PREVALENCE OF IgM ANTIBODIES AGAINST CYTOMEGALOVIRUS IN PREGNANT FEMALES

BY

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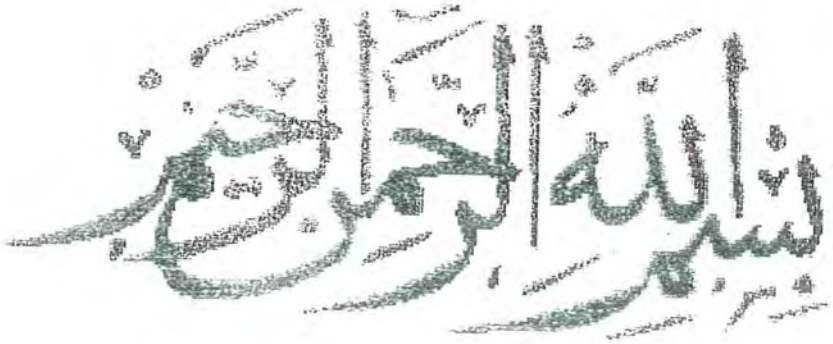
بسم الله الرحمن الرحيم

TO ALL THE LOVING ONES

CERTIFICATION

It is certified that contents and form of this thesis entitled by "**Prevalence of IgM antibodies against Cytomegalovirus in pregnant females**" submitted by **Zahid-ur-Rehman** have been found satisfactory for requirement of the degree of Master of Philosophy in Biology (Microbiology).

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(AMEEN)

ZAHID-UR-REHMAN

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LIST OF ABBREVIATIONS

AI	Avidity Index
CF	Complement fixing
CMV	Cytomegalovirus
COV	Cutt-off value
CTLs	cytotoxic T lymphocytes
DNA	Deoxyribonucleic acid
EBV	Epstein Bar Virus
HBV	Hepatitis B Virus
HCMV	Human Cytomegalovirus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
hr	Hour
HSV	Herpes Simplex Virus
IFA	Immunofluorescent Antibody
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IUGR	Intrauterine Growth Retardation
Kbp	Kilo base pairs
MACRIA	M-antibody capture Radioimmunoassay
MCH	Mother Child Health
min	Minute
MNC	Mean negative control
MPC	Mean positive control
NaCl	Sodium Chloride
NASBA	Nucleic Acid Sequence Based Amplification
NICU	Neonatal Intensive Care Unit
nm	Nanometer

PBL	Peripheral Blood Leukocytes
PCR	Polymerase chain reaction
PEI	Paul-Ehrlich-Institute
RIA	Radioimmunoassay
Rs.	Rupees
SEA	Socioeconomic Areas
TMB	Tetramethylbenzidin
tri	Trimester
WB	Western Blot
WBCs	White Blood Cells
Yr	Year
µl	Microlitre

Abstract

Cytomegalovirus (CMV) infection in pregnant females can be transmitted to the developing fetus. In fact primary CMV infections are transmitted more frequently to the fetus and are more likely to cause fetal damage than recurrent infections. The rate of vertical transmission was found to be 0.2 to 2.2% in previously seropositive mothers undergoing recurrent infection during pregnancy and 20 to 40% in pregnant women with primary infection. This vertically transmitted CMV infection is responsible for congenital abnormalities in the developing fetus or sequele which develop later in their lives. Primary infection in an individual can be diagnosed by detecting CMV specific IgM antibodies in the serum sample. In the present study 344 blood samples were collected from pregnant females visiting the antenatal clinic. These samples were screened for CMV specific IgM, by using ELISA. CMV specific IgM antibodies were found to be present in 6.1 % of the pregnant females. These females can actively transmit infection to the fetus. The effect of certain parameters like age, stage of pregnancy, parity, abortion, status of HBV, HCV and the socioeconomic status was studied on CMV IgM seropositivity. Our results show that age, stage of pregnancy, parity, status of HBV and HCV have no significant effect on CMV IgM seropositivity. While socioeconomic status and abortion contributes toward CMV IgM seropositivity i.e prevalence of IgM antibodies was higher in pregnant women belonging to low socioeconomic status and in women with history of abortion. From our results we conclude the primary CMV infection is common in our antenatal population. Congenital CMV infection can be prevented mainly by educating the antenatal population about the possible risk factors. With the absence of a safe vaccine, women at the onset of pregnancy should be advised to practice careful hygiene and to minimize contact with carriers and other sources of infection in order to decrease the chances of infection.

INTRODUCTION

Cytomegalovirus belongs to the herpesviridae family of viruses. The herpesviridae are a large group of double-stranded enveloped DNA viruses of 235 Kbp long. More than 150 members of the herpes family have been identified to date. In humans eight different herpes viruses are known. These are Herpes Simplex Virus type 1 and type 2 (HSV), Varicella Zoster Virus (VZV), Human Cytomegalovirus (CMV) and Epstein Bar Virus (EBV). Human herpes viruses 6 to 8 have no other names.

CMV can be transmitted by multiple means, including blood transfusion, organ transplantation, nursing, sexual contact, aerosol droplets, direct person-to-person contact, among toddlers in day-care centers and from pregnant mothers to fetuses (vertical transmission), (Hirsch et al.1994). And these fetuses are at risk of congenital defects, as they have undeveloped immune systems. The entry of CMV in target cells has been investigated in vitro in fibroblasts, endothelial cells and phagocytes (Compton,1995). First there is attachment and fusion of the virion with the cell membrane. Glycoproteins of the viral envelope (Compton, 1995), (Taylor and cooper,1990) play a determining role in this process. The capsid moves to the nucleus and the process of transcription and translation of early and late antigens starts, following a very strict timetable (Michelson *et al*, 1997), (Mocarski *et al*, 1996). Due to the cellular immune response, infected cells will be destroyed and further replication and dissemination of the virus will be stopped. The virus comes in a stage of latency and may reactivate during a state of decreased immunity e.g. by infection with HIV or immunosuppressive drugs those used during organ transplantation or steroid therapy.

Detection of immunoglobulin M (IgM) antibody to CMV has been proposed as a rapid method for diagnosis of primary infection with this virus. Studies have shown that IgM antibody to CMV in pregnant women generally signifies recent primary infection whereas women with reactivated or recurrent infection have seldom these antibodies (Grifths P.D *et al*, 1982), (Stagno *et al*. 1985). Pass and Boppana (1999) found that less than 5% of pregnant women with primary infection are reported to be symptomatic, and in more than 95 % of pregnant females this infection goes unidentified. Clinical findings observed in CMV

infection are fever, cervical adenopathy, sore throat, splenomegaly, hepatomegaly, and rashes.

Ganciclovir and foscavir are the two antiviral used in the Netherlands against CMV nowadays. The main goal of antiviral chemotherapy would be, to treat pregnant women with primary CMV infection in order to (hopefully) prevent transmission of the virus to the fetus. In this respect, the combination of hyper immune globulin with antiviral drugs of low or negligible toxicity could represent the best approach to preventing vertical CMV transmission in the future (Maine *et al*, 2001).

By passaging Cytomegalovirus through fibroblast cell lines, the attenuated virus strains Towne and AD169 have been developed for vaccination. Unlike wild-type CMV, the Towne strain does not produce reactivation or shedding, (Alder, 1994), and is therefore no danger to developing fetuses or transplant recipients if eliminated before the initiation of pregnancy/immunosupression (Adler *et al*,1998). The glycoprotein B has been selected for vaccine production because it is conserved in all known CMV strains and is the main target for neutralizing antibodies (Britt *et al*, 1995).

PCR was first used for HCMV DNA detection in the urine of congenitally infected babies at the end of the 1980s (Demmler *et al*, 1988). When compared with the standard tissue culture isolation procedure, the PCR assay followed by dot blot hybridization showed a sensitivity and specificity of 100%. More recently, determination of HCMV immediate-early mRNA in the blood of congenitally infected newborns by NASBA has been used to diagnose congenital HCMV infection (Revello *et al*, 2001). Similarly demonstration of IgM antibodies against CMV is also used for the detection of CMV infection.

CMV infection occurs world wide and it mostly causes asymptomatic infection. CMV can remain in dormant state in the host body for the whole life, so it mostly goes un noticed. In pregnant females it can be transmitted to the developing fetus (vertical transmission), and is responsible for congenital defects or sequele which develop later in their lives. It has been found that pregnant females having CMV specific IgM antibodies can actively transmit

infection to the fetus. In this study we will use ELISA technique for the detection of IgM antibodies against CMV infection in pregnant females. From these results we can evaluate the prevalence of primary CMV infection in pregnant women in rural areas around Islamabad. In different countries studies have been conducted to show the prevalence of CMV specific IgM antibodies in their populations. However the data regarding the occurrence of CMV infection in our population is not yet available. This data will be useful if any program regarding the control of CMV infection is undertaken. We will also study the effect, if any, of age, parity, stage of pregnancy, abortion, Hepatitis B virus, Hepatitis C virus and socioeconomic status on CMV prevalence and the association of CMV infection with individual risk factors during pregnancy.

Prabhakar *et al* (1992) studied the prevalence of antibodies to cytomegalovirus (CMV) by the indirect enzyme-linked immunosorbent assay in a selected population of 2655 in Jamaica. The overall prevalence rate was 95%, increasing from 56.2% in children 1-4 years of age to 90% in the 15-19 years age group and by 25 years of age 97% of subjects had been exposed to CMV. The prevalence rate in Sexually transmitted disease (STD) clinic attendants and pregnant women was also significantly higher than in blood.

The prevalence of antibodies to Cytomegalovirus (CMV) by ELISA in 19,043 subjects in the population of Parma (Northern Italy) was determined. The overall prevalence of 71.8% was found, Natali *et al* (1997). The age specific prevalence increases starting from 28% in two year-olds to 95.7% in 45-54 year-old subjects. A longitudinal study of CMV infection was undertaken in 1045 pregnant women and their babies. Rate of infection during pregnancy was 2.34% and rate of congenital infection was 0.57%. Results also indicate that mothers are the major source of perinatal infection (contaminated genital secretions and milk) and confirm the usefulness of monitoring antibody status and virus excretion of mother and neonate at birth.

One thousand and eighteen women were enrolled in a prospective study carried out by Gratacap-Cavallier *et al* (1998) in Grenoble, France. The overall rate of CMV seropositivity was 51.5 %, using a specific IgG ELISA test. Among a homogeneous population of 873 women born in France with high or middle socioeconomic status, CMV seropositivity increased with age and parity. The seroprevalence according to age was found to depend on parity. It increased with age in women with no children or with only one, it was higher but no more age-dependent in women with two children or more.

