



Original Research Article

Seroprevalence of Cytomegalovirus in Antenatal Cases with Bad Obstetric History at Warangal, Telangana, India

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ABSTRACT

The occurrence of foetal death and malformation is one of the tragedies that confront the physician providing obstetric care, his patient and her family as well. The pregnant woman and her fetus are susceptible to many factors affecting the outcome of pregnancy, notable among which are infections. Some of these infections may be quite serious and live threatening for the mother, where as others may have a profound impact on neonatal outcome by virtue of a high likelihood of fetal infection. Generally speaking, the pregnant woman is immune competent. Considering the possible effects of decreased immune surveillance during pregnancy, the rate of susceptibility to infection is high. Among the various infectious agents, viruses are most important followed by bacteria and protozoa. Among the viral infections in pregnancy, include Rubella, Cytomegalovirus, Herpes simplex, Parvovirus B, Varicella, Hepatitis B and Human Immunodeficiency Virus (HIV). Cytomegalovirus is reported to occur in high incidence. The causative agent Cytomegalovirus is transmitted to body through transplacental, intrapartum and through Human Milk. Infection in Pregnancy may cause abortion, still birth and physical defects, growth deficit and physical dysfunction. The Infected infant may be quite normal at birth, but later develop certain sequelae. Considering all the above factors, it is worthwhile to conduct a study to know the seroprevalence of Cytomegalovirus in antenatal women having bad obstetric history with increased incidence of fetal loss either by way of abortions, still births or congenital malformations. The present study is hence attempted to assess the Seroprevalence of Cytomegalovirus in high risk pregnancies in antenatal women attending the Government Maternity Hospital, Hanamkonda with a view to help the Clinician to arrive at a proper diagnosis which will in turn result in a better antenatal care and management.

Keywords

Cytomegalo - virus in antenatal cases, Pregnant woman

Introduction

A number of microbiological agents may produce pregnancy loss without significant evidence of maternal disease.

The true incidence of infection in pregnant women is not known and the contribution of infection to abortion, congenital

malformation and fetal disease can only be guessed.

The cytomegaloviruses (CMVS) are a distinct, widely distributed subgroup of herpes viruses that share common growth characteristics and induce acytopathology involving characteristic nuclear and cytoplasmic inclusions. Viruses in this group were generally known as salivary gland viruses until the common name cytomegalovirus was proposed by Weller to reflect both virus-induced cytopathic effects and the virus role in congenitally acquired cytomegalic inclusion disease.

Cytomegalo viruses are the principal members of the beta herpes virus subgroup and they share several characteristics with other herpes viruses, including virion structure and the ability to establish persistent and latent infection. The CMV's exhibit a number of distinguishing biologic characteristics common to the beta herpes viruses, including salivary gland tropism, strict species specificity, and slow growth in cultured cells, all of which have been reviewed.

In most areas of the world, human CMV spreads at an early age and infects a large majority of the population. This pattern has been altered by increased hygiene in developed countries, where this virus may reach only 40% to 60% of the population. In the developed world, a large proportion of the adult population remains susceptible to primary infection increasing the risk of congenital transmission and subsequent disease. The importance of human CMV as a pathogen has also a reason over the past 3 decades with the increase in organ allografting and immunosuppressive post transplant therapies and the increase in acquired immunodeficiency syndrome (AIDS). Their conditions predispose

individuals to a primary CMV infection or to reactivation of latent infection, which may lead to fulminant and life-threatening disease that can be difficult to treat despite available antiviral drugs.

Morphology

CMV is one of the herpiviruses with a genome of 240 kb and a virus diameter of 200nm. It contains double-stranded DNA in a 64nm core enclosed by an icosahedral capsid composed of 162 capsomers. The core is assembled in the nucleus of the host cells. The capsid is surrounded by a poorly defined amorphous tegument in which it is surrounded by a loosely applied, lipid containing envelop. The envelope is acquired during the budding process through the nuclear membrane in to a cytoplasmic vacuole which contains the protein components of the envelop. Mature viruses exit the cell by reverse pinocytosis. Serologic tests do not define specific serotypes. In contrast, restriction endonuclease analysis of CMV-DNA shows that, although all known human strains are genetically homologous, none are identical unless they were obtained from epidemiologically related cases.

It is the most common cause of perinatal infection and evidence for foetal infections is found in from 0.5 to 2% of all neonates.

