INTRODUCTION AND AIM OF THE WORK

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Human cytomegalovirus, a member of the herpesvirus group, remains a serious health problem . It is the most frequent cause of human congenital viral infections and leads to numerous birth defects (Hanshaw, 1966) .

Most infections are asymptomatic . Clinically apparent infections constitute a small proportion of infected individuals . CMV infection is an important cause of congenital brain damage (Stern, 1977), where congenital infection occurs in about 1% of the neonates each years (Stagno, et al., 1984) .

Weller and Hanshaw (1962), characterized the abnormalities found most frequantly in patients with cytomegalic inclusion disease as hepatomegaly, splenomegaly , microcephaly, mental retardation, motor disability, jaundice , and petechiae .

CMV infection is known to be prevalent in developing countries (Palacios, et al., 1983), where the Socioeconomic level plays an important role in virus circulation . Percentage of CMV complement fixing antibodies ranged from 40% in highly industrialized areas to 100 % in developing countries (Krech, 1973).

Effective therapeutic or preventive measures for CMV infection are not available at the present time and vaccination is under investigations .

The aim of this work is to evaluate the incidence of cytomegalovirus complement fixing antibodies in the sera of mentally retarded children .

Review of Literature

HISTORICAL REVIEW

The recovery of human CMV was first accomplished independently in three different laboratories in the united states and was reported in 1956 and 1957 .

Smith in (1956) isolated two CMV strains from the salivary gland and kidney of two dying infants, Both tissues contained cytomegalic inclusion bodies .

Rowe, et al. (1956) recovered three CMV strains from surgically removed adenoid tissue of three asymptomatic children .

Weller, et al.(1957) isolated three CMV strains from liver biopsy speimens and/or urine of three infants with congenital CMV disease .

The first description of the histologic features of infection is attributed to Ribbert in, 1904, who observed a large inclusion-bearing cells in the kidneys of a stillborn infants who died of syphilis in, 1881, He described the inclusion as a homogenous body in the nucleus separated by a clear area from the nuclear membrane. His observation was not reported until 23 years later when (Jesionek and Kiolemenoglou, 1904) published the first illustrations of these "protozoan-like" cells in the kidneys, lungs and liver of another stillborn infants.

Lowenstein in 1907, found inclusions in 4 of 30 parotid glands obtained from children 2 months to 2 years of age .

Initially, these cells were called protozoan-like cells and were thought by many workers to be a protozoa . However, over the next several decades, the similarities of these cells to those infected by varicella-zoster virus and herpes simplex virus and to the salivary gland inclusions of guinea pigs and mice led to the consideration of a viral cause (Good Pasture and Talbot, 1921, and Good pasture, 1929) .

The experimental evidence confirming the viral etiology of the disease was provided by Cole and Kuttner, in 1926, who induced the formation of inclusion bodies in guinea pigs with filtered material using a filter impermeable to bacteria .

Andrewes, in 1930, attampted to propagate rodent salivary gland viruses in vitro . Although he was able to demonstrate intranuclear inclusion body formation in the primary cultures, attampts at serial cultivation of the agent were unsuccessful .

Smith in 1954, succeeded in propagating the salivary gland virus of mice in the primary explant cultures of mouse embryonic tissue . Utilization of similar

techniques led to the independant isolation of human cytomegalovirus strains shortly there after by, Smith (1956), Row et al., (1956) and Weller et al. (1957) .

Farber and Wolbach, in 1932 , were the first to employ the term "Salivary gland virus disease " because of the property of the virus to induce characteristic nuclear inclusions in the lining cells of the salivary gland ductal epithelium .

Weller, et al. (1960) proposed the term "cytomegaloviruses " because the cytomegalic inclusion disease , salivary gland virus disease nomenclature were both unwidly and misleading in that the salivary glands are one of many possible sites of involvement . Further more, the term "salivary gland virus " had been used to designate unrelated agents obtained from bats .

Since the isolation of the virus in tissue culture and the subsequent development of antigens for use in a variety of serological tests, Weller and Hanshaw (1962), and Medearis (1964) have established that human cytomegaloviruses are significant pathogens of human fetus capable of including a wide spectrum of oculocerebral defects , as well as a variety of extraneural abnormalities . These observations have been extended and confirmed by numerous investigators in the last decade .

V I R O L O G Y

CMV is a member of the genus herpesvirus (which include herpes simplex virus type 1 and 2, varicella zoster virus, and Epstein-Barr virus) and belong to the family Herpetoviridae (Fenner, 1976).

Morphology :

Morphologically CMV is indistinguishable form other members of the Herpesvirus group . The complete virion is 150 to 200 nm in diameter, icosahedral in shape , and consists of an inner core, a capsid and an envelope . The inner core (genome) is 64 nm in diameter and consists of a linear double-stranded DNA molecule . The capsid is 110 nm in diameter, is made of protein and has 162 capsomers . The envelope contains lipoprotein and is comprised of a single or double membrane (Huang, et al., 1973).

There are at least 33 structural proteins some of which reside in the envelope and are glycosylated (Kim, et al., 1976) .

Physichal and Chemical Characters :

CMV is a labile virus and is inactivated by lipid solvents (20% ether for 2 hours), low pH (4), heat ($37^{o}C$ for 1 hour or $56^{o}C$ for ½ hour), and ultraviolet light (5 minutes).

Preservation of infectivity can best be achieved by freezing the suspension of the infected cells in Bicarbonate-Free diluent . The optimal temperature for storage is -190^{o}C (liquid nitorgen), but it can be kept at -70^{o}C for several months (Feldman, 1968).

Histopathology :

CMV infection may be localized in the salivary glands or generalized with involvement of many organs . CMV replication is relatively slow . It can replicates in epithelial, fibroblast, and endothelial cells and in macrophages . The process of CMV replication in cells, both in vivo and in vitro, results in greatly enlarged rounded cells 25-40 nm in diameter (Smith et al., 1974)

Cell enlargement is visualized within six hours after infection . A paranuclear easinophilic inclusion is seen within 24 hours . The basophilic nuclear inclusion appears 48 to 72 hs. after infection . Multinucleated giant cells with intranuclear inclusions are occasionally encountered with CMV (Diosi, et al., 1973).

Antigenicity :

CMV possesses a broad antigenic mosaic . However, antigens responsible for the production of various antibodies have not been well defined . Two complement fixing

anitgens have been described . The first antigen, which contains at least two polypeptides, is soluble, stable at 4°C and loses its potency at 37°C or with boiling . The other antigen, which is primarily made of nucleocapsids, is virus associated and is prepared by the treatment of infected cells with glycine buffer (Dreesman et al., 1967).

Sequential immunofluorescent antibody studies indicate that CMV replication produces pre-early (immediate) early or late CMV antigens . Pre-early antigens appear in the nucleus of CMV infected cells one to three hours after infection . Early antigens appear in the cytoplasm or membrane about three hours after infection and before DNA synthesis . Late antigen appears in the nucleus and cytoplasm within 6 to 24 hs after infection and after DNA synthesis (Giraldo et al., 1977).

Antibody Response :

The process of virus replication in the host results in the production of anti-CMV antibodies of the IgG, IgM and IgA classes . These antibodies may last from several weeks to several months or persist for a lifetime (Reynolds, et al., 1979) .

CMV specific IgG antibody may be detected by complement fixation (CF), neutralization, indirect hemagglutination (IHA), immunofluorescent antibody testing (IFA), radioimunoassay (RIA), immune adherence hemagglutination (IAHA), and enzyme-linked immunosorbent assay (ELISA) (Booth et al., 1982).

CMV and Animals :

CMV is highly host restricted . There is no experimental animal model for studying human CMV . All attempts to infect animals with human CMV have failed (Plummer, 1959).

EPIDEMIOLOGY

Knowledge of the epidemiology of CMV infection has developed in large part from serologic data and to a lesser extent from an analysis of viral isolates .