Socioeconomic status is the strongest predictor of seropositivity. Marshall *et al* (1993) studied seroprevelance of CMV IgG in woman of child bearing age in Jefferson country, Kentucky. Mean age of women in this study was 25.7 years; 76% were white, 60% were from middle and upper socioeconomic status, 64% were married, and 57% had other living children. Overall seroprevalence rate was 62%. Univariate analysis showed strong

associations between seropositivity and lower socioeconomic status, non-white race, and age younger than 25 years.

Seropositivity independently associated with increasing parity, older age, lower social class. Tookey *et al* (1992). Women (20,000) were prospectively studied to determine the prevalence of cytomegalovirus (CMV) antibodies, 54.4% of these women were CMV seropositive. Ethnic group was strongly associated with CMV status: 45.9% of white women were seropositive, 88.2% of Asian, and 77.2% of black women (African/Caribbean ethnic origin). Gambarotto *et al* (1997) studied 1101 women attending for antenatal care at Limoges University Hospital to determine the prevalence of Cytomegalovirus (CMV) antibodies: 47.9% of these women were CMV seropositive. Ethnicity is strongly associated with CMV status, such as, 42.6% of metropolitan and 94.5% of immigrant women were seropositive. Seropositivity is also associated with increasing parity and older age. Preiksaitis *et al.* (1988) conducted a seroepidemiologic study of cytomegalovirus (CMV) infection among 9,928 Inuit (Eskimo), Dene (Indian) and non-native inhabitants of the Northwest Territories (NWT) of Canada. Sera were screened for antibody to CMV by enzyme-linked immunosorbent assay (ELISA). The prevalence rates of CMV antibody increased with age in all ethnic groups.

Chandler *et al* (1985), studied Epidemiology of cytomegalovirus infection in a heterogeneous population of pregnant women. Logistic regression analysis showed that seropositivity correlated with lower socioeconomic status, birth outside North America, multigravidity, older age, history of abnormal cervical cytology, infection with Trichomonas vaginalis, a first pregnancy at less than or equal to 15 years of age, and greater total numbers of sex partners. Thus, past exposure to CMV relates both to sociocultural factors and to sexual behavior.

Three hundred and forty serum samples, collected from women of child bearing age, screened for the presence of IgG antibodies against CMV by ELISA test. The IgG antibodies were detected in 297 which gave prevalence rate of 87.4%. Significantly higher prevalence rates were observed with increasing age and with increase in parity.

There was significant difference in the antibody prevalence in different socioeconomic groups Sheevani *et al* (2005). Seroprevalence rate was also found to be more in women from rural area than those from urban area, although the difference was statistically not significant ($p > 0.05$).

Suarez *et al* (1994) studied the incidence of primary cytomegalovirus infection, in 939 pregnant women of a low socioeconomic level, and 123 pregnant university students by using ELISA. The initially seronegative women were tested again during the second and third trimester of pregnancy to identify primary infections. There was a higher prevalence of infection among low socioeconomic status women (95 v/s 69.9%). Two women (one student and one coming from a low socioeconomic status) had a primary infection. In the newborn of a student, congenital cytomegalovirus infection was detected. Women's socioeconomic condition is not a risk factor for cytomegalovirus primary infection during pregnancy.

Preliminary annual results of serologic and virologic researches of cytomegalovirus in 224 pregnant women showed that the incidence of seropositive (IgG) pregnant women was 21.42%. By indirect immunofluorescence cytomegalovirus was found in vaginal mucous of 8.03% pregnant women. Primary cytomegalovirus infection (viral antibodies of cytomegalovirus class IgM) was confirmed in 2.23% of them. In the other 5.80% of pregnant women it was a question of recurrent cytomegalovirus infection. This result indicated that pregnancy is not a risk factor for the appearance of primary cytomegalovirus in pregnant women. How ever it might be a factor of risk for reactivation of latent cytomegalovirus infection Mijanovic (1992).

Lazzarotto *et al* (2000) determined the avidity index (AI) of anti-cytomegalovirus (CMV) immunoglobulin G (IgG) and the anti-CMV immunoglobulin M (IgM) profile in 124 pregnant women. IgG avidity and blot for IgM were performed on two serum samples from each woman, at 6-18 weeks' gestation and at 20-23 weeks' gestation. Pregnancy outcomes were monitored. The results obtained showed that the determination of anti-CMV IgG avidity at 6-18 weeks' gestation can identify all women who would have an

infected fetus/newborn (100% sensitivity), whereas IgM detected by blot had poorer results (69% sensitivity). Interestingly, at 20-23 weeks' gestation, the sensitivity of IgM detection by blot was higher than that obtained by avidity (75 % and 63%, respectively) and the combination of IgG avidity and IgM by blot yielded the best results (81% sensitivity).

Kumar (1984) conducted a study on 3,253 pregnant adolescents, and 1,404 were found to be seronegative for cytomegalovirus. Specimen collection at each antenatal visit, including urine for viral culture and serum for complement-fixing antibody, allowed detection of primary CMV infection in 14 subjects (1%). Seven of 14 subjects delivered congenitally infected infants. These data shows that primary maternal CMV infection occurs in 1% of susceptible women and is associated with a 50% risk of intrauterine infection. Fetal infection, particularly if it occurs late in pregnancy, is not invariably accompanied by fetal damage.

The rates of abortion, congenital malformation, stillbirth, premature fetal death, and intrauterine growth retardation (IUGR) were all higher in the HCMV-IgM positive pregnant group than in the HCMV-IgM negative group. The infants of HCMV-IgM positive mothers also showed lower birth weight, high biparietal diameter, suboccipitobregmatic diameter, occipitofronatal diameter, occipito-mental diameter than the infants of HCMV-IgM negative mothers. Zhong *et al* (1993).

The demonstration of CMV-specific IgM during pregnancy is diagnostic of recent primary CMV infection, Kangro *et al* (1982). He studied Specific IgM class antibody production in different groups of patients with cytomegalovirus (CMV) infections using a radioimmunoassay (RIA). He showed in patients with symptomatic CMV infections, the appearance of IgM antibody is closely related to the onset of symptoms and coincided with production of complement fixing (CF) antibody. IgM antibodies were at maximum levels 3-4 weeks after infection but generally declined to low or undetectable levels by 3-4 months.

.Eggers *et al* (1998) used micro neutralization assay to distinguish b/w primary and recurrent CMV infection. The diagnosis of primary infection in pregnancy is very important, especially if sero conversion is not documented and follow-up sera with declining IgM-titers are not available. Employing a micro neutralization assay, it was found that neutralizing antibodies first appeared approximately 15 weeks after acute infection. However, serum samples of pregnant women with recurrent or past infection consistently displayed neutralizing activity. In conclusion, the neutralization assay can be used as a reliable method for discriminating acute primary infection from previous or recurrent infection in a single serum sample.

The sensitivity and specificity of direct antibody radioimmunoassay (RIA), M-antibody capture RIA (MACRIA), enzyme-linked immunosorbent assay (ELISA), and the immunofluorescent antibody (IFA) test were compared for the detection of CMV-specific IgM antiboby. RIA, MACRIA, and ELISA were of similar sensitivity with sera from adult patients, but ELISA was apparently less sensitive than RIA and MACRIA for the detection of CMV IgM in cord serum. By comparison IFA was significantly less sensitive than the other three tests, Kangro *et al* (1984). Stagno *et al* (1985) used commercially available ELISA kits and RIA to detect IgM antibodies against CMV. RIA was more sensitive in identifying primary CMV infection and less likely to measure CMV-specific IgM in recurrent infection, giving RIA a better specificity for primary infection. The RIA was superior for diagnosis of congenital CMV infection, with a sensitivity of 89% and a specificity of 100%. The lower sensitivity of the ELISA-IgM occurred in the category of congenitally infected infants born to mothers with recurrent infection. This commercially available ELISA-IgM could be used in combination with a CMV-specific IgG test for monitoring women during pregnancy for primary infection

Zeng *et al* (2003) evaluated ELISA, N-PCR and RT-PCR in clinical practice for pregnant women with HCMV. Total 5581 pregnant women were screened. Among them, 100 cases were positive for IgM (group 1), 69 for both IgM and serous DNA (group 2) and 69 for both IgM and mRNA (group 3). The infectious status, maternal-fetal transmission and pregnancy outcome were monitored. The maternal-fetal transmission rate in the group 1.

2 and 3 was 19.00%, 40.58% and 46.15%, respectively, with a significant difference found between group 2, 3 and group 1. Incidence of spontaneous abortion, fetal death, fetal abnormality and neonatal death in group 1, 2 and 3 was 10.00%, 15.94% and 30.77%, respectively. It was concluded that HCMV-IgM(+) can only be considered as an screening indicator for pregnant women with HCMV infection, while IgM(+) combined with serous DNA(+) or mRNA(+) indicates active infection and has a high incidence of maternal-fetal transmission and abnormal pregnancy outcome.

Revello *et al* (1999) investigated the diagnostic and prognostic value of HCMV load in blood of newborns/infants with congenital HCMV infection. Pp65 antigenemia, viremia and DNAemia was investigated in 116 sequential peripheral blood leukocytes (PBL) samples from 41 newborns/infants with congenital HCMV infection, and in 34 PBL samples from 34 uninfected newborn. Virus-specific IgM were determined in parallel on 145 sequential serum samples. When compared to virus isolation from urine, sensitivities of DNAemia, antigenemia, viremia, and IgM determination were 100, 42.5, 28.2, and 70.7%, respectively. Specificity was 100% for all assays. Antigenemia, viremia and DNAemia levels were significantly higher and persisted longer in newborns with symptomatic infection compared to sub clinically infected babies, whereas no difference was observed for virus-specific IgM antibody between the two groups.

Lazzarotto *et al* (1997) devised a novel Western blot (WB) test for anti-human cytomegalovirus (HCMV) immunoglobulin M (IgM) detection which contains viral structural polypeptides, significant portions of recombinant p150 (ppUL32), and a significant portion of the most immunogenic nonstructural protein p52 (ppUL44). And it was shown to be more sensitive and specific than traditional WB and conventional enzyme immunoassay for the detection of HCMV-specific IgM. Cappel *et al* (1978) used a simple solid phase enzyme immunoassay was used for the detection of immunoglobulin G and M to cytomegalovirus (CMV). Using this test IgM antibodies to CMV were detected in 0.7 per cent of newborns. For the determination of IgG, the enzyme immunoassay was more sensitive than the complement fixation test (CF) and the antibody titres were 4 to 8 fold higher.