The genome, surrounded by a tegment or matrix and enveloped in a lipid bilayer carrying a large number of virus encoded glycoprotein's such a

- a. Virion proteins
- b. Capsid proteins
- c. Envelop glycoproteins
- d. Tegment proteins
- e. Host proteins

Viral DNA

The genome of human CMV (235 Kbp) contains internal repeats in a class E genome structure and undergoes inversion of two genome components similar to HSV-1. The genomes of all other characterized beta herpes viruses, including all the animal CMVs, are linear and lack internal repeats. The human CMV genome has two unique components, UL and US flanked by inverted repeats b (TRL/TRL) and C (TRS/TRS).

Genome organization

The only human CMV strain that has been completely sequenced is a laboratory-adapted strain, AD 169.

The AD 169 strain of human CMV chosen for genome sequence analysis is a variant (AD 169 var UK) that was propagated under growth conditions to attenuate virulence for its use as a live attenuated vaccine. Its genome was plasmid cloned following 25 yrs. of serial passage in various laboratories. The reported sequence contains errors requiring corrections as well as omissions that complicate its use as a prototype.

Infectious agent

(Propagation and cytopathic effect in cell culture)

Although a variety of cell culture systems are permissive, human CMV is propagated best in fibroblasts of human origin. The growth of human CMV is characterized by a relatively prolonged replication cycle and distinctive focal cytopathic effect (CPE) with cell enlargement and rounding (OWL's EYE).

Human foreskin fibroblasts and the human embryonic fibroblasts, WI-38 and MRC-5

are commonly used for propagation and isolation of CMV.

Pathogenesis, persistence, and latency

Human CMV exhibits a ubiquitous pattern of distribution and efficient transmission via direct contact in all areas of the world regardless of socio-economic conditions. The usually benign primary infection by this virus is

Controlled by a combination of innate and adaptive immune control functions, after which latent infection continues for life. Primary infection, lifelong latency, and intermittent shedding resulting from reactivation commonly occur without any marked disease consequences. These characteristics underscore the remarkable balance this pathogen has developed with its host.

Primary infection typically starts with replication in mucosal epithelium as a result of direct contact with infectious secretions from an infected individual. A high incidence of infection in the general population and accumulating evidence that hand-washing prevents acquisition of CMV from infants who shed high levels of virus in urine and saliva provide excellent evidence that this virus reaches a large proportion of the population because it is efficiently transmitted. A systemic phase of infection disseminates virus in the host via a leukocyte associated viremia that may last for several months as assessed using assays for viral DNA or viral antigen. Cell-free infectious virus is not found in blood, although viral DNA, somehow protected from degradation, is detected in plasma from immune compromised individuals. Peripheral blood (PB) neutrophils, monocytes and endothelial cell have been observed to carry infectious virus in

immunocompromised host, but the role of particular leukocyte subsets as vehicles for dissemination during primary infection in the immunocompetent host is still unclear. The suggestion that neutrophils act as vehicles to disseminate infectious virus and viral products has been supported by cell culture studies on transmission of clinical strains in the absence of full replication. Peripheral Blood (PB) monocytes are vehicles for dissemination of CMV, and this interaction is controlled by viral tropism determinants. The systemic phase of primary infection in adults is accompanied by persistent viral shedding in urine, saliva, breast milk and genital secretions, which may be important sources of transmission between hosts. The distribution of the virus during acute infection is best understood from studies in the immunocompromised host, but a range of endothelial, epithelial and hematopoietic cells in tissues are also believed to be susceptible in immunocompetent individuals systemic replication on leads to a well-documented seeding of the ductal epithelia where replication releases virus in to secretions. Myeloid-lineage hematopoietic cells become important targets for lifelong latency. Viremia continues can first be detected, typically at least 6 months in adults and several years in young children. Although an initial persistent infection is often observed, clearance of acute infection is the norm in all immunocompetent individuals and it is correlated with a slow rise in cell-mediated immunity. The relatively long period of persistent shedding observed in infants correlates with their immune status and allows the virus an efficient means of transmission to uninfected individuals.

CMV is also transmitted as a result of transplantation of solid organs or bone marrow and transfusion of whole blood,

where blood leukocytes are an important reservoir for transmission. P.B mononuclear cells in the myeloid lineage support latent infection. Latently infected cells most likely originate from the bone marrow in healthy seropositive individuals and virus has occasionally been recovered from PB leukocytes subjected to long-term co-culture. Latent virus is strongly suspected to play an important role as a source for transmission with transplanted solid organs or transfused blood products.