A. Epidemiology in the General Population :

1. Prevalence of antibody :

CMV is a ubiquitous agent . Studies on general population of isolated parts of the world indicated that the majority of the world population experiences CMV infection during their lifetime (Stern and Elek, 1965 ; Sinha and Pauls, 1971; and Krech and Tobin, 1981).

Table (1) shows prevalence of complement fixing antibody to CMV in various population .

2. Prevalence of virus excretion :

i. Isolation from urine samples and oral secretion :

About 1% of new borns excrete CMV according to several surveys (Starr et al., 1968 & Leinikki et al., 1972).

Among older children, Hanshaw, et al., (1965) reported the incidence of CMV excretion to be 1 % in healthy children up to 14 years and among children of the same age admitted to hospital .

Stern, in (1968) found that 10% of unselected children (2 months to 5 years) excrete CMV in urine .

Rowe, et al. (1958) reported that 28% of children (8 months to 4 years) in an institution excreted CMV .

ii. Isolation from cervical secretions :

An average of 10% of non pregnant young women excrete CMV from the cervix (Knox, et al., 1979 & Stagno et al., 1975) . This rate increases in pregnant women and may reach 28% in the third trimester (Stagno, et al.1975).

iii. Isolation from semen :

CMV is found in semen samples of 5% of young adults (Lang, and Kummer, 1975 & Embil, et al., 1982), 10% of patients with suspected sexually transmitted disease, and 14% of homosexual men (Drew, et al., 1981).

iv. Isolation from breast milk :

CMV has been isolated from the breast milk of asymptomatic CMV seropositive women . The reported rate of virus shedding in milk ranges from 13% to 27% (Stagno, et al., 1980) .

v. Isolation from feces :

Rarely CMV has been isolated from feces of patients with mononucleosis and that of patients with CMV-induced gastrointestinal lesions (Cox and Hughes, 1974).

Eight per cent of CMV seropositive children studied by repeated culture of rectal swab have been found to excrete CMV in their feces, while the rate of virus excretion from the urine of these children was 89% (Cox and Hughes, 1974) .

vi. Isolation from tears :

CMV has been isolated from tears of patients with CMV mononucleosis or chorioretinitis (Cox, et al.,1975)

B. Epidemiology in Specific Groups :

1. Pregnant women :

Serological studies have shown that about 60% of women entering the child bearing years have antibody to CMV (Stern, et al., 1973) .

Maternal excretion of cytomegalovirus during pregnancy occur in 12% - 25% of the population (Gehrz, et al., 1981).

It has been hypothesized that transient depression of cytomegalovirus specific cellular immunity during pregnancy might facilitate reactivation of latent maternal virus (Moore, et al., 1983).

2. Homosexual men :

Homosexual men as compared to the general population or to heterosexual men, have a significantly higher prevalence of CMV antibody and CMV excretion in urine . A study in San Francisco indicated that the prevalence of CMV antibody in homosexual men (94 %) was significantly higher than that of male volunteer blood donors (43%) and CMV was isolated from the urine of 7.4 % of 190 homosexual men, but from none of 101 hetrosexual men attending the same clinic (Drew, et al., 1981).

3. Immunocompromised hosts :

Retrospective analysis suggests that a sharp increase in the infection rate with CMV coincided with the introduction of immunosuppressant drugs (Kanich and Craighead, 1966) .

4. Renal transplantation and cytomegabvirus :

There is double risk with renal transplantation, the first is the risk of transfusing CMV from the donor, the second is the risk of reactivating the latent virus in the recipient through immuno-suppressive regimens (Watson, 1984).

On autopsy evidence of CMV infection has been found in up to 91 % of patients receiving renal transplants (Watson, 1984).

Seasonal Incidence :

There is no clear evidence that congenital or acquired CMV infection has a well defined seasonal incidence (Hanshaw, 1971).

Table (1) : CMV prevalence by serological survey : General populations

Author	Date	Place	Population	CMV seropositive %
Hanshaw	1966	Rochester	0-5 mo.	35 %
			5-24 mo.	3 %
			2-6 yr	6 %
			6-10 yr	9 %
			10-17 yr	22 %
			27-40 yr	38 %
Rowe, et al.	1956	Washington	6-24 mo.	14 %
			5-9 yr	33 %
			>35 yr	81 %
Stern and Elek	1965	London	6-60 mo.	4 %
			5-10 yr	15 %
			>35 yr	54 %
Carlstrom	1965	Stockholm	6-24 mo	36 %
			5-10 yr	24 %
			>50 yr	63 %
Jack and McAuliffe	1968	Melbourne	6-36 mo.	22 %
			10-15 yr	40 %
			>35 yr	60 %
Evans, et al.	1974	Barbados	1-5 yr	62 %
			15-25 yr	77 %
Embil, et al.	1969	NovaScotia	6-12 mo.	12 %
			10-14 yr	14 %
			>40 yr	52 %
Krech and Jung	1970	Tanzania	6-18 mo.	80 %
			5-14 yr	100 %
			>20 yr	99 %
Shavrina, et al.	1973	Leningrad	7-12 mo.	67 %
			11-15 yr	63 %
			51-60 yr	80 %

Table (1) : Cont.

Author	Date	Place	Population	CMV seropositive %
Evans et al.	1979	St. Lucia	0-5 yr	83.5 %
			6-10 yr	94.9 %
			11-20 yr	92.5 %
			21-30 yr	96.2 %
			31-40 yr	100 %
			>40 yr	96.9 %
Wang, and Evans	1986	China	0-1 yr	100 %
			1-5 yr	100 %
			5-10 yr	89.5 %
			10-15 yr	90.5 %
			Total (average)	93.6 %
Ashraf, et al.	1985	Saudi Arabia	4 m-2 yr	69 %
			3 yr-5 yr	87 %
			6 yr-11 yr	95 %
			>12 years	98 %
			total (average)	87 %

MODE OF INFECTION

A. Natural :

1. Oral Route :

Close and prolonged contact with infected individuals or infectious secretions appear to be necessary for oral transmission of CMV (such as kissing in transmission by saliva, nursing in transmission by breast milk, and passage through the birth canal in transmission by cervical secretions .

Oral transmission of CMV in humans has not been shown experimentally but is supported by the following indirect evidence :

* Individuals with symptomatic or a symptomatic CMV infection excrete the virus in the saliva, urine and other body secretions for a long period of time (Weller, 1971).

* A higher prevalence of CMV antibody and shedding in children in boarding schools and daycare centers as compared to those attending day schools (Pass et al., 1982).

* High incidence of CMV infection in close contacts of children with viruria (Embil et al., 1970). And a higher incidence of CMV infection in breast-fed infants as compared to formula fed ones (Stagno et al., 1980) .

* The remarkable increas in the CMV viruria rate during the first year of life (8% to 60%) as compared to (0.4 % to 2.5%) at birth, suggests that CMV is acquired postnatally in most infants (Leinikki et al., 1972).

2. Genital Route :

There is strong epidemiologic evidence for genital transmission of CMV . Sexual transmission of CMV in adults is supported by several observations :

* A relatively high rate of CMV excretion in the semen of asymptomatic young men (5%) (Lang and Kummer, 1975) ; and in the cervical secretions of asymptomatic young women (10%) (Jordon et al., 1973).
* A higher incidence of CMV antibody, viruria, and symptomatic infection in homosexual men as compared to hetrosexual men (Drew, et al., 1981).

3. Transplacental Route :

The exact mechanism of transplacental transmission of CMV is not known. Infection may occur and be transmitted in any stage of pregnancy (Stagno et al., 1982). Direct transmission of CMV from the infected placenta to the fetus is possible but does not appear to be common (Monif, and Dische, 1972).

Viremia seems to be the major mechanism of transmission in the prenatal period . Local reactivation of infection in the cervix of the mother and contiguous spread is another potential mechanism of transmission (Griffiths et al., 1978).

B. Iatrogenic :

1. Transfusion :

Fresh or Stored blood or granulocyte transfusion can transmit CMV infection (Winston et al., 1980).