Genetic variability of 74 human cytomegalovirus (HCMV) clinical isolates was studied by Tanaka *et al* (2005) in japan. The hypervariable region of the HCMV genome, that is the sequence and UL144 region were analyzed using the polymerase chain reaction (PCR) and unrooted phylogenetic tree. Unrooted phylogenetic trees of a sequence and UL144 allowed the isolates to be grouped to 5 and 3 clades, respectively. Three gB genotypes were also determined. This study provides basic data on the genetic variability of HCMV in an Asian population and should help to determine the strains for vaccine candidates.

Polymerase chain reaction (PCR) and enzyme-linked immunosorbant assay (ELISA) were evaluated. Zhang *et al* (1998), for detection of human cytomegalovirus (HCMV) infection in pregnant women and fetuses. The positive rate of serum HCMV-IgM in pregnant women was 2.4%, and that of HCMV DNA was 12.0%. Fetuses or newborns (17.2%) from infected mothers had either positive HCMV-IgM or positive HCMV DNA or both of them. PCR had significantly higher detection rates than that of ELISA. So ELISA combined with PCR may raise the detection rate and diagnostic efficacy of HCMV infection in both pregnant women and fetuses.

Priya *et al* (2002) evaluated the diagnostic value of ELISA against polymerase chain reaction (PCR) in CMV disease. Anti-CMV antibodies were assayed by ELISA on the sera of 26 CMV PCR positive and 21 PCR negative patients and 35 normal healthy blood donors. Anti-CMV antibodies (IgG or IgG and IgM) were present in 20 (76.9%) of 26 PCR positive and 13 (61.9%) of 21 PCR negative patients. ELISA was negative in six (23.1%) of 26 PCR positive patients. Of the 28 paediatric patients, ELISA was positive in 14 (73.7%) of 19 PCR positive and three (33.3%) of nine PCR negative patients showing a statistically significant difference. Among the 19 patients having complications after organ transplant, ELISA showed anti-CMV antibodies in six (85.7%) of seven PCR positive and 11 (91.7%) of 12 PCR negative patients showing no significant difference. Results concluded that ELISA has no diagnostic value in the detection of CMV activation although it may help in the differential diagnosis of CMV infection in the pediatrics age group. Parmigiani (2003) assess the accuracy of serological/ELISA tests in comparison

with the polymerase chain reaction in maternal blood to diagnose cytomegalovirus infection. And he found that the serological tests had lower sensitivity in comparison with the polymerase chain reaction test when diagnosing cytomegalovirus infection. The consequences of positive polymerase chain reaction and negative immunoglobulin M in women remain unknown.

Luchsinger *et al* (1996) compared the incidence of congenital cytomegalovirus infections in two groups of newborns of differing socioeconomic status. Cytomegalovirus was isolated from urine or oropharingeal secretions in 218 children born in a private clinic and 471 born in a public hospital. Positive viral isolates were confirmed with indirect immunofluorescence using monoclonal antibodies. Infection was detected in 12 children (1.82%), four coming from the private clinic (1.86%) and 8 coming from the public hospital (1.81%). Study conducted by Al ali *et al* on pregnant women (60) with and without serological evidence of active cytomegalovirus (CMV) infection until delivery to detect the incidence and types of overt congenital CMV infection in neonates in Mosul, Iraq. CMV-IgM was detected in cord blood samples of six (10%) overtly sick infants (with different congenital malformations) born to mothers with active CMV infection. Central nervous system abnormalities were detected in all six cases (two with microcephaly and four with hydrocephaly. Yang *et al* (1994) studied perinatal cytomegalovirus (CMV) infection, using CMV-IgM ELISA, in 256 pregnant women at different periods and in the cord blood of 84 babies born by CMV positive mothers. There was a higher prevalence of perinatal morbidity, neonatal asphyxia, malformation, intrauterine death, and poor obstetrical outcome in the CMV positive mothers as compared with the CMV negative group ($P < 0.01$). This study showed that the presence of CMV-IgM indicated a recent or recurrent CMV infection during pregnancy and the babies should be carefully monitored.

Prevalence of cytomegalovirus (CMV) infection among neonatal intensive care unit and healthcare workers was studied. The aim of the work was to study the prevalence of CMV infection in NICU, to detect possible nosocomial transmission of CMV infection and to determine possible risk factors for neonatal CMV infection. This study was carried

on 175 neonates in NICU and 19 employees in the same unit. All members of the study were investigated for serum CMV-IgG and IgM by ELISA and CMV - DNA by PCR. The overall prevalence of CMV was 12.57%, 10 (5.71%) had congenital infection, while 12 cases (6.86%) had perinatal infection. On the other hand from the 19 employees, 2 (10.53%) were CMV-DNA positive by PCR, none of them was CMV-IgM positive and all of them were CMV-IgG positive. The risk factors related to CMV infection among neonates in NICU were, low birth weight, congenital anomalies and breast milk feeding, Morgan *et al* (2003).

Wen *et al* (1996) analyzed the state of human cytomegalovirus (HCMV) infection of pregnant women and the maternal-fetal transmission in three chinese metropolis, and studied the methods of early prenatal diagnosis for intrauterine infection. Cases (301) of active infection were selected to detect HCMV DNA by polymerase chain reaction (PCR) technique. The overall HCMV infection rate was 88.93% in the three metropolis and they were 96.74% and 91.42% in Shenyang and Shanghai respectively, which were significantly higher than that (79.53%) in Wuhan. The active infection rate was 5.42% generally while they were 11.23% and 10.89% in Wuhan and Shenyang respectively, which were significantly higher than that in Shanghai. In addition, the active infection rate of women with history of abnormal pregnancy was significantly higher. Results concluded that the vertical transmission frequently occurs from the actively infected mother. ELISA combined with PCR techniques is a valuable method for early prenatal diagnosis of HCMV congenital infection.

Spano *et al* (2004) determined human cytomegalovirus (HCMV) cervical reactivation in both pregnant and non-pregnant women. Clinical specimens were obtained from 40 pregnant and 62 non-pregnant women. Specimens under investigation were blood samples submitted to seroprevalence determination, antigenemia assay, HCMV-DNA detection, and vaginal secretion, submitted for HCMV-DNA detection. Viral seroprevalence was found in 98% of the women investigated, two of whom were found to be IgM positive. HCMV gB gene amplification was found in 5.1 and 8.5% of WBCs and

in 10 and 14.5% of vaginal secretion from pregnant and non-pregnant women, respectively.

Odland *et al* (2001) studied to assess the prevalence of different viral infections in relation to late abortions, stillbirths, and congenital malformations in sera from Russian pregnant women. In this study one group consist of normally pregnant women (Group 1; n=182) and one group of recurrent aborters (Group 2; n=127) were evaluated, including demographic, medical, clinical, and serological data. The mean age of the two groups was 27.1 and 28.2 years, respectively. The mean number of deliveries was low (0.4 and 0.5, respectively). 31.6% of Group 1 and 41.9% of group 2 were daily smokers. There was little difference in total antibodies to cytomegalovirus (CMV) (78.0% and 81.1%, respectively) or B19 IgG (75.3% and 66.9%, respectively) between the groups.

Wong *et al* (2000) studied seroprevalence of cytomegalovirus (CMV), toxoplasma and parvovirus infection in local antenatal population, and to see the effects, if any, of age, race, parity and nationality on its seroprevalence. The sera of 120 consecutive antenatal women were screened for cytomegalovirus (CMV) IgG, toxoplasma IgG and parvovirus B19 IgG and IgM. A total of 87.0% of patients were tested seropositive for CMV IgG, 17.2% seropositive for toxoplasma IgG and 30.0% seropositive for parvovirus IgG. There seemed to be a trend of increasing seropositivity with age in all three groups. The incidences of all three infections were higher among the Malays, Indians and other races compared to the Chinese. Results indicate that CMV is endemic in there population and hence the most common infection. Alanen *et al* (2005) studied seroprevalence, incidence and fetal transmission of varicella zoster virus (VZV), cytomegalovirus (CMV), herpes simplex virus (HSV) types 1 and 2 and parvovirus B19 infections during pregnancy. Sera of five hundred and fifty-eight parturient women were obtained. IgG and IgM antibodies against VZV, CMV, HSV-1 and -2, and parvovirus B19 were measured from maternal serum in the first trimester and at delivery and from cord serum. Seroprevalences were 96.2% for VZV, 56.3% for CMV, 54.3% for HSV, 46.8% for HSV-1, 9.3% for HSV-2 and 58.6% for parvovirus B19. Results show that parity was associated with CMV seropositivity. Ghazi *et al* (2002) determined the seroprevalence rates of IgG to common

TORCH agents in pregnant Saudi women using indirect enzyme-linked immunosorbent assay. A total of 926 samples of sera were tested for antibodies to TORCH agents. Toxoplasma IgG antibodies were detected in 35.6%, CMV total IgG antibodies were found in 92.1%, rubella IgG antibodies in 93.3%, HSV-1 IgG antibodies in 90.9%, HSV-2 IgG in 27.1%, and VZV IgG antibodies in 74.4%. A 0% seroprevalence rate for HIV-1 and -2 was found.

Pathogenic factors of human cytomegalovirus (HCMV) infections were investigated. Total 36 serum samples were obtained from early pregnant woman and examined with ELISA for anti-HCMV antibody IgG and IgM. After artificial abortion,chorionic villus and decidua were also examined with polymerase chain reaction (PCR) for HCMV-DNA. When the results of PCR were positive, pathological changes of these chorionic villus and decidua were analyzed. The results showed that only 10 samples were PCR positive while IgG and/or IgM antibody to HCMV was positive. After infection with HCMV, different changes occurred in chorionic villus and decidual trophoblastic cells placental villus were hyperplasic and decidua cells degenerated and necrotized followed by lymphocytes infiltration. Li AB *et al* (2003).

Numazaki *et al* (2000) investigated the role of the sexual transmission of human cytomegalovirus (CMV) as a cause of congenital infection. Serum samples were collected from 756 pregnant women and from there husbands. CMV from neonatal urinary specimens was isolated according to a standard tissue culture technique, using MRC-5 cells. At 10 to 12 weeks of gestation, 634 of the 756 pregnant women (83.9%) had IgG antibody to CMV. At 32 to 36 weeks of gestation, 642 of the 756 women (84.9%) had IgG antibody to CMV. A meaningful rise of serum IgG-antibody titer (seroconversion) occurred in 8 women (1.1%). CMV was isolated from the urine of an infant born to a seroconverted woman within a week after birth. The prevalence of IgG antibody to CMV was significantly higher in the husbands of women who seroconverted during pregnancy than in the husbands of the women who were seronegative during pregnancy ($P < 0.01$).

