Asian Pac J Trop Med
2009;2(6):43-47

Original article

Incidence of human cytomegalovirus in pregnant women attending pre-natal clinic in Northern Nigeria

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Received June 8, 2009; *Accepted July* 7, 2009

Abstract

Objective: To investigate the incidence of human cytomegalo virus (HCMV) in pregnant women. **Methods**: One hundred and twenty two (122) blood samples and 80 umbilical cord fluids were tested for IgG and IgM respectively using the enzyme linked immunosorbent assay (ELISA). **Results**: Of the 122 samples screened for IgG and IgM only 56 (47.1 %) tested positive for IgG while 63 (52.9 %) were positive for IgM with 3 samples non-specific. Pregnant women with signs of normal pregnancy made up 60% of the positive results while 20 % had a history of ectopic pregnancy and 20 % with a history of miscarriages. Of the total of 80 umbilical cord fluids tested, only 59 (73.8) tested positive. Eleven of the selected 40 umblical cord fluid was positive to IgG to human cytomegalovirus along with mother and child. Antibody titration result gave diagnostic titre for both IgG and IgM from the 40 umbilical cord fluids. There was a significant relationship between mother, child and umbilical cord fluids ($\chi^2 = 1.360$, CI = 99 %, $P = 0.568$). **Conclusion**: There could be a possible neonatal infection, and the infection is common among toddlers and children of pre-school age.

Keywords: Human cytomegalo virus; Herpes viridae; Neonatal infection; Persistent infection; Recurrent infection

INTRODUCTION

Cytomegalovirus a member of the herpes viridae which are ubiquitous agents that normally infect many animals including human. Cytomegaloviruses are a group of host-specific viruses. Human cytomegalovirus (HCMV) is an ancient virus closely linked to its natural host-human. As with other herpes viruses, primary cytomegalovirus infection is usually followed by persistent or recurrent infections[1]. When HCMV comes in contact with the fetus, it may be limited by various host defense mechanisms acquired by the fetus from the maternal circulating antibodies. However, result of an infection with the fetus may range from subtle abnormalities not detectable at birth to severe generalized disease in the new born. The later more recognizable from the infection has been better documented than the milder documentation[2]. HCMV has since emerged as an important opportunistic pathogen in the immune compromised hosts[3].

HCMV is usually characterized by large homogenous inclusion bodies referred to as cytomegalic inclusion bodies[3].

It has been reported that HCMVs are significant pathogens of human fetus, capable of inducing a wide range of oculocerebral and extra-neural abnormality; these observation have been confirmed by numerous investigators over the past three dec-

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ades[3,4]. During the 1970s and 1980s, the molecular biology of HCMV was explored and continues to be investigated by many researchers. When the human cytomegalovirus was first reported to replicate successfully in vitro, techniques became available to study the events surrounding recognized clinical infection by these agents. Most initial researches focused on the known entity of salivary gland inclusion disease and cytomegalic inclusion disease and congenital infection disseminated from the infection[5]. In this classic infection form CID is characterized by enlargement of the liver and spleen, hyperbilurinemia, petechiae due to thrombocytopenia and various form of central nervous system including microcephaly, chorioretinitis, cerebral calcifications, deafness and mental retardation. Most frequently no manifestations of the disease are present in early infancy and the infant is born with clinically silent infection[3].

The infection involves about 1 % to 2 % of newborns and 2 % to 5 % of pregnant women[6]. The prevalence of infection which rises with age is higher in developing countries than among developed nations. Sources of infection include saliva, milk, cervical and vaginal secretions, urine, faeces and blood. The spread of CMV requires close contact because it is labile. Transmission occurs by direct person to person contact but direct transmission is possible by contaminated formitis such as toys[3]. In recent years an acquired form of infection was identified; children were identified with asymptomatic and symptomatic infections and associated abnormalities of liver function that persisted for several weeks. About the same time a CMV associated form of fever abnormal value of liver function tests and typical lymphocystisis was described. The syndrome is similar to infectious mononucleosis and occurs in previously healthy individuals[2]. Pneumonia can also occur in infants who have acquired CMV infection. Gastrointestinal disease causing esophangitis, gastroenteritis, small bowel obstruction, colitis, proctitis, pancreatitis and haemorrhage has been associated with CMV infection in immunocompromised patients especially patients with AIDS and those who have bone marrow renal liver transplantation[7,8]. CMV hepatitis in recipients of bone marrow, lung or heart transplants patients with malignant disease or AIDS or healthy individuals with a primary CMV infection is usually manifested by mild hepatic enlargement and mild elevation of serum liver enzymes levels accompanied by fever and lymphopenia or lymphocytosis. Jaundice and hyperbilirubinemia usually do not occur and severe hepatitis of cirrhosis is rare. Hepatic necrosis and liver failure due to CMV hepatitis have not been well documented[9]. Central nervous system involvement is well known in congenital CMV meningoencephalitis appears to be rare, yet well documented[10]. There is growing recognition of CMV encephalitis in patients with AIDS. Up to 50% of such patients may show evidence of CMV infection in the central nervous system at autropy[11,12]. Myocardiatis has been described as a rare complication of both congenital CMV disease and CMV the CMV mononucleosis syndrome.