CMV can be isolated from the mononuclear, polymorphonuclear, or both fractions of peripheral blood leukocytes and not from washed erythrocytes or plasma (Armstrong, et al., 1971) .

2. Organ transplantation :

CMV can be transmitted by organ transplantation when an allograft from a seropositive donor is given to a seronegative recipient .

Following kidney transplantation, several centers have reported that CMV-seronegative recipients given kidneys from seropositive donors have a greater incidence of CMV infection than whose kidney allografts were from seronegative donors (Ho M, et al., 1975). Similar results have

been reported following cardiac transplantation (Preiksaitis, et al., 1981).

The failure to isolate CMV from donated kidney suggests that the virus is present in the kidney in a latent form. However, attempts to unmask the latent virus by the tissue explantation technique from kidney tissue have been unsuccessful . It is possible that blood leukocytes that are trapped in the allograft organ and not the allograft parenchyma are the source of virus transmission (Naraqi , et al., 1978).

Incubation Period :

The exact incubation period of CMV is unknown . The incubation period may be estimated from the interval between the time of exposure to CMV and development of symptoms or detection of viruria in individuals with no prior exposure to CMV . This period is four to eight weeks, and is similar in infants exposed to CMV in the genital tract at the time of delivery, in breast-fed infants exposed to CMV in mothers milk, and in adults with CMV mononucleosis following blood transfusion (Stagno et al., 1980; Reynolds et al., 1973; and Lang and Hanshaw, 1969).

PATHOGENESIS

After entering the human body, it is widely blieved that CMV spreads via the blood stream to various organs . The vehicle of the virus transportation in the blood is blood leukocytes . Both mononuclear and polymorphonuclear leukocytes have been implicated . Viremia and virus excretion from various sites occur in the presence of high titer neutralizing antibody (Rinaldo, et al., 1977).

CMV infection may occur in individuals without prior exposure to the virus (primary infection) or in those with prior exposure to the virus (recurrent infection). Recurrent infection may result from reactivation of endogenous latent virus or from reinfection with an exogenous virus (Huang, et al., 1980) .

Pathogenesis of Fetal Infection :

There are a variety of possible end-effects that may result from infection of pregnant women . Mothers who are infected systemically (as evidenced by cytomegaloviuria) do not necessarily produce infected off spring .

Hanshaw, et al. (1973) did not find infection in any of eight new borns born to mothers with viruria. The surrounding membrans and separate circulation of the fetus

provide effective barriers to passage of most infectious agents . The manner in which cytonegalovirus is able to circumvent barriers that protect the fetus from the great majority of infectious agents is not understood .

Just as CMV is able to cross the placental barrier, it is able to gain access to the central nervous system accross the blood brain barrier . There is recent evidence that this occurs in the adult central nervous system as well as in the more readily penetrated fetal central nervous system (Chin et al., 1973; and Dorfman, 1973).

Most of the clinical abnormalities seen in CMV infection are the result of inflammatory changes occuring relatively late in pregnancy . These include hepatosplenomegaly, thrombocytopenia with petichae and purpura, hepatitis, pneumonitis and chorioretinitis . Abnormalities resulting from faulty organogenesis secondary to infection include microcephaly, optic atrophy, aplesia of various parts of the brain, and microphthalmia (Hanshaw, 1970).

Post Transfusion Infection :

The pathogenesis of CMV infection following transfusion is not clear . The major questions are whether this represents a primary or a reactivated infection and if the latter, whether the source of virus is the recipient or blood of blood donors (Diosi, et al., 1969).

Seronegative infants who receive blood from seropositive donors have an incidence of CMV infection of 13.5% (Kumar, et al., 1980); and transfusion of CMV-seronegative blood to seronegative infants has not been associated with post transfusion syndrome (Yeager et al., 1981).

I M M U N O L O G Y

CMV affects humoral as well as cell-mediated immune responses in both normal and immunocompromised hosts .

A. Humoral Response :

Normal and immunocompromised hosts develop IgG, IgM and IgA antibodies to CMV following active infection. Antibody production against CMV is relatively intact in immunosupressed patients (Rasmussen et al., 1982).

CMV disease and viremia occur in the presence of high titers of neutralizing antibody (Fiala, et al.,1973).

Maternal antibody does not prevent transplacental transmission of CMV infection (Stagno, et al., 1973) , Furthermore, there is a higher incidence of CMV infection in organ allograft recipients who are CMV seropositive perior to transplantation than in those who are not (Fiala et al., 1975) .

Congenitally infected infants born to CMV immune mothers have a lower incidence of brain damage and CMV disease . In one series, five of 33 infants born after a primary infection had CMV disease while none of the 27 born after recurrent infection were ill (Stagno, et al., 1982).

In bone marrow allograft recipients, administration of cytomegalovirus immune plasma did not prevent CMV infection but significantly reduced the rate of CMV pneumonia or illness (Winston, et al., 1982).

Patients with organ allografts have a high fatality rate from CMV infection (Simmons et al., 1976) . These observations indicate some protective role for humoral antibody .

B. Cellular Response :

Cell mediated immunity (CMI) has a crucial role in limiting the spread of CMV infection in patients with mononucleosis and in preventing disease caused by reactivated infection in immuno compromised hosts (Ho, M., 1981). All the conditions associated with a high prevalence of CMV infection involve depressed CMI (organ transplantation , Malignancy and pregnancy) .

Impaired T cell proliferative responses to CMV after congenital or neonatal infection have been reported (Hayward et al., 1984). Also CMV is reported to interfere with monocyte function (Loh, and Hudson, 1982).

In congenitally infected children and their mothers during pregnancy and shortly after delivery, CMI to CMV is depressed . This depression is transient . Whether CMV

infection is the result or the cause of this immunosuppression in neonates and pregnant women is not known (Gehrez, et al., 1981).

CMV a major human herpesvirus preoduces severe disease in individuals with immature or impaired immune responses (Weller, 1971) . The virus can also suppres the immune system in normal individuals (Carney, et al., 1983) , and neonates (Pass et al., 1981), and probably potentiates immunosuppression in allograft recipients (Glenn, 1981) and in patients with AIDS (Stahl et al., 1982).

The mechanism of CMV induced immunosuppression in compromised hosts is not clear . It is suggested that the macrophages as well as T cells that are present in normal individuals with CMV mononucleosis may be deficient in allograft recipients . This depression further predisposes the patients to severe and fatal secondary infection (Pass et al., 1981).

Kapasi and Rice in (1986) provided the evidence that infection of the monocyte by CMV accounts for the invitro immunosuppression and that CMV can directly alter important , specialized functions of the monocyte .

Tanaka et al. (1986) reported that cessation of the virus excretion in inapparent CMV infection among healthy infants and children probably results from the specific cell-mediated immunity.

Immunologic Abberations :

A variety of immunologic abnormalities has been observed in association with CMV infection . These include circulating immune complexes, cold agglutinins, rheumatoid factor, antibody to nuclear antigen, positive coombs test , cryoglobulin, and monoclonal gammapathy of IgA , IgM, or IgG types (Kantor et al., 1970; and Vodopick et al., 1974). These abberations are seen in congenital or acquired infection . All these abnormalities are transient and disappear with the resolution of CMV infection . A high incidence of skin rash is also observed in patients with CMV mononucleosis who receive ampicillin, but the mechanism of this reaction is unknown .

PATHOLOGICAL FINDINGS

Most infants who die in the neonatal period are premature or small for dates, indicating that intrauterine growth retardation has taken place . Of the organs affected, the kidney, the liver and lung . Less often the pathologist may find inclusion bearing cells in the pancreas, thyroid and brain . Rarely, inclusions are found in the intestine, ovary, pituitary, parathyroid and thymus . Fosi of extramedullary hematopoiesis are seen in the liver, spleen and kidneys . The microscopic and gross appearance of the liver may be identical to that of neonatal giant cell hepatitis. It is not uncommon for this pathologic appearance to occur in the absence of typical inclusion bearing cells (Hanshaw, and Dudgeon, 1978).