Whether CMV antibodies play an important role in the defense against CMV is still unclear. The importance of the humoral immunity is suggested by the clinical observation that without humoral immune response the patient will not clear the virus. If this is caused by the absence of humoral response or by absence of cellular response that causes also absence of humoral response by not functioning CD4 T helper cells remains unclear. Another argument for a role of antibodies in the clearance of the virus is the effectiveness of prophylactic administration of CMV immunoglobulin in seronegative recipients of kidneys from seropositive donors in preventing morbidity and mortality associated with CMV (Snydman *et al.* 1993), (Metselaar *et al.* 1989). The importance of the humoral immune response is also demonstrated by the finding that CMV specific antibodies reduce the generation of pp65 positive granulocytes by inhibiting uptake of pp65 by granulocytes from infected endothelial cells in vitro (Kas-Deelen *et al.* 2001).

The cellular immune response by CD4 helper T-lymphocytes, CD8 cytotoxic T-lymphocytes and Natural Killer (NK) cells is important in the defense against Cytomegalovirus (Venema *et al.*1994), (Rentenaar *et al.* 2000), (Gamadia, *et al.* 2001). But Cytomegalovirus has developed several mechanisms to evade cellular immune response (Riddell and Greenberg,1997), (Ploegh, 1998). For example the virus can prevent HLA class I loaded with viral peptides to be delivered to the cell surface. This makes the infected cell invisible to CD8 cytotoxic T-lymphocytes. Also the virus encodes a glycoprotein homologous to class I MHC antigens to prevent attack by NK-cells.

Given the prevalence of CMV-induced pathology in neonates, AIDS patients, and transplant recipients, a vaccine is desperately needed. Not surprisingly, different strategies must be used to protect these different populations. Attenuated virus strains Towne and AD169 have been developed by passaging Cytomegalovirus through fibroblast cell lines. Unlike wild-type CMV, the Towne strain does not produce reactivation (latent infection) or shedding, (Alder, 1994), and is therefore not a danger to developing fetuses or transplant recipients if eliminated before the initiation of pregnancy/immunosupression (Adler *et al.* 1998). Genomic studies reveal that Towne was attenuated by several deletions, making reversion to virulent CMV unlikely.

Human trials have demonstrated that the Towne strain is safe and capable of inducing lymphoproliferative responses to CMV in 100% of subjects, CTL responses to CMV in 75% of subjects with persistence for over 6 months, and neutralizing antibodies in titers comparable to those induced by natural CMV infection (Adler *et al*, 1998), (Starr *et al*, 1991). Additionally, vaccination protected against acute mononucleosis-like symptoms in vaccinated individuals who were initially CMV-seronegative (Jonjic *et al*, 1988). Towne strain has also been shown to be safe and effective in prevention of disease caused by CMV infection in renal transplant recipients (Starr, 1992). Interestingly, vaccination doubled the probability of graft acceptance, (Wang *et al*, 1996), suggesting that CMV infection can contribute to organ rejection. In the highest risk group, seronegative recipients from seropositive donors. Towne vaccination reduced the incidence of severe CMV-induced disease by 85% although infection rates were similar (Wang *et al*, 1996).

The virus surface protien glycoprotein B has been selected for vaccine development because it is conserved in all known CMV strains and is the main target for neutralizing antibodies (Britt *et al*, 1995). The adjuvant saponin (QS-21) was used due to its ability to stimulate CTLs and induce antibody class switching (Britt *et al*, 1995). Together, glycoprotein B and QS-21 administration to mice have been shown to cause the production of high-affinity neutralizing antibodies, IgG subclass-switching with significant production of IgG2a (the analog of human IgG1 involved in complement fixation and ADCC), and the production of cytotoxic lymphocytes in mice (Britt *et al*, 1995). Human trials have demonstrated that this vaccine induces both the production of mucosal IgA (unlike natural infection or Towne) and IgG. (Starr.1992), and may therefore, unlike the Towne vaccine (which protects against infection but not disease), be able to prevent both infection (via mucosal immunity) and disease (via IgG and CTLs). Additionally, gB has been inserted into an attenuated yet replication-competent adenovirus and a similarly innocuous poxvirus, the latter currently being tested in phase I clinical trials (Jonjic *et al*, 1988).

Revello *et al* (2002) discussed the following controversial issues in the light of the most recent advances in the field. The actual perception of the problem; universal serologic screening before pregnancy; the impact of correct counseling on decision making by the couple involved, the role of prenatal diagnosis in ascertaining transmission of virus to the

fetus, the impact of preconceptional and periconceptional infections on the prevalence of congenital infection, and the prevalence of congenitally infected babies born to mothers who were immune prior to pregnancy compared to the number born to mothers undergoing primary infection during pregnancy.

MATERIAL AND METHODS

Blood sample were collected from pregnant women visiting the Gynae and obstetric department of Federal Government Services Hospital Islamabad and Mother Child Health centre Aabpara, Islamabad. Blood samples were collected by approved medical techniques. Blood sample (3-5 ml) from each of the 344 pregnant women was collected. After collection and labeling, samples were placed in ice box to prevent spoilage. Information were gathered from females enrolled in this study regarding their age, parity, previous medical history, monthly income, gestational month and onset of any kind of signs and symptoms of any disease during the last 6 months or after being pregnant. All of the patients were asymptomatic to CMV at the time of sampling, but have suffered from previous obstetric complications like, abortion, recurrent abortion, intrauterine death, and premature deliveries. Results of HCV and HBV were available of some of the patients. These tests are routinely performed of the pregnant women visiting the antenatal clinic. These blood samples packed in ice box transported to National Institute of Health Islamabad. Samples were centrifuged at 3000 rpm for 4-5 min, serum separated from RBC was stored at -20 °C for 3-4 months and thawed for 2-3 times during this period.

The entire serum specimens from pregnant women were tested by ELISA for CMV specific IgM. The kits used for the detection of IgM were. HUMAN-ELISA-IgM-TEST, and procedure was followed as recommended by the manufacturer

The principal of classic ELISA is as follow:

The Human CMV IgM ELISA is based on the classical ELISA technique. The polystyrene microtiter strip wells as a solid phase are coated with cell culture derived CMV antigens (CMV Ag). If corresponding specific antibodies (CMV–IgM-Ab) present in patient specimens or controls they are bound to the antigens at the solid phase. The sample dilution buffer contains anti-human IgG to prevent rheumatoid factor (RF) interference and competition from specific IgG present in the specimen. After a washing step to remove unbound material, anti-human IgM peroxidase conjugate is added, this

binds specifically to IgM class antibiodies resulting in the formation of sandwich complexes.

CMV Ag* anti-CMV IgM* anti-IgM Ab-HRP

After a second washing step to remove unbound conjugate the enzyme linked complexes are detected by incubation with TMB/substrate which results in the development of blue color which is changed to yellow by stopping the enzymatic reaction with sulphuric acid. The intensity of color is directly proportional to the amount of anti-CMV IgM antibodies in the specimen. Results for patient samples are obtained by comparison with a cut-off value. Absorbance at 450 nm is read using an ELISA microtiter plate reader.

Reagents and Contents;

- **Dilution Buffer IgM** **(100ml)**
  Phosphate buffer
  NaCl
  Albumin
  Anti-human-IgG (goat)
- **Anti-IgM Conjugate** **(11 ml)**
  Anti-human IgM, peroxidase-conjugated (rabbit),
- **Washing Solution** **(50 ml)**
  Concentrate for about 1000 ml
  Tris buffer
  NaCl
- **TMB solution** **(1.5 ml)**
  3.3', 5.5'- tetramethylbenzidin
  Benzalkonium Chloride
- **Substrate Buffer** **(25 ml)**
  Potassium citrate
  Hydrogen peroxide

- **Stop Solution** (11 ml)
  Sulphuric Acid
- **CMV IgM Negative Control** (2 ml)
- **CMV IgM positive control** (2 ml)
  Calibrated against Paul-Ehrlich-Institute (PEI)
- **Microtiter Strips**
  8 well snap off strips
  coated with CMV antigen (cell culture derived)
- **Strip holder** (1)
- **Strip sealer** (24)

The reagents are stable, when stored at 2...8 °C

Bring all reagents to room temperature (15...25 °C) before use.

Reagent preparation;

- **Working wash solution**
  Dilute Washing solution (1 + 20) with fresh and germ free distilled or deionised water e.g. 50 ml WS + 1000 ml deionised water = 1050 ml,
  Stable for 1 week at 2...8 °C
- **TMB Working Solution**
  Prepare the necessary amount of TMB working solution by diluting TMB solution 1+ 20 with substrate buffer e.g. 100µl TMB sol. + 2 ml substrate buffer,
  Use only a clean plastic vial previously rinsed with distilled or deionised water. Store the solution at room temp. 17...25 °C protected from bright light.
  Stability; 8 h at 17...25° C
  A slight color may develop in the solution during prolonged storage. The substrate blank should have an absorbance reading at 450 nm of A < 0.15. if the absorbance is higher, then discard solution and prepare a fresh TMB working solution.

Test procedure:

Step 1

- One of the well left blank.
- 100 µl of positive control and negative control were dispensed in there specified wells.
- Diluted samples (100 µl) were put into there appropriate well.
- The plate is then covered with adhesive strip and incubated for 30 min at 17…25 °C.
- After incubation the wells were washed with prepared washing solution described previously. The washing procedure was repeated 4 times to ensure complete washing of the wells, by dispensing 350 µl of washing solution in each well.
- Remaining liquid was removed by tapping the strip on tissue paper.

Step 2

- Anti-IgM (100 µl) conjugate was added in each well except for the substrate blank.
- Again wells covered with adhesive strip.
- And incubated for 30 min at 25 °C
- Repeat the washing five times as described in step 1.

-Step 3

- Prepared substrate (100 µl) was added in each well.
- The wells again incubated for 15 min at 25 °C.
- After incubation 100µl of stop solution was added in each well and mixed carefully.

All reagents must be dispensed using fresh tips and reagent reservoir. Specially designed microtubes may be suitable for this purpose.

After termination of reaction absorbance of wells measured at a wave length of 450 nm. The absorbance should be taken soon after termination of reaction or with in 30 min.