Pathogenesis of CMV disease is directly linked to the immune status of the host. Primary infections as well as reactivation from latency, contributes to pathogenesis. Innate and adaptive immune mechanisms control acute virus infection, maintain latent infection, and modulate virus during reactivation. CMV infection directly perturbs immune functions, leaving the host open to disease such as during transplant procedures, bone marrow or stem cell allografting and PB stem cell-autografting. This virus has been shown to down-regulate MHC-class-I protein expression to reduce the impact of cytotoxic t-lymphocyte (CTL) immunity.

Antigenic structure

- a. There are several families of glycoproteins in CMV, and these are important antigenic targets. One potential glycoprotein gene has homology with the cellular class I HLA-*a*-chain gene. HCMV binds to the host cell protein *B2*-macroglobulin (which associates with HLA class-I) and can use the class-I HLA molecule as an additional receptor for cell attachment and infection.
- b. Virions of CMV excreted in urine are coated with *B2*-microglobulin, which

appears to be attached to the tegument protein. Like HSV, CMV induces a receptor for the FC portion of human IgG on cells.

Vertical transmission of CMV

CMV is unique among the human herpes viruses in that transmission from mother to fetus or newborn not only occurs but is common and plays an important role in maintaining CMV infection in the population. Human CMV is spread from mother to baby by three (3) routes:

Trans placental,
Intra partum and
Human Milk

Serology

Serology is of secondary importance in the diagnosis of cytomegalovirus infection and disease.

Serology does play an important role, however, in assessing risk for recipients of transplants. Infection with cytomegalovirus, which is often asymptomatic, may be accompanied by DNA in peripheral blood for many months after primary infection even in the presence of a vigorous antibody response. Recrudescence of this latent virus may be accompanied by IgM response, so this usual marker of primary infection is flawed in this situation. Problems in valid detection of IgM class antibodies complicate the picture. In one careful study of intrauterine CMV disease only 10% of women with IgM antibody as determined by an enzyme immunoassay delivered a congenitally infected fetus. When the presence of IgM antibody was confirmed with a specific immunoblot assay, however, the risk was similar to documented infection during the first trimester.

The highest risk for serious CMV infection developing is transplantation of an organ from a seropositive donor into a seronegative recipient. When possible, attempts are made to avoid this combination.

Materials and methods

One hundred and eighty two samples for the present study were collected from Government Maternity Hospital, Hanamkonda during October 2001 to December 2001. All the patients are in the Child bearing age and have bad obstetric history during previous pregnancies. Criteria of selection of cases is high risk pregnancies, repeated abortions, missed abortions, intrauterine death, pre-term deliveries, intra uterine growth retardation, congenital malformations and perinatal deaths. All the patients are examined clinically, Laboratory investigation are performed to exclude other causes of fetal wastage like hormonal deficiencies, diabetes mellitus, renal disease, HIV, syphilitic infection etc. All the pregnant women subjected into investigations, represented different strata from both rural and urban segments.

As control study 50 cases of normal pregnancy of different ages were taken. Care is taken to see that the test and control groups are age matched. From each patient 5ml of venous blood samples are collected aseptically into sterile bottle, serum is separated and stored at -20°C till processed.

All the test samples and control sera are tested for cytomegalovirus IgG antibodies by ELISA test using kit (Sera Quest) supplied by the immunodiagnosis people AMAR, Banjarahills, Hyderabad. The technique of ELISA test is performed as per standard by Naot and Remington (1980).

List of infections in pregnancy

Group	Causative agent	Clinical picture in the neonate
I Protozoa	<i>Toxoplasma gondii</i>	Chorioretinitis, deafness seizure, hydrocephalus, microcephaly
II Viruses	1. CMV	Cytomegalic inclusion disease, microcephaly, chorioretinitis, mental retardation.
	2. Herpes simplex	Microcephaly, vesicular skin lesions.
	3. HIV-1	AIDS
	4. Parvovirus 13,9	Hydropsfetalis, congenital anaemia
	5. Rubella	Congenital rubella syndrome, cataract, heart disease and deafness
	6. Varicella-zoster	Fetal varicella
	7. Coxsackie virus	Myocarditis and meningoencephalitis
	8. Measles	Neonatal measles
	9. Hepatitis B	Symptomatic carrier state
III. Bacteria	1. <i>Treponema pallidum</i>	Congenital syphilis
	2. <i>Listeria monocytogenes</i>	Abortions and meningitis
	3. <i>Borrelia burgdorferi</i>	Congenital heart disease, hyperbilirubinaemia, blindness