Brain :

The most extensive changes in the brain involve the subependymal mantle and adjacent periventricular areas . The ependyma can appear rust-coloured and become iregularly thickened to form either coarse rugae or fine granulations. Calcifications occur in discrete clusters in the subependymal mantle and adjacent white mantle .

Although the lesions of CMV encephalitis of congenital infection are usually confined to the limbic system, other malformations outside this area have been reported.

These inlude micropolygyria, cerebellar aplasia and cerebellar hyperplasia . Occasionally calcification occurs in the region of the fourth ventricle . In some patients, an obstructive hydrocephalus develops, presumably on this basis (Hanshaw and and Dudgeon, 1978).

Kidneys :

The kidneys show no gross alteration. Microscopically, inclusion-containing cells are seen especially in the epithelium of the proximal convluting tubules. They may also be present in the distal and collecting tubules, the interstitial tissue and the glomeruli . Affected cells may desquamate into the lumina of the tubules and appear in the urinary sediment . This phenomenon was the basis for a diagnostic test prior to the development of methods for the isolation of virus . Some tubular cells may show cloudy swelling . Mononuclear cell infiltration may be present in the peritubular zones of the kidney (Hanshaw, Dudgeon,1978)

Lungs :

Pulmonary CMV lesions are similar in the adult and the newborn . The pneumonitis may be bilateral or unilateral and generally involves the lower lobes. On gross examination there may be well-defined rounded areas 2mm to 6 cm in diameter located just beneath the pleura . This

nodular character may be lost as the disease process advances and becomes more confluent . Microscopically, the alveolar cells appear large, and some contain intranuclear inclusions . An asphyxial barrier to gas exchange may be present in the form of PAS-Positive hyaline membranes adjacent to the septal wall . These membranes are found less often in young infants than in older children and adults . Numerous mononuclear and plasmacytic inflammatory cells may appear focally or more diffusely throughout the septal walls (Hanshaw, and Dudgeon, 1978).

Liver :

The gross changes in the liver are variable . Some infants dying in early infancy have yellow fatty changes, others have evidence of cirrhosis . Microscopically,there may be disintegration of the normal lobular architecture with areas of necrosis in the parenchyma, peripherally as well as in the center of the lobule . When inclusions are present, they are often in the parenchyma, kupffer's cells and the biliary duct epithelium . The latter observation has led to the speculation that intrauterine CMV infection may be one cause of biliary duct reduplication . Regenerative activity of the hepatic cells in often present (Hanshaw, and Dudgeon, 1978).

CLINICAL MANIFESTATIONS

Although CMV infection is quit common, CMV disease is rare . The majority of infections are asymptomatic and can be detected only by laboratory study . The frequency and nature of clinical response appear to depend on the age at which infection, on the route of infection, and on the immune status of the host . The commonest forms of associated clinical syndroms are :

Congenital CMV Infection :

There are many clinical varients seen in congenital CMV infection, ranging from silent infection to the more severe classic cytomegalic inclusion disease . The more adversely affected infants represent less than 5 percent of all infected newborns . Typical clinical features include hepatomegaly, splenomegaly, jaundice, petechial or purpuric rash, microcephaly, chorioretinitis and cerebral calcifications (Medearis, 1964) .

Neurological Manifestations :

Areas of periventricular calcification in the subependymal region are characteristic of severe congenital cytomegalovirus encephalitis occuring relatively early in pregnancy . Severe microcephaly is frequently associated with calcium deposition . Rarely, obstruction of the fourth

ventricle follows and hydrocephalus develops, a condition that is usually fatal . Chorioretinitis, optic atrophy and strabismus are frequently seen in patients with cerebral calcifications . Although less than 1 percent of all CMV infected infants have calcifications, their appearance in a periventricular distribution is strong presumptive evidence of the severest form of congenital CID .

Microcephaly :

Microcephaly usually defined as a head circumference of less than the third percenticle, was found to be present in 14 of the 17 patients reported by, Weller, and Hanshaw (1962), and in all of seven patients studied by, Medearis (1964) .

Although there are reports of infants with microcephaly who later were judged to have normal intelligence (Berenberg, and Nankervis, 1970); the great majority of infants with disproportionate growth in head circumference have associated psychomotor retardation .

Microcephalic patients were compared with normocephalic children with respect to the prevalence of CMV-CF antibody, Nine of 25 (36%) children, six months to eight years old, were sero-positive in contrast to 7 of 157 (5%) normocephalic control children (Hanshaw, 1966) . Subsequently, younger patients (6 to 24 months of age) with microcephaly

were studied and a similar percentage (35%) was found to be seropositive (Hanshaw, 1968).

Baron et al. (1969) found a higher prevalence of CMV-CF antibody in microcephalic than in normocephalic children five months to five years of age . These differences were not significant, however, Stern et al. (1969) found antibody in 14 of 64 (22%) microcephalic mentally retarded children and in (6.5%) of 154 normal controls . These data are statistically significant .

Encephalitis :

Encephalitis with residual neurologic impairment is the most devastating aspect of congenital cytomegalovirus infection, but, fortunately, is a rare event in postnatally acquired infection . A single case report by Dorfman, describes fatal cytomegalovirus encephalitis in a patient with chronic hepatitis following a short course of steroid therapy (Dorfman, 1973)

Polyneuritis :

An association between acute idiopathic polyneuritis (The Guillain Barre Syndrome) and cytomegalovirus infection has been suggested by several investigators . Klemola et al. (1967) described a patient who developed viruria and a fourfold rise in cytomegalovirus complement fixing

antibody during his neurologic illness . Dowling et al. (1977) reported significant increases in cytomegalovirus complement fixing antibodies in 21 of 92 patients with Guillain-Barre syndrome . Viral cultures were not done routinely in this study, although the authers isolated cytomegalovirus from urine of the 4 patients.

Mental Retardation :

Of the original group of 17 infants described by Weller and Hanshaw (1962), 14 infants were mentally retarded and in six of these the retardation was severe . In United States CMV infection is frequent (33,000 infected infants each year), one thousand infants are born with the severst form of the infection causing marked mental retardation and cerebral palsy . Another 5,000 infants are affected by less severe, but nevertheless important abnormalities such as mild mental retardation, deafness and perhaps learning and behavioral difficulties . An other larger survey in which cord blood samples were screened for CMV-specific IgM antibody identified lower I.Q. scores and higher percentage of school failures in children with CMV infection (Hanshaw, et al., 1976).

Ocular Defect :

The principal abnormalities related to the eye are Chorioretinitis, Strabismus, and optic atrophy, although

microphthalmia, cataracts, retinal necrosis and calcification, blindness, anterior chamber and optic disc malformation, have been described in association with generalized congenital CID (Hanshaw, 1970).

Hearing Defect (Impairment) :

Cytomegalovirus has the potential for damaging the inner ear, and deafness and hearing impairments occur in a significant of children with congenital cytomegalovirus infections . Post natally acquired cytomegalovirus infections have not been associated with hearing impairments. Histologic examination of temporal bones from two congenitally infected infants revealed abnormalities in the cochlear duct epithelium, characterized by inflammatory changes with sparing of the organ of corti (Meyers, et al., 1968 and Cox, et al., 1975) .

Other Manifestations :

Pulmonary involvement (pneumonitis):

Pulmonary disease occurs rarely in congenital infection but is a frequent manifestation of postnatally acquired infections in children and adults . Cytomegalovirus should be considered in the differential diagnosis of pneumonitis in infant typically presenting at 2 to 3 months of age with cough, tachypnea, and rales. Abdallah et al. (1976) reviewed 16 cases of cytomegalovirus pneumonia in

immunologically compromised hosts . All patients presented with fever, dry non productive cough, and dyspnea. Predominantly interstitial infiltrates were present on the X-ray film in 9 of the 16 patients, with variable abnormalities in the remaining 7 patients .