The cutt-off value was calculated as follow;

Cutt-off value = MNC + 0.2 × MPC
(COV)

Where;

MNC = Mean negative control

MPC = Mean positive control

According to manufacturer criteria;

1. Substrate blank should be less than 0.150
2. Negative control ≤ 0.250
3. Positive control ≥0.400

Approximately four human IgM ELISA kits were used to apply the test on 344 samples from pregnant females. The absorbance of substrate blank, negative control and positive control were in the range as recommended by the manufacturer.

Now the interpretation of results was according to these criteria;

A 450 patient ≥ Cov ± 15% → Anti-CMV-IgM-positive.

A 450 patients < Cov- 15 % → Anti-CMV-IgM-negative.

We divide the patients into different groups to, "study the prevalence of CMV IgM in a specific group" or "to study the effect of a particular group on CMV IgM seropositivity". From this, we can easily point the conditions and stage of life when pregnant females are more susceptible to CMV infection.

We divide the patients into two groups, to study, in which group of age CMV infection is more common.

a. Age less than or equal to 25 years, (age ≤ 25 Yr)
b. Age above 25 years, (age > 25 Yr)

To study the influence of parity on CMV IgM positivity, females were divided in two groups.

a. Primiparous
b. Multiparous.

We divide the females into three groups with respect to their stage of pregnancy, to detect, in which stage of pregnancy CMV is more prevalent.

a. Patients in first trimester of pregnancy, (1^{st} tri).
b. Patients in second trimester of pregnancy, (2^{nd} tri).
c, Patients in third trimester of pregnancy, (3^{rd} tri).

Although all samples were of females belonging to low socioeconomic group. But to study, the effect of monthly income or socioeconomic status on CMV IgM positivity, we divide the females in two groups;

a. Females with monthly income less than and equal to 5000 or low socioeconomic group, (monthly income $\leq$ Rs 5000).

b. Females with monthly income above 5000 rupees or high socioeconomic status group, (monthly income > Rs. 5000).

Effect of HCV positivity and negativity was also studied on CMV prevalence. For this, we divide the females into three groups on the basis of their of HCV status.

a. Females positive for HCV.
b. Females negative for HCV.
c. females with unknown status of HCV.

Similarly effect of HBV status was also studied on CMV prevalence. For this we divide the females into three groups on the basis of HBV status.

a. Females positive for HBV.
b. Females negative for HBV.

c. Females with unknown status of HBV.

In the last we also studied effect of abortion on prevalence of IgM antibodies against CMV. Females were divided in two groups on the basis of abortion.

a. Females which never had experienced abortion.

b. Females which had experienced one or multiple abortions.

RESULTS

Serum samples (344), collected from pregnant females, processed using ELISA technique for the detection of IgM antibodies against Cytomegalovirus infection and 6.1% of these were found positive. All the women were asymptomatic at the time of sample collection, and no previous history of cytomegalovirus infection was available of any female. Information's were gathered regarding there age, parity, stage of pregnancy, monthly income and history of abortion. There status of Hepatitis B virus and Hepatitis C virus was seen from their pregnancy cards (pregnant females are normally screened for hepatitis B and C during there antenatal visit to hospital). Our main focus was to study the prevalence of IgM antibodies against CMV infection in our local antenatal population and then to study the effect, if any, of different parameters like age, parity, stage of pregnancy, abortion, socioeconomic status and status of HBV and HCV on the prevalence of cytomegalovirus. The mean age of the 344 patients enrolled in this study was 26.29 years, mean parity was 2.76 mean gestational length was 2.68 months and mean monthly income was 4712.5 rupees

To study the effect of different parameters, as described early, on CMV IgM seroprevalence we divide the samples into different groups. Samples were collected randomly from pregnant females, so the number of samples is different in different groups. By comparing the percentage of CMV IgM positive samples in each group, we can find which group is more susceptible to CMV infection.

Blood samples were taken from pregnant females irrespective of there age. We were having blood samples of pregnant females with age from 17 years (minimum) to 40 years (maximum). To study which age group is more susceptible to CMV infection, we divide the samples into two groups i.e. 1st group with age below or equal to 25 years and 2nd with age above 25 years (Fig 1).

a. We were having 164 samples of pregnant females with age less than or equal to 25 years and the mean age of the females in this group was 22.5 years. When these females

were screened for IgM against CMV by ELISA 11 of these was found to be positive for CMV IgM, showing IgM prevalence of 6.7 % in this group.

b. There were 180 samples of pregnant females with age above 25 years. Mean age of the females in this group was 29.6 years. When tested for CMV IgM 10 were found positive, showing CMV IgM prevalence of 5.6 % in this group.

To study the effect of parity on CMV IgM seroprevalence, we divide the females into two groups i.e. primiparous and multiparous (Fig 2).

a. We were having 64 blood samples of primiparous pregnant females and 4 samples were found positive for CMV IgM, means that 6.25 % of females in this group are positive for CMV IgM.

b. There were 280 blood samples of pregnant females which were multiparous. When these samples were screened for CMV IgM by ELISA technique, 17 were found positive, showing IgM prevalence of 6.07 % in this group.

To study the correlation of CMV IgM seropositivity with the stage of pregnancy we divide the females into three groups on the basis of their stage of pregnancy i.e.first trimester, second trimester and third trimester (Fig 3).

a. There were samples of 15 females which were in 1st trimester of pregnancy, and among these no woman found positive for CMV IgM.

b. There were 79 samples of pregnant females which were in 2nd trimester of pregnancy and among these 5 were found positive for CMV IgM, showing prevalence of 6.3 %.

c. There were 250 samples of pregnant females which were in 3rd trimester of pregnancy and 16 were found positive, means 6.4 % of samples positive for CMV IgM.

To study the effect of socioeconomic status on CMV IgM seropositivity, we divide the females into two groups i.e. low socioeconomic status group and high socioeconomic group. We were having blood samples of pregnant females, with gross monthly income from 1400 rupees (minimum) to 20000 rupees (maximum) (Fig 4).

a. The females lying in this group were categorized as low socioeconomic status group, there were 274 samples of females which lie in this group. When these samples were

screened for CMV IgM by ELISA method, 18 samples were found positive, which shows that 6.5 % of samples are positive in this group.

b. Females lying in this group are categorized as high socioeconomic status group, we were having samples of 70 pregnant females which lie in this group. When these samples were tested for the prevalence of CMV IgM by ELISA technique, 3 samples were found positive, means 4.2 % of samples are positive for IgM antibodies against CMV in this group.

To study either prevalence of HCV effects on the prevalence of CMV we divide the females into three groups i.e. females positive for HCV, females negative for HCV and females with unknown status of HCV (Fig 5).

a. There were 93 samples of pregnant females which were positive for HCV, and among these 6 samples were found positive for CMV IgM, means that 6.4 % of these samples are positive for IgM against CMV.

b. There were 135 samples of pregnant females which were negative for HCV and out of these 8 were found positive, showing that 5.9 % of these samples are positive for CMV IgM.

c. The remaining 116 samples were of pregnant women with unknown status of HCV, and among these 7 were found positive for CMV IgM, means that 6.03 % of samples are positive for CMV IgM.

Similarly to study the effect of HBV on CMV prevalence, we divide the females into three groups i.e. females positive for HBV, females negative for HBV, and females with unknown status of HBV (Fig 6).

a. We were having 10 samples of pregnant females which were positive for HBV and no sample was found positive for IgM against CMV.

b. There were 218 samples which were negative for HBV and 14 of these were found positive for CMV IgM i.e. 6.4 % of these found positive for CMV IgM.

c. Samples (116) were with unknown status of hepatitis B, and 7 were found positive for IgM against CMV, i.e. 6.03 % samples are CMV IgM positive.

To study the effect of abortion on CMV IgM seroprevalence, we divide the females into two groups i.e. females who had experienced abortion previously, especially natural abortion. and females who never had experienced abortion (Fig 7).

a. We had 244 samples of pregnant females which never had experienced abortion and 13 of these samples were positive for CMV IgM i.e. CMV IgM is positive in 5.3 % of females which never had experienced abortion.

b. There were 79 samples of pregnant females who had experienced abortion for one time, and among these 6 samples were positive for CMV IgM. 18 samples of women who had experienced abortion 2 times and among these 2 samples were positive for CMV IgM. Similarly 2 samples of females who had experienced abortion for 3 times and 1 sample of woman who had experienced abortion for 4 times, but no sample was found positive for CMV specific IgM in these females. But if we see the prevalence in all the aborted women, there were total 100 samples of women that had experienced abortion and 8 samples were positive for IgM against CMV i.e. prevalence is 8 % in females who never had experienced abortion.

All the groups with number of samples in each group, number of CMV IgM positive sample and there percentage is shown in Table 1.

TABLE 1. Percentage of CMV IgM positive sample in each group of age, stage of pregnancy, parity, abortion, monthly income, HBV and HCV.

Parameters		Frequency	No. of CMV positive sample	Percentage
Age	≤ 25 years	164	11	6.7 %
	> 25 years	180	10	5.6 %
Stage of pregnancy	1st trimester	15	0	0 %
	2nd trimester	79	5	6.3 %
	3rd trimester	250	16	6.4 %
Parity	Primiparous	64	4	6.25 %
	Multiparous	280	17	6.07 %
Abortion	No abortion	244	13	5.3 %
	1 &multiple abortion	100	8	8 %
Monthly income	≤ 5000 Rs./month	274	18	6.5 %
	> 5000 Rs./month	70	3	4.2 %
HBV	Unknown status	116	7	6.03 %
	+ ve sample	10	0	0 %
	- ve sample	218	14	6.4 %
HCV	Unknown status	116	7	6.03 %
	+ ve sample	93	6	6.4 %
	- ve sample	135	8	5.9 %