Evidence of involvement of the organs of the endocrine system is well described in both congenital and postnatal acquired disseminated CMV infections. Involvement of the adrenal, pituitary, thyroid and parathyroid glands has been encountered. Cutaneous manifestation of CMV infections are uncommon but have been described[13]. These are most notable in infants with symptomatic congenital infection who exhibit non-palpable petechiae or purpura, usually as a result of thrombocytopenia[6].

Ganciclovir (DHPG) is the first antiviral approved specifically for the treatment of life – threatening and sight-threatening infections with CMV. It is effective against all herpes viruses and similar to ancyclovir[14]. Ganciclovir is virus-static and suppresses active CMV but does not produce a cure. It is indicated for treatment of CMV in renitis in immunocompromised patients and it may also be effective against colitis, esophangitis, hepatitis and meningoencephalitis due to CMV. Because of high mortality and morbidity associated with congenital symptomatic infections, therapeutic trials of ganciclovir in newborns with evidence of central nervous system involvement are in progress[15]. Foscarnet is another investigational drug however the drug gets deposited in bone, teeth and cartilage which is an important consideration when treatment of children is contemplated[15]. Live attenuated vaccines have been developed by Plottin and his co-workers using a strain that was originally isolated from the urine of a congenitally infected infant. The vaccine induces both humoral

and cellular immunity and thereby protects against the disease.

MATERIALS AND METHODS

Blood samples ($n = 122$) were collected aseptically from women attending pre-natal clinics in Kaduna Nigeria. The umbilical cord fluids ($n = 80$) were also collected from 80 of these women during delivery and also their new born infants. Sera were separated from whole blood by centrifuging at 3 000 rpm (hetutich) and stored in −20 ℃ refrigerator until used. The competitive enzyme linked immunosorbent assay (ELISA) to detect IgM and IgG to HCMV using the alkaline phosphate-conjugated rabbit anti-human IgG and IgM antibodies. All the weakly positive samples were regarded as positive. The ELISA technique was then carried out using the method of Krivoshein (1998) using the HCMV antigen. Forty randomly selected subjects were screened for HCMV IgG and IgM, the screening included the mothers, child and umbilical cord fluids. All the 40 individuals had their antibody titerated for the purpose of comparing the titre level of mother child and umbilical cord fluids. Staistical analysis was carried out using the epi-info software. The chi and regression was used to determine the relationship between the umbilical cord fluids collected as well as those of the new born infants.

RESULTS

Of the 122 women attending ante-natal clinics in Kaduna Nigeria and screened for HCMV 119 were positive (97.5 %). From the 119 positive samples included 56 (47.1 %) that were positive for IgG and 63 (52.9 %) that were positive for HCMV IgM (Table 1). From the 80 umbilical cord fluids 59 (73.8 %) were positive to HCMV IgG while 37 (46.3 %) new born infants had positive results. Age group distribution of the women reveals that HCMV was more prevalent in the age group 26 − 30 as well as 21 − 26 (Table 1). There was the presence of IgM in nearly all the age groups screened. The new born infants presented with no IgM except with those children with ages below 5 years of age. The umbilical cord fluids had no IgM while IgG was present in most of the umbilical cord fluids. However, of the 40 umbilical cord fluids titerated for HCMV IgG and IgM along with mother and newborn child, 11 of the 40 samples were positive for both mother child and umbilical cord fluids (Table 2). There was a significant association between mother, child and umbilical fluids ($\chi^2 = 1.360$, CI = 99%, $P = 0.568$).

Table 1 Age group distribution of patients testing positive to HCMV IgG and IgM .

Age groups	Number tested	IgG	IgM
1 − 5	20	15	8
6 − 10	15	9	3
11 − 15	27	9	7
16 − 20	10	0	8
21 − 25	13	6	9
26 − 30	20	10	16
31 − 35	15	6	11
36 − 40	2	1	1
Total	122	56	63

From the table 1 above, it shows that some patients have both IgG and IgM.

Table 2 Relationship between mother, child and umbilical cord positivity using their antibody titres.