Hepatomegaly :

The liver edge is smooth and non tender and usually measures 3 to 7 cm below the right costal margin . The larger the liver, the greater the probability that hyperbilirubinaemia will be present . Liver function tests are often abnormal but usually not markedly so. The serum glutamic oxalacetic transaminase (SGOT) level rarely exceeds 800 and is usually below 500 units.

Weller and Hanshaw (1962) isolated CMV from the urine or liver of five of ten infants with the histological changes of "neonatal hepatitis". In most instances, other findings characteristic of cytomegalic inclusion disease were also present.

The association of cytomegalovirus with liver abnormalities has been established in both congenital and acquired CMV infections. In (1965), Hanshaw, et al. reported the association of hepatomegaly and abnormal liver function tests (Particularly alkaline phosphatase and SGOT) or both

in 9 of 20 children with a symptomatic viruria .

Although some patient with congenital CMV infection have developed cirrhosis of the liver (Lysaught, 1962),there is no good evidence that chronic active hepatitis is associated with acquired CMV infection (Toghill, et al., 1969). Recently, Reller (1973) presented evidence that acquired cytomegalovirus infection may be responsible for granulomatous hepatitis.

The persistence of hepatomegaly is variable . In some infants, enlargement diappears by the end of the second month of life . In others, significant hepatomegaly persists throughout the second year of life . Viruria may continue long after the diminution in liver size . Massive hepatomegaly extending beyond the first 12 months of life is quite uncharacteristic of CID (Handshaw, and Dudgeon, 1978).

Splenomegaly :

Enlargement of the spleen is found to a greater of lesser degree in all the common congenital infections of newborn and is especially frequent in congenital CMV infection (Handshaw, & Dudgeon, 1978). It may be the only abnormality present at birth . In some instances, splenomegaly and a petechial rash coexist as the sprime manifestations of the disease . Occasionally the spleen size is beyond

5 to 6 cm. Rupture of the spleen, a complication of EBV-induced infection mononucleosis in older persons, has not been reported in CMV mononucleosis or in congenital disease (Hanshaw, and Dudgeon, 1978).

In some patients dying of CID, there may be direct evidence of CMV infection of splenic tissue. Virus is often directly isolated from this organ, and large inclusion-bearing cells may be seen (Hanshaw & Oadgoen, 1978).

Hyperbilirubinaemia :

This is a common manifestation of congenital CMV infection, occuring in more than half of symptomatic infants in the first week of life . The patterns of hyperbilirubinaemia may take several forms, ranging from the high levels on the first day that require exchange transfusions, to undetectable jaundice, with gradual elevation of the bilirubin level to clinically apparent jaundice (Weller, and Hanshaw, 1962).

Petechiae, Purpura, and Thrombocytopenia :

There is evidence that CMV has a direct effect on the megakaryocytes of the bone marrow, resulting in a depression of the platelets and a localized or generalized petechial rash, which may be transient, disappearing within

48 hours, It is rarley present at birth but often appears within a few hours thereafter . The petechiae may be the only clinical manifestation of CMV infection . More often, However there is associated enlargement of the liver and the spleen . The petechiae may persist for weeks after birth and in some instances are present well into the first year of life (Hanshaw, and Dudgeon, 1978).

Although purpuric phenomena have not been clearly established as a manifestation of acquired CMV infection, we have observed one sibship of two brothers who had cytomegaloviruria and chronic petechiae (Hanshow, and Dudgeon, 1978).

Platelet counts in the first week of life range from less than 10,000 to 125,000 . Some infants with petechial rashes do not have associated thrombocytopenia (Hanshaw, and Dudgeon, 1978).

DIAGNOSIS

There are several methods that may be used to make a diagnosis of CMV infection.

1. Clinical :

The diagnosis can almost never be made with certainty on purely clinical grounds . This would require a rather classic presentation and, particularly, the presence of periventricular calcifications . It is highly improbable that this type of calcification would be found in toxoplasmosis, rubella, or congenital syphilis . Clinically, CMV infection should be suspected in :

i. Newborn infants with microcephaly, jaundice, hepatosplenomegaly, and/or purpura.

ii. In individuals with mononucleosis syndrome.

iii. In immunocompromised hosts with fever, pneumonia, or other known syndromes caused by CMV.

Even in the most typical case of cytomegalic inclusion disease, it is advisable to confirm the diagnosis through one or more laboratory tests .

2. Virological :

Isolation of virus from a fresh urine specimen, a throat swab, a liver biopsy specimen, or another organ at autopsy, will establish that CMV infection is present but

does not necessarily establish that the disease observed is due to CMV . This is particularly true when the virus is isolated several months after birth, a time when an asymptomatic infection could have been acquired . If the urine culture for CMV is positive, the probability is high (over 90%) that an infant has a systemic cytomegalovirus infection . The cells required for cultivation of the virus are human fibroblasts.

Recently, Wanner and Weller (1974) have cultured human CMV in simian and bovine fibroblastis cells . The urine obtained for culture should be a clean-voided specimen . If transfer to the virus laboratory is not immediate, the specimen should be refrigerated or held in water ice . This is to prevent bacterial overgrowth, as well as to avoid fall of virus titer caused by warmer temperatures. It is important to inoculate all cultures as soon as possible after collection, preferably within six hours .

Before inoculation of the urine it is advisable to centrifuge the specimen at 2500 rpm for 20 to 30 minutes. An aliquot of 0.25 ml of urine supernatant is then pipetted into two or more tissue culture tubes containing 1.5 ml of minimal essential media with 10 percent newborn calf serum . Antibiotics (Penicillin, gentamicin and amphotericin B) are added to the medium to avoid bacterial contamination . There are a variety of satisfactory methods for

virus isolation (Benyesh-Melnick, 1969). Some workers allow the urine to be absorbed on to the cell sheet for 30 minutes, remove the urine, and then add the medium . Satisfactory results have been obtained with both techniques.

Once the cultures are inoculated, the tubes are placed in an incubator at 35°C . Cultures may be rolled or incubated in stationary Racks . The appearance of Viral cytopathic effect (CPE) is accelerated by the rotation of the tubes . Tubes are observed under the light microscope one or two times per week and compared with control tubes prepared at the same time . Cultures may become positive as early as the first day or, more commonly between 7 and 14 days . Most positive cultures show a cytopathic effect by the end of the third week . Rarely, cultures become positive after a 3o day observation period . An early cytopathic effect (within 24 hours) is often generalized, a more delayed cytopathic effect is typically focal in character with small clusters of five to ten rounded, refractile, elongated fibroblasts appearing in a sheet of less conspicuous fibroblastic cells . This appearance is characteristic enough in most cultures to warrant specific identification of the agent as a cytomegalovirus (Hanshaw,1969).

Recently, Schuster, et al. (1986) showed that human CMV can be easily detected in urine specimens by hybridization with a peroxidase-labeled human CMV probe consisting

of the Hind III L DNA fragment or its in vitro-synthesized RNA transcripts . Both probes are able to detect a few Pico-grams of homologous, cloned human CMV DNA and are highly specific for human CMV DNA .

Inoculation of Other Specimens :

In addition to upper respiratory tract swabs, CMV has been recovered from many organs, particularly liver , lung, spleen, kidney and brain, as well as faces, tears (Cox, and Hughes, 1973), milk (Hayes, et al., 1972) and semen (Lang, and Kummer, 1972). Virus can occasionally be isolated from cerebrospinal fluid . If the specimen is a piece of tissue, it should be ground in a mortar contain-ing media in abrasive substance such as sterile alundum . A 10 percent suspension of the ground tissue should be allowed to settle in the mortar before it is inoculated with 0.2 ml of the supernatant .

3. Serological Tests :

There are several methods available for measuring CMV antibody.

These include :

1. The complement-fixation test (C.F.).
2. The indirect fluorescent antibody test.

3. Indirect hemagglutination test (IHA).
4. Platelet agglutination .
5. Neutralization tests.
6. Recently, Radioimmunoassay (RIA), and Enzyme-linked immunosorbent assay (ELISA) tests.