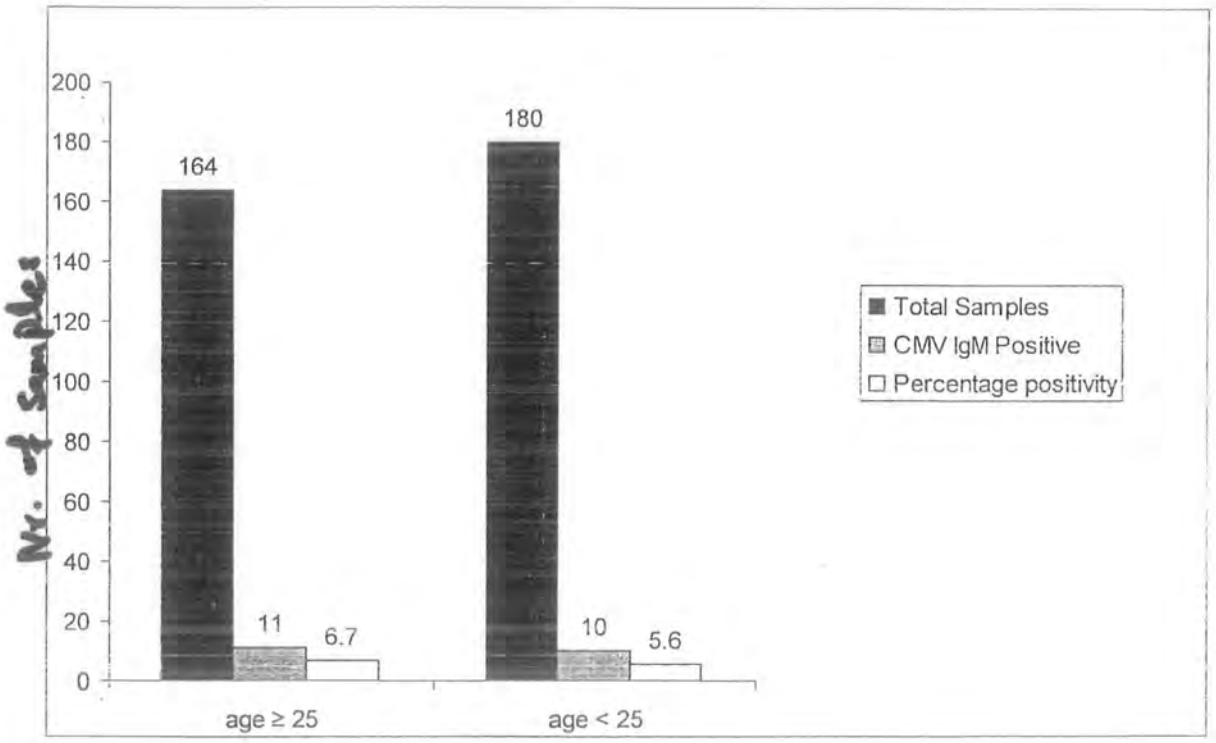

Fig 1. Effect of age on CMV IgM seroprevalence.

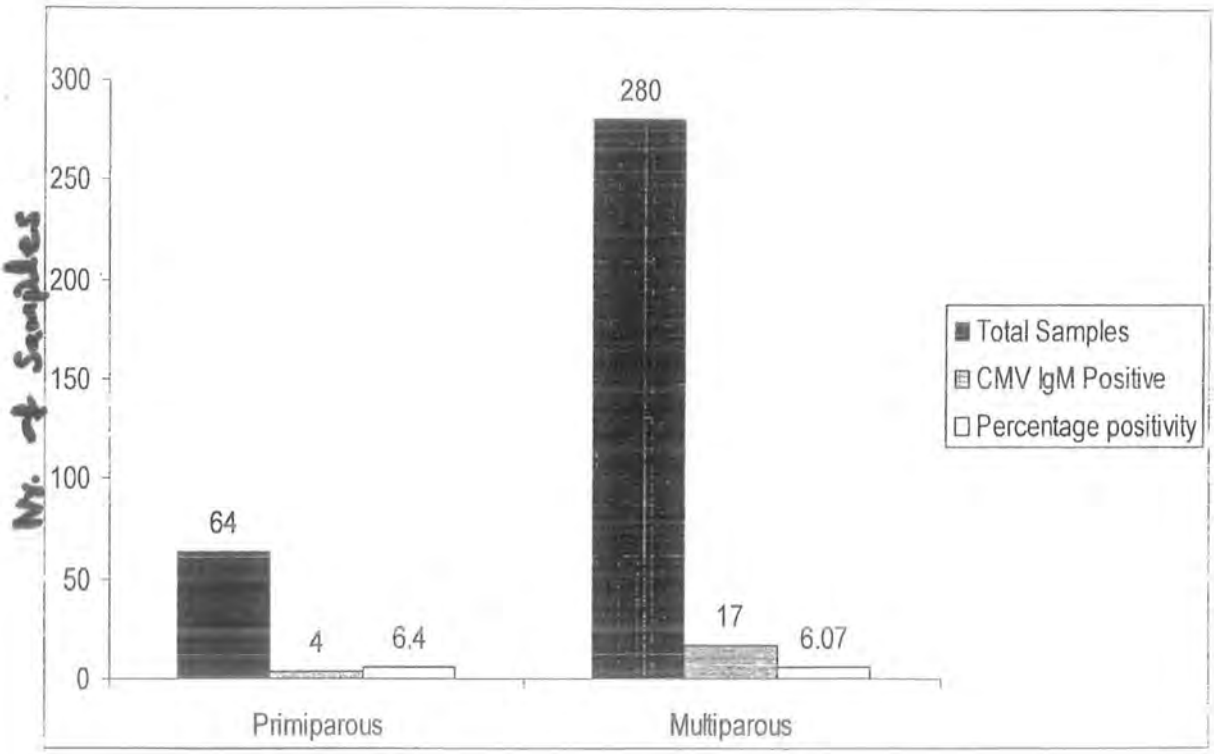

Fig 2. Effect of parity on CMV IgM seroprevalence.

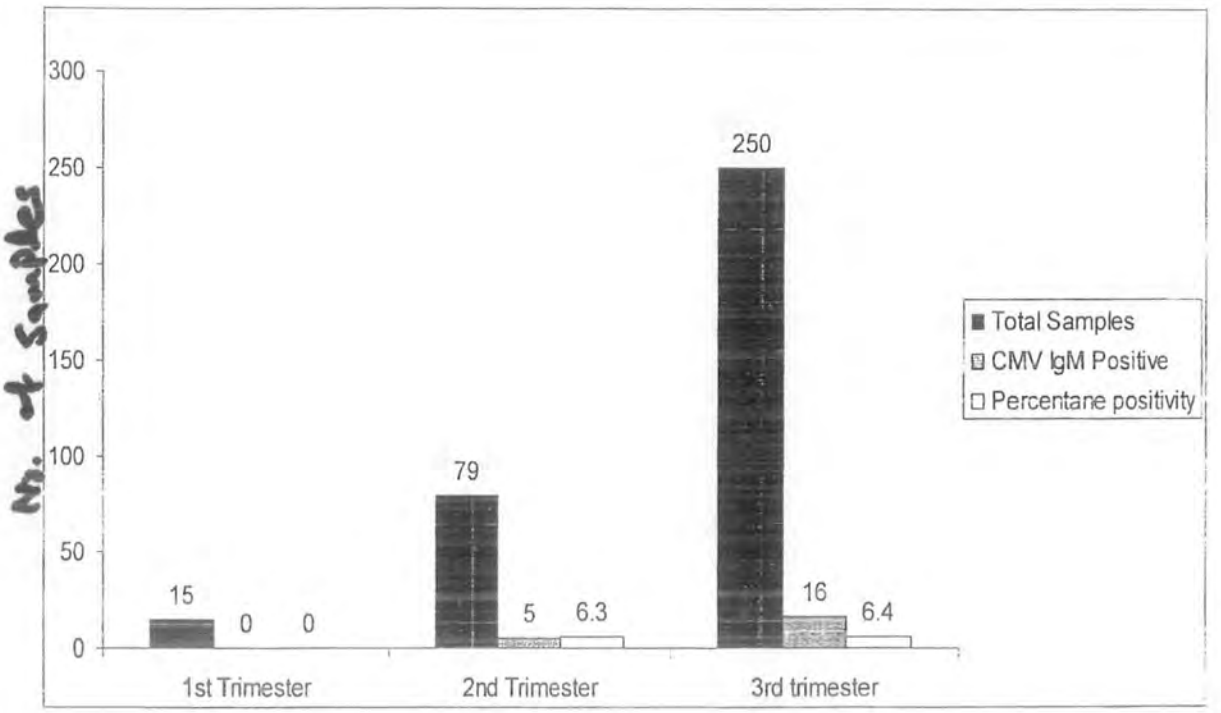

Fig 3. Effect of stage of pregnancy on CMV IgM seroprevalence.

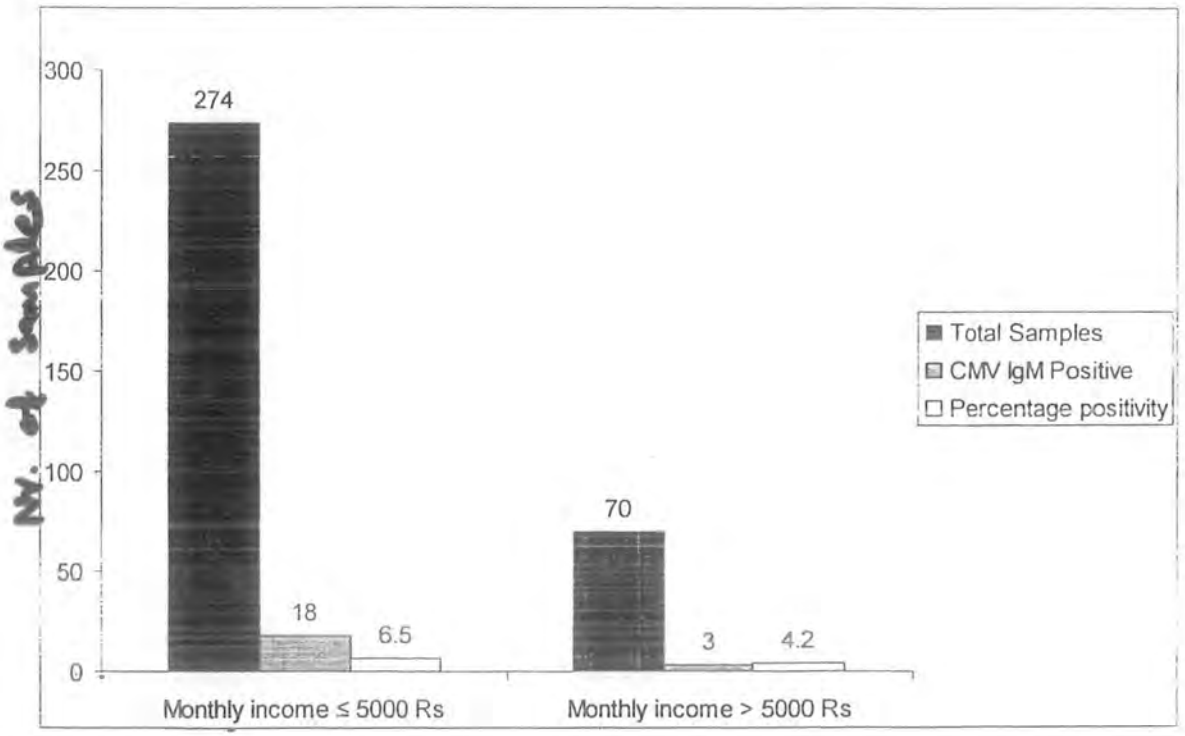

Fig 4. Effect of monthly income on CMV IgM seroprevalence.