Patients Ref. No.	Antibody titres					
	Mother		Child		Umbilical cord fluid	
	IgG	IgM	IgG	IgM	IgG	IgM
NKD 01	1: 64	1: 64	1: 64	–	1: 32	–
NKD 02	1: 128	–	1: 64	–	1: 64	1: 16
NKD 05	1: 32	–	–	–	–	–
NKD 06	–	1: 32	–	–	–	1: 16
NKD 07	1: 256	1: 64	1: 128	–	1: 64	1: 16
NKD 08	1: 16	1: 16	–	–	–	–
NKD 09	–	–	1: 8	–	1: 8	–
NKD 13	1: 32	1: 64	1: 8	–	1: 32	–
NKD 14	1: 32	1: 8	1: 8	–	1: 16	–
NKD 15	1: 8	–	–	–	–	–
NKD 22	1: 128	1: 256	1: 64	–	1: 32	1: 8
NKD 23	1: 32	1: 32	1: 32	–	1: 16	1: 8
NKD 24	1: 16	1: 16	1: 8	–	–	1: 8
NKD 25	1: 8	–	–	–	–	–
NKD 27	1: 8	1: 16	1: 8	–	–	1: 8
NKD 28	1: 8	–	–	–	–	–
NKD 29	1: 16	1: 16	1: 16	–	1: 16	–
NKD 32	1: 32	1: 16	1: 16	–	1: 16	1: 8

NKD 03, 04, 10 – 12, 16 – 21, 26, 30, 31, 33 – 40 were negative.

DISCUSSION

The high number of positive individuals in this study shows that HCMV infection is endemic in the population and despite this endemicity, the infection still thrives unchallenged. This trend might be due to its latency that brings about most asymptomatic infections. Such asymptomatic infections go without recourse to diagnosis especially in the population under study where diagnostic and research materials are limited. It is such non-diagnosis of this infection that makes treatment impossible and allows the infection to thrive well in the population as earlier reported[11]. HCMV have been reported to be treated with some antiviral drugs and been prevented by vaccination however, this depends on rapid diagnosis and the availability of epidemiological data. Such epidemiological data is been reported in this study and which also justify this study in the population which virtually lack epidemiological data in relation to HCMV. The high prevalent of infants (1 – 5 years of age) shows that the infection is common among toddlers and children of pre-school age and thereby pointing to neonatal infection[5]. There is the possibilities that infants may have contacted the infection from there mothers either congenitally or during breast feeding although literature have not yet pointed to breast milk as a major vehicle of transmission of HCMV but there are few reports[5]. Infection could also be contacted from their mothers via formitis as it has earlier been reported that infection could be contacted via formitis especially in children attending toddlers. It has also been reported that children in nursery homes or school could also contact the infection via formitis on toys used in schools by other school mates who might be infected with HCMV[2]. Infected children who now become carriers might give the virus to unsuspecting school mates when they use the same toys or via person to person contact. The detection of IgG in the children may also not mean that they have contacted infection, but IgG may have been transferred from mother to child as IgG can cross the placenta and still remains in the child after birth for sometime where they might be

protective. This also points to the risk of congenital infection as earlier reported. As the child grows it develops its own antibodies to infection. Hence the detection of HCMV IgM means that the child has started to produce her own antibodies to HCMV infection. This then signal to the fact that the child has gotten in contact with the virus. To monitor congenital infection in this study, 40 umbilical cord fluids were collected from women during birth and the result shows that there was a possibility of congenital infection. Since some of the samples from the umbilical cord fluids were positive along with their mothers and child. The absence of IgM in all the newborns does not rule out congenital infection but is of the fact that IgM does not cross the placenta. This accounts for why IgG was seen in most of the samples from the infants because IgG can cross the placenta. This makes IgG a parameter to monitor congenital infection. The fact that some umbilical cord fluids had IgM does not mean that it came from the placenta but may have been from other surrounding fluids. Another confirmation of congenital infection is that 8 of the positive umbilical cord fluids had the mother and the child as been positive to HCMV IgG.

The result in this study also shows that most of the pregnant women tested positive (97.5 %) to HCMV. It is possible that the women may have contacted HCMV in their unhygienic environments and since the infection is asymptomatic may have remained unnoticed. This study suggests that like in other herpes virus infections, HCMV may have been reactivated by pregnancy as a result of certain hormonal changes. This is more so that most herpes viruses are found of latency and are reactivated by certain conditions like pregnancy and if HCMV is found of latency and also a member of the herpes family, could also be reactivated by pregnancy. This reactivation may have been responsible for the high positive number of individuals testing positive to HCMV. Since all these women were pregnant there are chances that they also transmit the infection to the foeteus as well as the newborns infants. This is confirmed by statistical analysis when the number of infants positive was compared to positive women and this confirms the umbilical cord fluids testing positive along with mothers and children.

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Executive Editor: Beijia Tan