1. Complement-Fixation test (CF) :

This test is simple, reliable and specific and was the most widely used routine serologic assay before the fluorescent antibody technique become available . The CF test has certain limitations . False-negative results are encountered rarely.

CF test, first developed by Rowe, et al. (1956) , was modified by Medearis (1964) and Sever et al., (1963).

Most laboratories now employ the micromethod, which is accurate, requires small amounts of serum and is convenient for large scale testing . The variable antigenic composition of the cytomegaloviruses may account for the fact that as many as 10 to 15 percent of infants with cytomegaloviruria may not have antibody to any one CMV antigen , such as the commonly used AD 169 strain . The percentage of seropositive specimens can be increased if other strains such as Kerr and Davis also are used . This is not usually done, however, in routine diagnostic work because of the greater technical effort required for a relatively small number of "false negatives " and because of a general lack

of appreciation of antigenic heterogenicity in the CF test. Huang, et al. (1974) have presented evidence that at least two different strains of CMV are closely related or identical .

The complement-fixation test may be used alone or in conjunction with virus isolation or CMV-IgM tests to determine whether an infant is congenitally infected. More important than the presence of antibody in a young infant with suspected infection is the persistence of a titer of 1:8 or greater during the four to six months after birth . An infant of two months of age with a CF titer greater than the maternal titer is probably actively infected . It is rare for transplacentally acquired maternal CF antibody to persist beyond six months . It has been estimated that the half-life of most maternally derived IgG antibody is approximatly 23 days . Thus , a relatively high cord serum titer of 1 : 64 might be expected to be 1 : 8 or less at three months of age (Hanshaw, 1969).

Wanner, et al. (1973) followed the CMV-CF antibody titers in 50 adult patients over an 18-months period. The AD 169, Davis, and Esp strains were used as antigens. Their data suggest that CMV-host relationships are more dynamic than had been appreciated . Some patients responded to one antigen at certain times and to all three at others . Wide fluctuations were noted in 20 of the 39 CF-reactive donors.

Some previously positive sera declined to levels below 1 :8 and subsequently rose to titers of 1 : 64 or higher . These important observations suggest that CMV-CF titers must be interpreted with caution, especially in the diagnosis of primary infection in adults.

2. Fluorescent Antibody Tests (FA) :

Hanshow, et al. (1968) developed the CMV-IgM test following the successful domenstration of toxoplasma-IgM antibody by, Remington, et al. (1968). The main advantage of the immunofluorescent method is the ability to measure specific immunoglobulins by using conjugates directed against IgM, IgG and IgA . This is particularly useful in the detection of congenital infection because of the large amount of masking IgG antibody of the maternal origin. Thus, specific CMV-IgM and CMV-IgA antibodies in the neonate can be interpreted as evidence of congenital CMV infection . The presence of significant and persisting CMV-CF antibody or, preferably, the isolation of the virus from the newborn patient is further proof that the IgM antibody is specifically CMV . As noted above, there is evidence that herpesvirus, varicella, and EBV are antigenically related to CMV and that infections with these agents may produce a positive CMV-IgM test (Hanshaw, et al., 1972) . If the CMV-IgM test is employed as the only means of identifying CMV

infection in symptomatic infants, it will be approximately 95 per cent accurate . We were able to domonstrate this antibody in all of 50 infants with virologically documented cytomegalic inclusion disease (Hanshaw, et al., 1968). In one recent experience, we were able to find CMV-IgM antibody in a virus - positive infant with congenital cytomegalic inclusion disease (CID) . The test was positive, however when the Kerr CMV was substituted for the AD 169 agent as the CMV antigen source . The efficiency of FA method is less than that of employing virus isolation techniques to detect excretors . Once CMV-IgM is detected, the level may remain elevated for weeks or months after birth . Although there is widespread belief that the presence of IgM antibody is strong evidence of a primary infection, IgM antibody may fluctuate in patients tested over a period of several months (Hanshaw, 1973) , suggesting that one can not necessarily equate the presence of specific macroglobulin with recently acquired primary infection.

3. Indirect Hemagglutination Test (IHA) :

A specific indirect hemagglutination test (IHA) described for herpes simplex virus type 1 and 2 by, Fuccillo et al., (1970) has been applied successfully to the detection of CMV indirect hemagglutinating antibody by the same group of workers (Fuccillo et al., 1971), as well as by

Bernstein, and Stewart (1971) . The specificity of CMV antibody response detected by the IHA test correlates well with the standard neutralization test . The method is somewhat more sensitive than the CF test in detecting antibody in congenitally infected newborns, although this difference is minimized if potent CF antigens are prepared . Bernstein, and Stewart, (1971) found the test to be highly sensitive and reproducible . Because of the hemagglutination reaction can be inhibited by small amount of homologous antigen, it is possible to use this technique to identify virus isolated from diagnostic specimens.

4. Platelet Agglutination (PA) :

Penttinen et al. (1970) in Finland have described a new method for the measurement of CMV antibody, based on the sedimentation patterns of washed platelets used as CMV carriers .

5. Neutralization Tests :

The neutralization procedures, which include the more traditional virus serum tube-dilution method (Weller, et al., 1957) and the more recently described plague-reduction neutralization test (Plummer, and Benyesh-Melnick, 1964), are not used for routine CMV diagnostic serology.

6. Radioimmunoassay (RIA) and Enzyme-linked Immunosorbent Assay (ELISA) Tests :

These are newer serological methods, were found to be comparable in sensitivity and specificity for detecting CMV IgG antibody, and 10 to 100 times more sensitive than complement -fixation, anticomplement immunofluorescence (ACIF), and passive haemagglutination (PHA) (Booth et al. 1982).

In screening tests for antibody, the frequency of false-positive and false-negative results was 0.6% for RIA and ELISA, 1.5 % for CF; 1.6 % for ACIF, and 3.6 % for PHA. PHA was the least satisfactory test, largely because of technical problems (Booth, et al., 1982).

Detection of CMV-specific IgG antibody in neonates or adults does not mean active infection because of passive transplacental transfer and life-long persistence of specific IgG following infection . Detection of CMV-specific IgA in serum or body secretions is not sensitive and does not have practical diagnostic or prognostic value (Sarov, et al. 1981), on the other hand, measurement of CMV-specific IgM antibody is a useful tool for the diagnosis of CMV infection. Specific IgM antibody has the potential value of confirming the diagnosis in a single serum specimen . Furthermore, IgM antibodies do not cross the placenta, and their presence in the cord blood of neonates suggests congenital infection.

Detection of CMV-specific IgM antibody has been found useful in diagnosing CMV infection in pregnant women and in patients with mononucleosis . The specificity and sensitivity of CMV-specific IgM detection is dependent on the technique used (Griffiths, et al., 1982).

The sensitivity of the fluorescent antibody technique is 50% of that of virus isolation, and false-positive reactions may occur in the presence of rheumatoid factor. The RIA technique appears to be very useful, and recent studies suggest 100% specificity and almost 90% sensitivity as compared to the virus isolation (Stagno, et al., 1975; and Rasmussen et al., 1982).

4. Antigen Detection :

Detection of viral antigen in the infected tissue or urine using ELISA, RIA, or fluorescent antibody (IFA) methods may be of value in rapid diagnosis of CMV infection . IFA is a highly sensitive, specific and relatively simple method for this purpose . Thin sections (4 nm) or exfoliative cells may be used . The practical usefulness of antigen detection needs further study (McIntosh et al., 1980).

5. Electron Microscopy :

Detection of the virus particles in the urine by electron microscopy has been found useful for the rapid diagnosis of CMV infection in neonates with congenital

infection . These patients have a high titer of cell-free virus in the urine . The sensitivity of this method has been reported to be 95% as compared to virus isolation . Other body secretions and tissues may also be used . This method is not specific and herpes simplex virus and other herpesviruses would be confused with CMV (Lee, et al.,1978).

PREVENTION

Prevention of CMV infection is highly desirable, However, there is currently no effective method for prophylaxis against CMV infection.