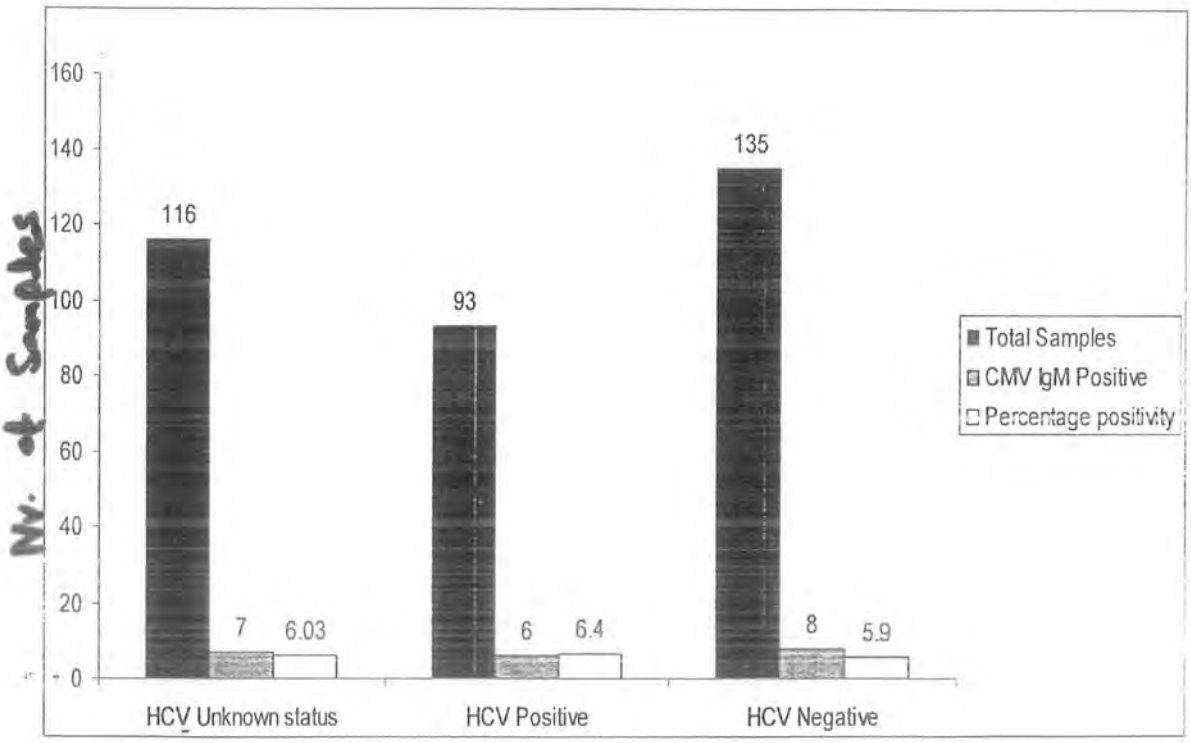

Fig 5. Effect of Hepatitis C virus status on CMV IgM seroprevalence.

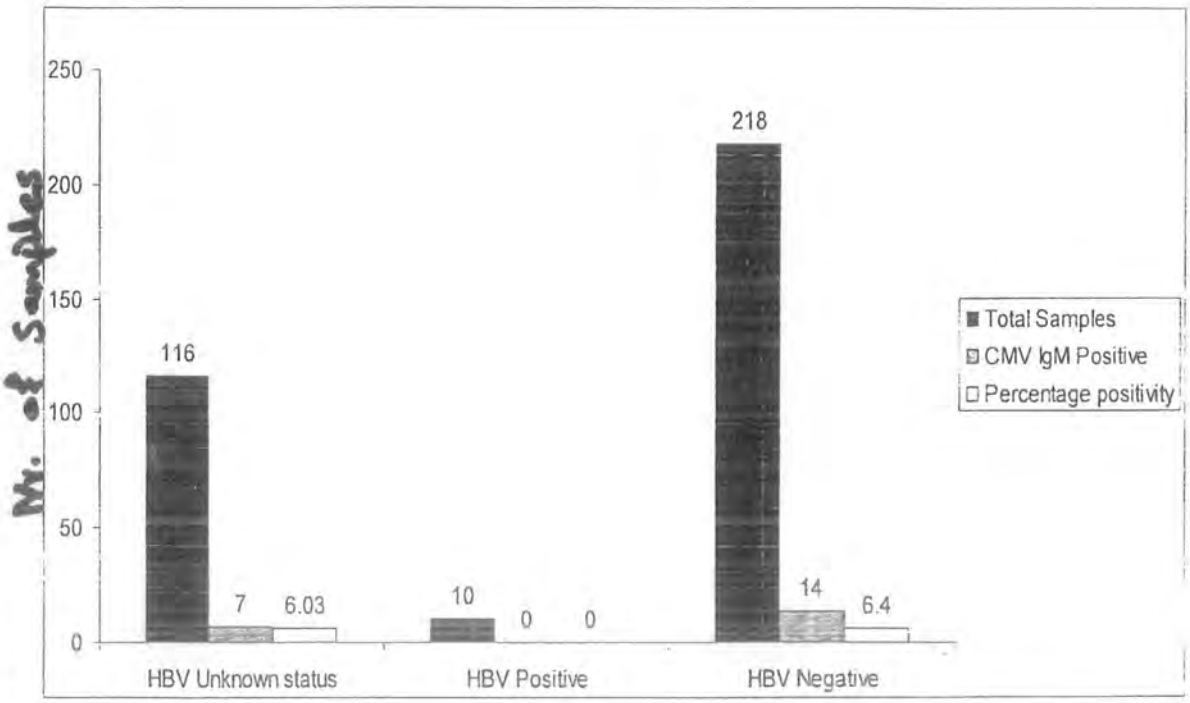

Fig 6. Effect of Hepatitis B virus status on CMV IgM seroprevalence.

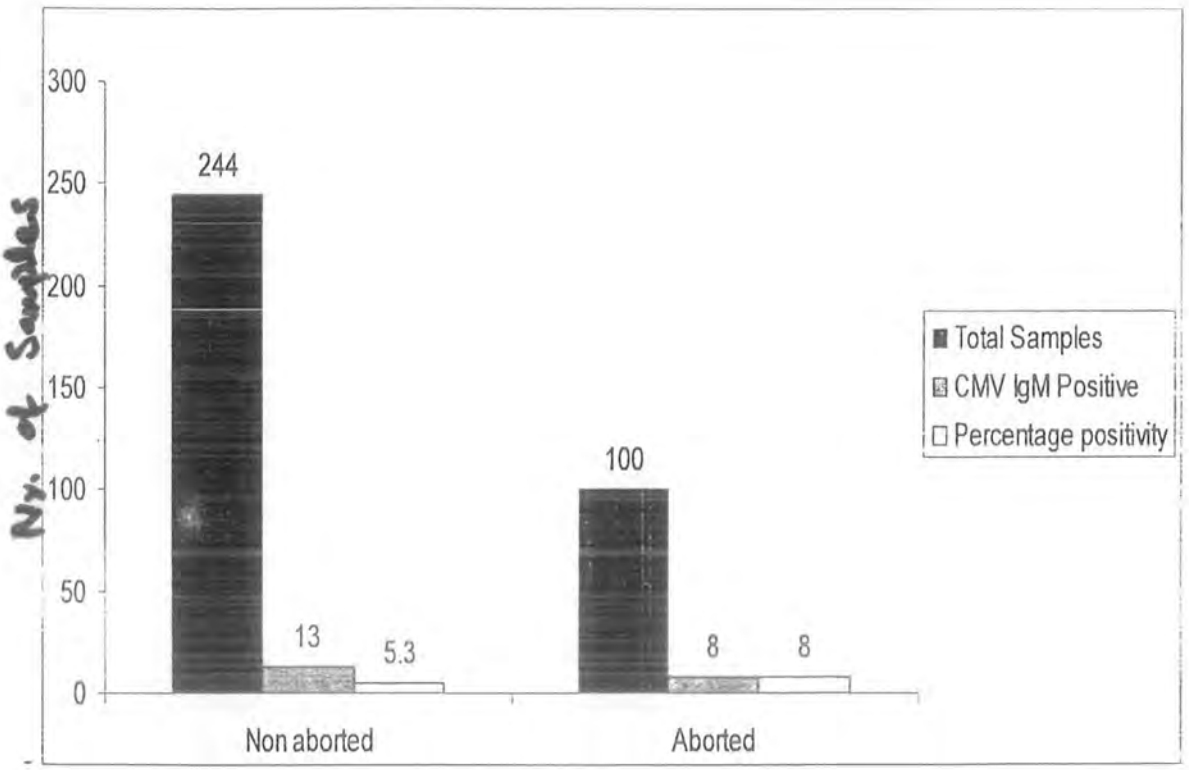

Fig 7. Effect of abortion on CMV IgM seropprevalence.

DISCUSSION

Primary infection in an individual can be diagnosed by detecting CMV specific IgM antibodies (Paul *et al*, 1982). Although the IgM antibodies are also produced in case of recurrent infections but sensitivity of IgM in recurrences is low in HCMV infections, perhaps only 10%. Thus 90% of recurrences may remain unidentified.

It has been proved that primary HCMV infections are transmitted more frequently to the fetus and are more likely to cause fetal damage than recurrent infections (Fowler *et al*,1992). In addition, it seems that primary infection occurring at an earlier gestational age is related to a worse outcome (Demmler, 1991),(Stagno *et al*, 1986). By far the major role in transmitting HCMV infection to the fetus is played by primary infections of the mother during pregnancy. In fact, the rate of vertical transmission was found to be 0.2 to 2.2% in previously seropositive mothers undergoing recurrent infection during pregnancy (Boppana *et al*, 1999),(Stagno, 2001) and 20 to 40% in pregnant women with primary infection (Stagno, 2001), (Stagno *et al*, 1985). Thus, the ratio of transmitting to non transmitting mothers is on the order of 1:100 between those with recurrent and those with primary infection. In this respect, diagnosis of primary infection during pregnancy is a major task of the diagnostic virology laboratory. It may be achieved in the majority of cases through concurrent analysis of the following factors: serum antibodies, virus detection in blood, and clinical signs and symptoms.

