of CMV in a sample. The nuclear inclusion has the appearance of an owl's eyes because it is typically surrounded by a clear halo that extends to the nuclear membrane (17).

CLINICAL MANIFESTATIONS OF CMV

Following a first exposure to CMV, the virus lies dormant in the body for life and can be reactivated. Usually a reactivated infection causes few or no symptoms. CMV disease may manifest in any of the under listed ways.

A. Congenital infection:

Congenital CMV infection is defined as the isolation of CMV from the saliva or urine of the neonate within 3 weeks of birth (18). The virus may also be transmitted from the mother to the infant in breast milk¹⁹. CMV is now the commonest cause of

congenital infection and affects around 0.3%-1% of all live births²⁰. A total of 5-10% of congenitally infected infants have symptoms at birth while fatal disease occurs in 20% of these infants (20). Ninety percent of the symptomatic survivors have long term sequelae while 15% of the asymptomatic survivors also have long term sequelae. CMV is now the second commonest cause of mental retardation after Down's syndrome and causes more cases of congenital damage than Rubella (21).

Cytomegalic inclusion disease refers to any group of diseases caused by cytomegalovirus infection, and marked by characteristic inclusion bodies in enlarged infected cells. The classic disease is congenital, being acquired *in utero* from the mother and typically presents with intrauterine growth retardation, jaundice, hepatosplenomegaly, thrombocytopenia and encephalitis, with or without microcephaly. It is often difficult to differentiate, on clinical grounds between the several agents that may cause intrauterine infection. The severe thrombocytopenia, hepatitis, pneumonitis and myocarditis attributed to the virus may be life threatening. CNS involvement may lead to seizures, focal neurological signs, and mental retardation. Unlike rubella, there is no evidence that CMV is teratogenic. Most of the damage is caused by destruction of target cells once they have been formed, and unlike rubella, the fetus can be damaged by infection during any stage of pregnancy. In 20% of cases (1% of those who are congenitally infected), the infection is so severe that mortality occurs during infancy, while the rest are likely to sustain serious abnormalities for the rest of their lives. Brain damage is by far the commonest abnormality on follow up, and thus may manifest as microcephaly, mental retardation and seizures. The spectrum of brain damage varies from mild to severe. Optic atrophy, deafness, and blindness may also be present (22).

The effects of congenital CMV infection are as follows ; CNS abnormalities - microcephaly, mental retardation, spasticity, epilepsy, periventricular calcification, eye- choroidoretinitis, and optic atrophy, ear - sensorineural deafness, liver - hepatosplenomegaly and jaundice which is due to hepatitis., lung - pneumonia, heart - myocarditis, haematological - Thrombocytopenic purpura, haemolytic anaemia, neutopenia and lymphocytosis. Late sequelae include damage to the enamel forming organ of the teeth, resulting in yellow discoloration of the teeth and brittleness. This occurs in 40% of infants (22).

B. PERINATAL INFECTION

Despite the continued excretion of high titers of virus in the urine for many months, the vast majority of perinatally infected infants do not develop acute symptoms, although few cases of infantile pneumonitis have been reported (21, 23).

This appears to be an exceedingly rare event.

C. POSTNATAL INFECTION

The incubation period for CMV is thought to be 4-8 weeks. Primary CMV infection in the postnatal period is usually mild or asymptomatic. Occasionally, primary infection may be accompanied by the syndrome of infectious mononucleosis, with atypical lymphocytosis. This is similar to the syndrome produced by Epstein barr virus (EBV) except that lymphadenopathy is uncommon, and Paul-Bunnell test is negative. CMV- induced mononucleosis can be symptomatically indistinguishable from EBV-induced mononucleosis (24). Malaise, fever of up to 39.4°C, chills, sore throat, headache, and fatigue can be the predominant features of both viruses. Many of the same clinical manifestations that are typical of EBV-induced mononucleosis (e.g. lymphadenopathy, splenomegaly, pharyngeal erythema) also can occur with CMV, although less frequently. Presentation of acute cytomegalovirus infection in an immunocompetent host may be in form of mononucleosis.

Patients with mononucleosis may present with nonspecific skin rashes such as; generalised maculopapular, urticarial, and scarlatiniform rashes. These rashes are not a direct cause of CMV proliferation within the skin, but are the result of an immunologic response to the virus. The classic hypersensitivity drug rash which is associated with ampicillin therapy given to patients with EBV-induced mononucleosis also can occur with CMV-induced mononucleosis. The post perfusion syndrome is essentially CMV mononucleosis acquired by blood transfusion (25). Sometimes the hepatitis picture predominates so that a diagnosis of non-non-B hepatitis is made.