1. General Preventive Measures :

Person-to-person transmission of CMV needs intimate and close contact with the infected patients . Isolation of patients may not be necessary but is practiced by some centers to prevent person-to-person spread of the disease in neonate nurseries.

Blood transfusion and organ allograft transplantation are the major sources of primary CMV infection in hospitalized patients . In organ allograft recipients, CMV can be prevented by giving organs from CMV-seronegative donors (Glenn, 1981).

2. Chemoprophylaxis :

Chemoprophylaxis with low-dose adenine arabinoside or acyclovir was found ineffective in prevention of CNV pneumonia following bone marrow transplantation (Kraemer, et al., 1978, and Saral, et al., 1981).

Interferon and CMV immune sera have been shown to have some prophylactic effect in bone marrow or renal

allograft recipients. These agents delay the onset of virus shedding, decrease viremia, and decrease the severity of infection (Winston et al., 1982).

3. Vaccination :

In recent years, two different strains of live human CMV vaccine have been prepared and tested in normal volunteers, prospective renal allograft recipients, and medical personnel. Both vaccins are immunogenic and have not been associated with ilness or virus excretion in the urine or throat of vaccinees (Lang, 1980, and Glazer et al., 1982).

A live tissue-culture-adapted CMV vaccine was prepared and tested in CMV-susceptible normal volunteers by, Eleck and Stern, in (1974), while the vaccine was not effective following oral or intradermal inoculation, it induced CF and neutralizing antibodies following subcutaneous inoculation . Side effects were minimal, and a delayed hypersensitivity reaction was observed at the site of injection. Immunogenicity of this vaccine has been confirmed by (Lang, 1980 and Osborn, 1981).

Plotkin, and Coworkers (1976) developed a live, attenuated vaccine using a virus strain (Towne-125) that was isolated from a child with congenital CMV infection . The virus has been shown to possess immunogenicity (both humoral and cell mediated) in normal volunteers and in patients

with endstage kidney disease who are candidates for kidney transplantation . It is not effective by the intranasal route of administration but induces CF, neutralizing, and immunofluorescent antibodies following subcutaneous injection . It has minimal side effects and stimulates the production of IgM and antibody to early antigen . It also causes a booster response in CMV seropositive individuals.

Two major problems are associated with CMV vaccination. First, unlike measles virus and polio-virus, once CMV is administered, the virus persists in the host in a potentially infectious state (latency) and may become reactivated later . Second, the oncogenic potential of CMV is still unsettled and must be weighed against the benefit obtained from vaccination (Glazer et al., 1982).

TREATMENT

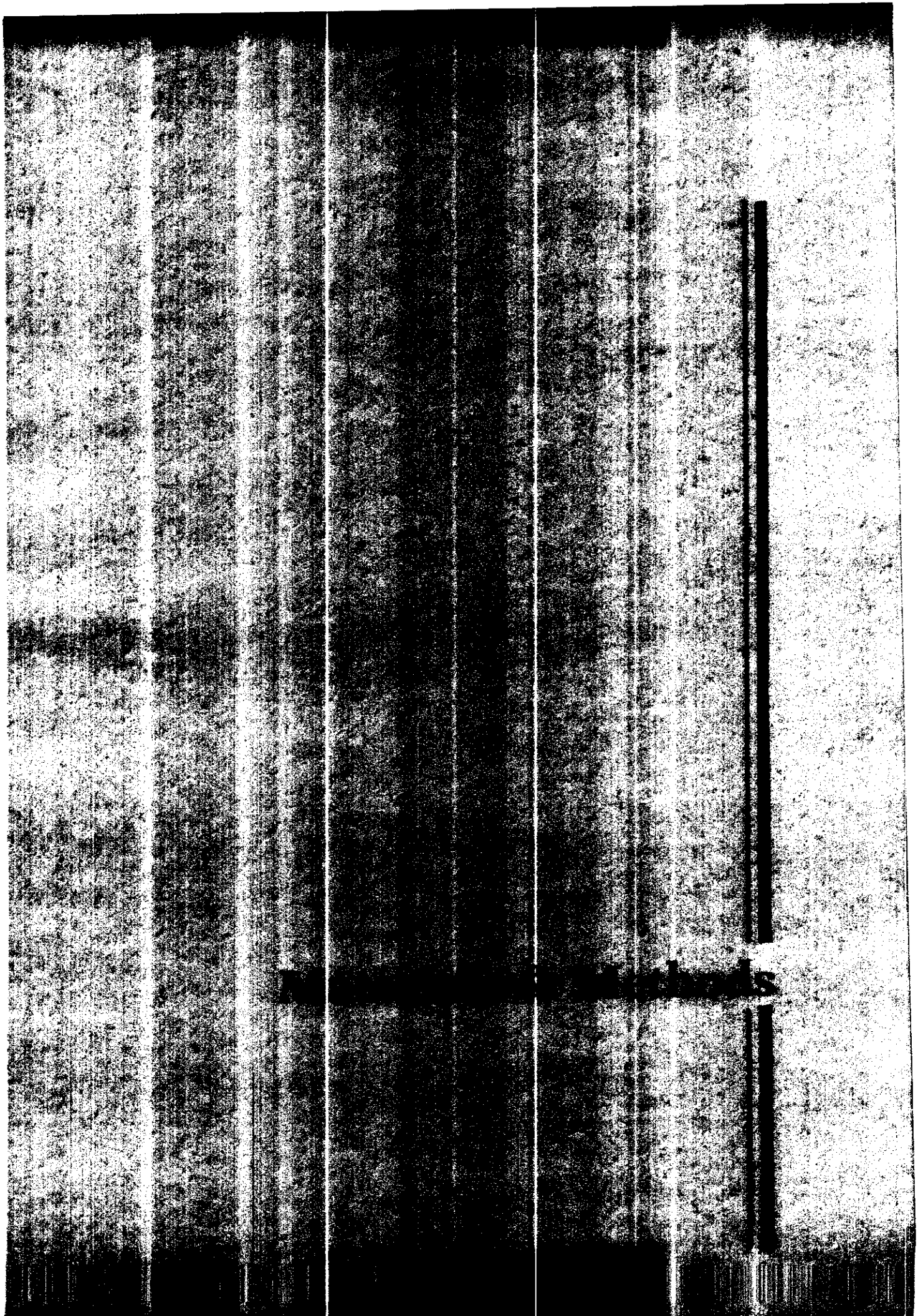
Specific Therapy :

There is no specific therapy for congenital CMV infection that has been shown to be clearly helpful . Although several drugs, such as adenine arabinoside (Ara-A), cytosine arabinoside (Ara-C), idoxuridine (IDU),floxuridine (FUDR) and interferone inducers (poly I.C.), have been used experimentally in patients, there is no evidence that these antiviral agents have any lasting effect on the progression of the disease . Both arabinosides and IDU can induce a transient diminution in virus excretion in some patients. There is no evidence that this effect alters the clinical course or long-term prognosis (Hanshaw, and Dudgeon, 1978).

Non Specific Therapy :

Most infants with symptomatic infection do not require therapy . Exchange transfusion is rarely indicated for hyperbilirubinemia, much of which is direct . Neonatal sepsis, an unusual complication of CID, may be due to enteric organisms or a streptococal infection . Thus, the therapy would not be different from that used in other newborns with sepsis . Since CID can be a cause of cerebral palsy, mental retardation and obstructive hydrocephalus,

long-term measures for dealing with these specific chronic problems must be planned on an individual basis (Hanshow, and Dudgeon, 1978).



MATERIAL AND METHODS

MATERIAL :

This work was carried out on 60 child, 40 of them diagnosed by developmental assessment as mental retardation, and the other 20 child with normal intelligence as a control. From Benha University Hospital Pediatric Department .

Complete history was taken from each case including, personal history; perinatal, natal and postnatal history ; Family history (+ve consangous marrage, same condition,and sibs), past history; Nutritional and Vaccination.