Route	Maternal situation	Rate of CMV in infant
Trans placental	Seropositive before conception	0.2%–2.2%
	Primary gestational infection	20%–40%
Intra partum	Seropositive	5%
	Virus in genital tract at term	50%
Breast milk	Seropositive mother	25%
	Vairolactia	65%–70%

Results and Discussion

Out of 182 test sera collected from patients with bas obstetric history, 177 have shown seropositivity for cytomegalovirus by Elisa (IgG), compared with 14% seropositivity found in the control groups of 50 Sera collected from Government Maternity Hospital, Hanamkonda that showed to be 14% seropositive.

All the test sera age grouped into different age groups and percentage seropositivity is calculated (Table 2).

Table 3 shows the incidence of cytomegalovirus seropositivity in rural; and urban group. The percentage seropositivity is slightly higher (98%) in urban group where as it is 96% in rural group.

Out of 182 test sera collected from patient with bad obstetric history, 177 have shown seropositivity for cytomegalovirus by ELISA (IgG), compared with 14% seropositivity found in the control groups of 50 sera collected from Government Maternity Hospital, Hanamkonda that

showed to be 14% seropositive. Results were shown in table 1. The control group of 50 sera collected from pregnant women with normal obstetric history.

Table 4 shows the relation between cytomegalovirus seropositivity and the number and type of pregnancy.

As per table 5, there is a gradual increase of cytomegalovirus seropositivity with increasing parity. The highest percentage seropositivity 98% in gravida IV and above and it is still lower in (96%) gravida II.

The present study is undertaken to examine the incidence of seropositivity of Cytomegalovirus in pregnant women with bad obstetric history. The following are the various groups contributing for disease process in Cytomegalovirus infected persons.

- No.1 – Abortions
- No.2 – Abortions + IUD
- No.3 – Abortions + Preterm deliveries
- No.4 – Abortions + Congenital malformations

Table.1 Comparison of seropositivity in control and study group

Group	No. of sera tested	No. of +ve	Percentage
Study	182	177	97%
Control	50	14	28%

Table.2 Different age groups and percentage of seropositivity

Group	No. of sera tested	No. of +ve	Percentage
< 20 Yrs	146	142	97%
21 to 30 years	36	35	97%
	182	177	97%

Table.3 Incidence of cytomegalovirus seropositivity in rural and urban groups

Group	No. of sera tested	No. of +ve	Percentage
Rural	130	126	96%
Urban	52	51	98%
	182	177	97%

Table.4 The relation between cytomegalovirus seropositivity and the number and type of pregnancy

Type of pregnancy wastage	No. of sera tasted	No. of +ve	percentage
Abortions	124	120	97%
Abortions + IUD	16	16	100%
Abortions +Pre- Mature Labour	26	25	96%
Abortions + congenital Defects	16	16	10%
	182	177	97.6%

Table.5 Seropositivity with increasing parity

Gravida	No of Sera Tested	No. of +ve	Percentage
II	99	96	96%
III	59	58	96.5%
>IV	24	23	98%
	182	177	97%

Samples were collected from different age groups and parity wise belonging to different social-economic strata.

The occurrence of abortion, abortion + IUD, abortions + preterm deliveries and abortion + congenital malformations have been reported by several authors.

Experimental and clinical evidence indicate that humoral immunity to CMV take place a Key role in protecting the host from disease.

In conclusion, the present study indicates the

high prevalence of seropositivity of CMV constitutes to the high risk groups of women who needs to be Identified and monitored during pregnancies.

The high levels of seropositivity of CMV may be due to vertical transmission of virus from mother to fetus or from mother to newborn in maintaining CMV infection in population.

The acquisition of CMV prior to sexual maturity is also an important factor as most of the congenital CMV infections are caused

by reactivation of endogenous latent virus (Schopfer *et al.*, 1978).

The observation that congenital CMV infection rates are higher in population with high rates of maternal seropositivity has been offered as evidence that in many populations, most congenital CMV infections are caused by reactivity of endogenous latent virus (Stagno, S., Ritt, 2006).

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