D. CMV INFECTION IN THE IMMUNOCOMPROMISED PATIENTS

Primary CMV infection in immunocompromised individuals is far less likely to be asymptomatic. These patients develop spiking pyrexia, which resolves within a few days (25). Some may develop a viraemia with septicemia - like syndrome in the presence or absence of hepatitis. Pneumonitis may develop, which is associated with grave prognosis. The virus may disseminate to involve the retina, causing CMV retinitis. CMV retinitis is the commonest ocular opportunistic infection, and the most common cause of visual loss in people with AIDS (23). CMV may disseminate to the gut, where it may cause an asymptomatic infection or ulceration or haemorrhage by the erosion of nearby blood vessels. In patients with CMV - induced immuno suppressive syndrome the patient becomes unable to deal with opportunistic infections such as Pseudomonas. AIDS patients may develop low grade encephalopathy and CMV adenitis. In addition, kaposi sarcoma has been associated with

past CMV infection. CMV is by far the most common infection in bone marrow transplant recipients. Infection by this virus occurs in over one-half of patients. The virus is an immunomodulator and CMV disease exacerbates an ongoing immunosuppression in transplant recipient, thereby increasing the risk for bacterial and fungal infection such as *Nocardia asteroides*, *Mycoplasma*, and *Pneumocystis carini*.

LABORATORY DIAGNOSIS OF CMV INFECTION

CMV should be suspected if a person has symptoms of infectious mononucleosis but has negative test results for mononucleosis and Epstein Barr virus, or negative heterophile antibody test and shows signs of hepatitis, but has negative test result for hepatitis A, B, and C (26).

MICROSCOPY

Electron microscopy - Virions in the urine of congenitally infected infants may be visualized by electron microscopy in up to 80% of cases (27, 28). The pseudoreplica method of electron microscopy can be used to detect the virus in urine and oral specimens of congenitally infected infants. Positive results can be obtained from almost all specimens that have infectivity titers of $> 10^4$ /ml of urine.

Tissue immunofluorescence - Infected tissue cells may be stained by specific anti CMV antibody. This is based on the principle that viral specific antibody conjugated to fluorescent labeled is allowed to react with the cells obtained from the clinical specimen. Preparations from bronchoalveolar lavage specimens can also be examined in this manner, and results of high sensitivity and specificity are possible. Cytomegalic inclusions can be recognized from biopsy materials by the typical 'owl's eyes appearance" (29).

SEROLOGICAL DIAGNOSIS OF CMV INFECTION

ENZYME LINKED IMMUNOSORBENT ASSAY

A variety of tests are available for serologic diagnosis of CMV infection. The choice of method to be used depends on; factors such as the patient population, cost of test, turnaround time, equipment needs and ease of performance. Several commercial kits are available for detection of CMV IgG and IgM antibodies in body fluids. ELISA has replaced other traditional methods for detecting antibodies to CMV. The ELISA is based on the principle that Cytomegalovirus IgG and IgM specific antibodies are detected by addition of diluted patients 's sera to wells coated with purified CMV antigen. The IgG and IgM antibody, if present, binds to the antigen. An enzyme conjugate is added to bind the antigen-antibody complex, if present. A substrate to the enzyme is added, and a hydrolytic

reaction between the enzyme and its substrate results in a change in the colour of the end product, whose intensity can be measured at 450 nm and is directly proportional to the amount of IgG specific antibody in the patient's sera.

The main advantages of the ELISA are that; it is rapid, sensitive and specific. They are also useful in detection of CMV antibodies in urine. The limitation of ELISA is that they have low sensitivity when used to detect CMV antibodies in urine; this is because CMV is complexed to beta 2 microglobulin in the urine and may result in false positive results³⁰.

PASSIVE LATEX AGGLUTINATION TEST

This provides a simple and rapid means of detecting antibodies to CMV in human sera and plasma. This method is highly sensitive and specific, and it may serve to determine the immune status of patient or blood donor populations. The procedure detects both IgG and IgM antibodies but does not differentiate between the two classes of immunoglobulins (30, 31).

COMPLEMENT FIXATION

The complement fixation (CF) test has been used for many years in clinical laboratories and is suitable for detecting rise in antibody titers. The CF test with glycine - extracted antigen is more sensitive for distinguishing sero-positive from sero-negative specimens.

ANTIGEN DETECTION TESTS

CMV ANTIGENEMIA TEST

This test is based on the detection of pp 65, a structural protein expressed on the surface of infected polymorphonuclear leucocytes (32). The number of infected leucocytes present has been reported to correlate with the severity of infection. The main advantage of this test is that it is very rapid such that a result can be available within the same day.

Detection of early antigen fluorescent foci (DEAFF)

This is a method used for the early diagnosis of CMV infection. The specimen is inoculated into cell culture which is examined 24 hours later by immunofluorescence for expressed CMV encoded early proteins. The monoclonal antibodies must be able to cover most, if not all strains of CMV. Rapid culture methods other than the DEAFF tests are also available.

VIRAL CULTURE

Human CMV has been cultured in human cells only and previous claims that it could be grown in other animal cells is yet to be substantiated. Human fibroblast cells best support the growth of CMV and therefore can be used for diagnostic purposes. Acceptable fibroblast cultures include those prepared from human embryonic tissues or

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