General examination was done to every case including anthropometric measurments and local examination of all systems including chest, heart, abdomen, and neurology .

Growth and developmental assessment sheet was done to each child to evaluate his intelligence .

METHOD :

Every case was subjected to complement fixation test (Lennette, and Schmidt, 1964) by Kolmer technique .

Under aseptic condition 5 c.c. of venous blood was collected by a sterile syringe from each child and transfered into sterile centrifuge tubes . After centrifugation sera were separated into sterile containers and stored in

the deep freez at -20°C untill testing.

Reagents :

" Stored at + 2 to + 8°C)

A. Cytomegalo-virus antigen.

B. Cytomegalo virus control sera positive .

C. Cytomegalo control antigen negative.

D. Guinea pig complement .

E. Amboceptor 6000 (hemolysin).

F. Veronal buffer (Hoechst Orient S.A.A.).

Preparation of the Reagents and Patients Samples for Kolmer Technique (Lennette, and Schmidt, 1964) :

A. Cytomegalovirus antigen was dissolved in one ml of distilled water and adjusted to 2 antigenic units with a diluent (Veronal buffer)*.

B. The control antigen was diluted in the same manner as the pertinent antigen .

C. Guinea Pig complement dissolved in the accompanying solvent.

D. Amboceptor, 1 : 100 stock dilution were prepared by mixing 1 ml each of amboceptor and glycerol, 94 ml of diethyl barbiturate buffer solution, and 4 ml of 5% phenol solution.

E. 2 % suspension of sheep erythrocytes.

F. Patient's serum and positive control serum were inactivated at 56-60°C for 30 min.

* Veronal buffer :

NaCl	85.00 g
Diethyl barbituric acid	5.75 g
Sodium diethyl barbiturate	3.75 g
$MgCl_2 . 6 H_2O$	1.68 g
$CaCl_2$ (anhydrous)	0.28 g
Re-distilled water to	2000 ml.

Mix and autoclave for 20 min. at 115°C and store at +2 to + 8°C . Before use, dilute 1 : 5 with distilled water.

Steps :

1. Sensitized Cell Suspension :

Cells : Defibrinated or citrated sheep red blood cells washed in saline solution for 5 minutes at 2,000 rpm for three successive times and packed for 10 min.,then 2 % sheep cells suspension was made (2 ml packed cells + 98 ml saline).

Hemolysin : Stock solution of 1 : 100 hemolysin was prepared as described previously .

Titration of Hemolysin

0.5 ml of 1 : 100 hemolysin + 4.5 ml saline = 1 : 1,000 dilution
1.0 ml of 1 : 100 " + 5.0 ml " = 1 : 6,000 "
1.0 " " " " + 7.0 ml " = 1 : 8,000 "
1.0 " " " " + 9.0 ml " = 1 :10,000 "
1.0 " " 1 : 10,000 " + 0.5 ml " = 1:15,000 "
1.0 " " " " + 1.0 ml " = 1:20,000 "
1.0 " " " " + 1.5 ml " = 1:25,000 "
1.0 " " " " + 2.0 ml " = 1:30,000 "

0.2 ml hemolysin dilutions (1:6000 to 1:30,000)
+ 0.1 ml 1 : 30 complement (0.2 ml complement + 5.8 ml saline).
+ 0.2 ml of 2.0 per cent sheep cells
+ 0.5 ml of saline.

Cell control : 0.2 ml cells + 0.8 ml saline.

Shaked and incubated in water both at 37°C for ½ hr. and read. The highest dilution of hemolysin showed complete hemolysis represented one unit and was equal to 1 : 20,000 dilution, there for two units would be contained in 0.2 ml of 1 : 10,000 dilution of hemolysin.

The dilution 1 : 10,000 hemolysin was prepared in a volume equal to the amount of 2.0 percent cells to be used in the test .A portion of the cells sufficient for the complement titrations was sensitized by pouring diluted hemolysin into an equal volume of cell suspension and by rapidly

pouring the mixture back and forth several times, Allowed to remain at room temperature at least 10 min. before used.

2. Titration of complement :

Reagent	Amount of reagent (in ml)to be added to tube							
	1	2	3	4	5	6	7	8
Antigen	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Complement (1 : 30)	0.12	0.11	0.10	0.09	0.08	0.07	0.06	0.05
Saline	0.28	0.29	0.30	0.31	0.32	0.33	0.34	0.35

Shaked and incubated in 37°C water bath for ½ hr. and 0.4 ml of sensitized cells was added to all tubes. Shaked and incubated in 37°C water both for ½ hr. and read. The tube contained the least amount of complement showed complete hemolysis equaled to 0.09 ml of 1 : 30 dilution of complement represented one unit . Two exact units contained in 0.2 ml were used in the test, calculated as follows :

0.09 ml of 1:30 dilution of complement represents one unit

So 0.18 ml of 1:30 dilution of complement represents 2 units

So,the dilution of complement used in the test $= \frac{30}{0.18} \times 0.2 = 1:33$ dilution . (i.e. 1 ml complement + 32 ml saline).

3. Titration of Antigen :

Performed by testing serial twofold dilutions of antigen against serial twofold dilutions of immune serum to obtain the optimal dilution of antigen that gives fixation.

Antigen dilution	Immune serum dilution				
	1 : 8	1 : 16	1 : 32	1 :64	1:128
1 : 4	*4	4	4	4	0
1 : 8	4	4	4	2	1
1 : 16	4	4	4	2	0
1 : 32	4	4	4	1	0
1 : 64	4	1	0	0	0

* Degree of complement fixation .

We transfered 0.2 ml of each dilution of the serum in the titration tubes, then 0.2 ml of serial dilution of antigen was added to appropriate tubes, then 0.2 ml of complement was added . Shaked and incubated over night at 4-6 ^{o}C.

The tubes were warmed for 10 min. in a water bath at 37^{o}C, then 0.4 ml of the hemolytic system (sensitized cells) was added to the tubes and incubated again at 37^{o}C for 15-30 min.

The highest antigen dilution showing 3 + or 4 + fixation with the highest dilution of serum was 1 : 64 and represented one unit . Two units in 0.2 ml were used in the test.

2 units in 0.2 ml

64 units in ? ml

$$\frac{64 \times 0.2}{2} = 6.4 \text{ ml}$$

i.e. 1 ml antigen + 5.4 ml veronal buffer, this results in presence of 2 antigenic units in each 0.2 ml.

Principle of the Test :

The tested serum was added to cytomegalovirus antigen, then the complement was added . If the serum contains antibodies specific to the added antigen, they will unit together and fix the complement, but if the serum is negative for specific antibodies, no antigen antibody complex is formed and the complement left free .

In order to visualize this reaction, an indicator system (sheep red cells + antisheep red cells), was added if the complement was free it would cause hemolysis of the red cells and this means negative serum (-ve) But if the complement was fixed no hemolysis would occur as in positive serum (+ve).

i.e. : hemolysis means - ve serum

no hemolysis means + ve serum

Table (2) : Schema for performance of the complement-fixation test

Tubes	Serum (ml)	Saline solution (ml)	Antigen (ml)	Non specific antigen (ml)	Complement (ml)		Sesnitized (ml)		
Serum under test	0.20 (1:8 dil.).	-	0.20	-		0.20		0.40	
Serum control	0.20	0.20	-	-		0.20		0.40	
Reagent controls:									
1. Complement control :									
Type No. 1	-	0.35	0.20	-	Shake	0.05	Over night	0.40	Incubate
Type No. 2	-	0.30	0.20	-	and	0.10	incubation at 4-6°C	0.40	15-30 min in 37°C
Type No. 3	-	0.25	0.20	-	add	0.15	followed	0.40	water both
Type No. 4	-	0.20	0.20	-		0.20	by 10 min. in 37°C	0.40	
2. Hemolytic ontrol	-	0.40	-	-		0.20	water both	0.40	
3. Sheep cell control	-	0.60	-	-		-		0.40	
4. Non specific antigen control	-	0.20	-	0.20		0.20		0.40	