The commercially available ELISA-IgM could be used in combination with a CMV specific IgG test for monitoring women during pregnancy for primary infection,(stagno *et al*,1985). However humoral response to CMV of IgM class measured by RIA is restricted to women with primary infections.

What ever the case may be, women with IgM positive against CMV are more active in transmitting infection to foetus than IgM negative. In 1994, a study conducted in Malaysia involving 1688 infants with congenital abnormalities were screened for evidence of congenital CMV infection and this was detected in 11.4% of the infants, which was much higher than other intrauterine infections like congenital toxoplasmosis

(1%) and congenital rubella infection (2.7%), (Balasubramanium *et al*, 1994). CMV infection is endemic in Iraq, the prevalence rates of cytomegalovirus IgM and IgG antibodies in non-pregnant women have been reported to be 1% and 84% respectively, and 2.5% and 90% in pregnant women, (Ali *et al*, 1992).

In our study we demonstrated CMV specific IgM antibodies, in pregnant females by ELISA, our results shows that 6.1% of pregnant females have IgM antibodies against CMV in rural areas around Islamabad city. IgM antibodies are produced in case of primary infection, and persist for three to four months. A study conducted by Rubina lone *et al* (2004) in Kashmir valley, shows that CMV specific IgM antibodies were detected in 15.98% of the 1918 pregnant women studied. Similarly a study conducted in Wuhan in 1993 showed HCMV IgM positive rates were 8.8% in maternal sera. Similarly Mijanovic D. in 1992 conducted the same study in Belgrade, and found Primary cytomegalovirus infection (viral antibodies of cytomegalovirus class IgM) in 2.23% of 224 women screened. From these findings we conclude that CMV infection in our antenatal population is not as high as in Kashmir valley and Wuhan. But CMV IgM prevalence in our antenatal population is significantly higher than Belgrade. As Pakistan is a developing country and most of the population is illiterate and living under poor hygienic conditions, so this infection can be transmitted more frequently in our population. This will result in increase seroprevalence rate of CMV IgM in our population and hence in the antenatal population, which will eventually results in increase birth rates with congenital defects.

Once an acute or recent primary HCMV infection is diagnosed, the woman should be given complete information about the risks of transmission, possible clinical outcome for the child, therapeutic possibilities in the case of symptomatic disease at birth, as well as prenatal diagnosis (if gestation time allows this option). All information should be given within a framework that is as neutral as possible and in an unhurried fashion. If the mother has an acute or recent infection and is still viremic, the possibility of vertical transmission should also be discussed. The woman should also be informed about the

possibility of terminating the pregnancy, but she should be referred to her obstetrician for specific counseling.

Finally, if the woman undergoes prenatal testing and the fetus is found to be infected, results of prenatal diagnosis are discussed during an additional counseling session in order to provide the woman with the most accurate picture of fetal conditions based on biochemical/hematological, virological, and ultrasound findings. The woman (or the couple) then makes the final decision about continuation or termination of the pregnancy.

Beside studying the prevalence of IgM antibodies in local antenatal population we studied the effect of some factors on the prevalence of IgM against CMV like age, parity, gestational length, prevalence of hepatitis B and C, history of abortion and socioeconomic status. Effect of all these factors is discussed one by one.

Our results indicate that age have no effect on CMV IgM seropositivity. The difference in percentage of positive sample between the two age groups is not significant i.e. below or equal to 25 years of age, 6.7 % of samples were positive and in age group above 25 years, 5.5 % samples were positive (Fig 1). Our results are supported by Echaniz-Aviles *et al* (1993), whose results show that the CMV IgM prevalence is not significantly different among women of different ages. Similarly Rubina lone *et al* (2004), also studied the effect of age on the seroprevalence of CMV IgM. Her results show that there is no statistically significant relationship between seropositivity and age. Hao *et al* (1995) found positive rate of 1.1% of IgM against CMV in Henan province of China and conclude that women of different ages, had the same positive rate. These findings indicate that exposure to CMV at any stage of life can result in infection, so there is a possibility that any one can acquire CMV at any age. We can not limit the CMV infection to a certain age group. However certain age groups are frequently exposed to CMV infection and can acquire infection easily, like children at the day care centre can acquire the infection more easily by exposure to contaminated toys and other toddlers. Similarly females having more sex partners can acquire the infection easily.

We also studied the effect of parity on CMV seropositivity. Samples of pregnant women were divided into two groups according to their parity i.e primiparous and multiparous. Samples (6.25 %) were positive for CMV specific IgM antibodies in primiparous pregnant women and 6.07 % were positive in multiparous pregnant women (Fig 2). Our results show no significant relationship between CMV seropositivity and parity. Rubina lone also studied IgM prevalence with relation to parity and her results shows that there is no significant relationship between IgM seropositivity and parity. Hao *et al* (1995) finds the same, that women with different parities have the same positive rate.

The effect of stage of pregnancy on CMV IgM seropositivity was also studied. In our study 15 of the pregnant were in the first trimester, and none of these were found positive for CMV IgM. Of the 79 women in second trimester, 6.3 % of these were positive. And 250 women were in third trimester. 6.4 % were positive for IgM against CMV (Fig 3). Our results show no significant relation ship between IgM seropositivity and the stage of pregnancy. Studies conducted by Hao *et al* (1995) show that females in different stages of pregnancy have the same positive rate. However it is reported that females having CMV IgM in the late gestation can transmit the infection to the developing fetus more frequently. And women with CMV IgM positive in early pregnancy can deliver fetus with more serious complications

Our next step was to find the effect of prevalence of hepatitis C on CMV IgM seropositivity. We were having 93 samples positive for hepatitis C, when these samples tested for the prevalence of IgM against CMV by ELISA, 6.4 % of these samples were found positive. And out of 135 samples negative for HCV, 5.9 % were positive for CMV IgM (Fig 5). These results show no significant relationship between HCV positivity and CMV IgM positivity. Any research work related to effect of HCV on CMV IgM seropositivity was not found. So more research work with large number of samples is still needed to study the effect of prevalence of hepatitis on CMV IgM seropositivity.

Similarly we had 10 samples positive for hepatitis B virus, and no sample was found positive for CMV IgM (0%). Out of 218 samples negative for hepatitis B virus 14 (6.4 %)

were found positive for CMV IgM (Fig 6). The difference between numbers of samples in two groups is too large, so the results obtained are not comparable. Research work with large number of samples is required to study the effect of HBV on CMV IgM seroprevalence.

In this study we had 100 samples of pregnant females that had experienced abortion, and 8 % of these were positive for CMV IgM. And most of these women had experienced aborted at 2nd or 3rd month of pregnancy. Out of 244 women who never had experienced abortion 5.3 % were positive for CMV IgM (Fig 7). Results show a significant difference in prevalence of CMV specific IgM between these groups, but the reason for this difference is unknown. Similarly Guo *et al* (1992) have reported no relation of CMV IgM positivity with abortion especially natural abortion.

The last factor which we study, effecting CMV IgM seropositivity was socioeconomic status. A lot of research work is available showing the effect of socioeconomic status on CMV positivity. Almost all of our samples were of women belonging to low socioeconomic status, but to study the effect of monthly income on CMV seropositivity we divided the women in two groups. Group one with females having monthly income less than or equal to five thousand rupees, called as low socioeconomic status. Second group of females with monthly income above five thousand rupees, are called as high socioeconomic status. Out of the 274 samples with monthly income below or equal to Rs. 5000, 6.5 % samples were positive for CMV IgM. And out of 70 samples with monthly income above Rs. 5000, 4.2 % were positive (Fig 4). Our results show that CMV IgM seropositivity increases, with the decreasing monthly income or with poor socioeconomic status. Guo (1992) describes that high positivity rates of CMV IgM antibody were correlated with lower socioeconomic status. Similarly Mustakangas (2000) find that low socioeconomic environment seems to be the most powerful factor, predicting both high CMV seroprevalence and recurrences during pregnancy, Tookey *et al*, (1992) concluded that seropositiviy against CMV was independently associated with lower socio economic class. Our results show that low socioeconomic status contributes a lot toward CMV seropositivity. And that, pregnant women belonging to low socioeconomic class are

seems to be more CMV IgM seropositive than with high socioeconomic status. Adverse living conditions, low education, poor hygiene and high frequency of close contact between women of lower socioeconomic class make them susceptible to CMV infection, which increases the possibility of acquiring the infection more frequently.

In our country pregnant women are never screened for primary CMV infection. Similarly child born with congenital defects are never screened for CMV, and traditional assumptions are made in spite of performing proper tests to diagnose the defect. These all factors are contributing a lot in spread CMV infection. CMV can cause a wide and varying pattern of neonatal infections, and it is important for obstetricians and physician to consider CMV infection in dealing with infants born with congenital anomalies, especially which have symptoms like microcephaly, vision and hearing impairment, and mental abnormalities. Such infants and their mothers should be screened for evidence of active CMV infection.

All these findings indicate that CMV infection is not uncommon in our local population. This high seroprevalence reflects the low hygienic conditions are practiced in our population. Also CMV can lead to substantial damage to the fetus and as the damage done in utero cannot be reverted, control of intrauterine CMV infection is important. Hence prevention of CMV infection, especially in the pregnant women is essential. Screening of pregnant women, although, cannot change the outcome of the disease but may be useful in alerting the physician for possible infection to the baby. Hence screening of pregnant females for CMV infection is desired in order to reduce the fatal outcome of the pregnancy occurring due to the CMV infection.

Recommendations for individuals providing care for infants and children:

1. Female employees should be educated concerning CMV, its transmission, and hygienic practices, such as handwashing, which minimize the risk of infection.

2. Susceptible nonpregnant women working with infants and children should not routinely be transferred to other work situations.

3. Pregnant women working with infants and children should be informed of the risk of acquiring CMV infection and the possible effects on the unborn child.

4. Routine laboratory testing for CMV antibody in female workers is not recommended, but can be performed to determine their immune status

Universal screening of pregnant women for CMV infection during an early prenatal visit is not yet recommended worldwide. With the absence of a safe vaccine, seronegative women at the onset of pregnancy should be advised to practice careful hygiene and to minimize contact with carriers and other sources of infection in order to decrease the chances of infection. Since no treatment is available for pregnant women with active primary CMV infection, and the effect of the infection on pregnancy is unpredictable, regular follow-up of these women is advised. Data are insufficient to recommend therapeutic abortion if fetal infection is discovered in early pregnancy, and 90% of infected babies are asymptomatic at birth, (Margaret, 1993).

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