International Journal of Pharmaceutical Chemistry

ISSN: 2249-734X (Online)
Journal DOI:10.7439/ijpc
CODEN:IJPCI4 (American Chemical Society)

Research Article

Seroprevalence of cytomegalovirus among blood donors in local population

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Abstract

Cytomegalovirus (CMV) infection is more common in developing countries and considered as the major cause of morbidity and mortality among neonates, immune compromised individuals especially in organ transplant recipient and other immunosuppressive drug users. Blood transfusion is a major source of infection in such individuals. This study was conducted to estimate the seroprevalence of CMV antibodies among healthy blood donors of different age groups in local population of Islamabad. Total 175 serum samples were taken from completely healthy male donors and all were tested negative for HBsAg, HIV 1/2 antibody, HCV antibody, Malaria and Syphilis. These samples were analyzed for anti-IgM CMV and anti-IgG CMV using the ELISA technique. From 175 samples, 3.4% tested were positive for CMV IgM antibody and 96.5% were positive for CMV IgG antibody. None of the donor was positive for both IgG and IgM antibodies. There was no statistically significant difference in seropositivity of CMV IgG based on distribution of age. As about 96.5% of blood donors were seropositive for CMV, it should be mandatory in blood banks to screen donor's blood for CMV. Furthermore there is need to maintain record of CMV negative blood bags and also of CMV negative blood donors and these should only be transfused to immunocompromised patients and to neonate to avoid complications of CMV.

Keywords: Seroprevalence, Cytomegaloviruses, Congenital Infection**,** ELISA, Microparticle Enzyme Immunoassay, Hematological Parameters

1. Introduction

Cytomegaloviruses (CMVs) are highly species-specific; that is, they have a very limited range of hosts[1]. CMV has emerged in recent years as the most important cause of congenital infection in the developed world, commonly leading to mental retardation and developmental disability[2].

CMV is a globally-occurring infection with a high prevalence (50-90%) in all human populations. In developing countries, the rates are generally higher, and the infection is acquired at younger age. Virus transmission occurs via body fluids (blood, saliva, breast milk, semen, and cervical secretions), and since the virus is labile, intimate contact with a person with primary or reactivated infection is needed. Therefore, transmission mainly occurs within families, between sex partners, and in groups of small children. Children acquiring CMV at a pre-school age shed the virus for extended period and therefore constitute a major societal reservoir of virus[1]. Around the world the mean seropositivity rate varies with location, race, and socioeconomic status. However in any location, almost all the individuals eventually became infected, ranging from 60-70% in urban Asian cities to 100 % in Africa[3]. Breastfeeding is probably one of the most important routes of transmission. Although it is often contracted during childhood, primary CMV infection is a life-long event[4].

Little is known about the molecular mechanisms responsible for the pathogenesis of tissue damage caused by CMV, particularly for congenital CMV infection. Although the CNS is the major target organ for tissue damage in the developing fetus, culturing CMV from the cerebrospinal fluid of symptomatic infants with congenital infection is surprisingly difficult. Because CMV can infect endothelial cells, some authors have postulated that a viral angitis may be responsible for perfusion failure in the developing brain with resultant maldevelopment. Others have postulated a direct teratogenic effect of CMV on the developing fetus. Observation of CMV-induced alternations in the cell cycle and CMV-induced damage to chromosomes supports this speculation; however, this hypothesis has been difficult to experimentally verify.

In clinical specimens, one of the classic hallmarks of CMV infection is the cytomegalic inclusion cell. These strikingly enlarged cells (the property of "cytomegaly," from which CMV acquires its name) contain intranuclear inclusions that have the histopathological appearance of owl's eyes (Figure 1). The presence of these cells indicates productive infection, although they may be absent even in actively infected tissues. In most cell lines, CMV is difficult to culture in the laboratory; however, in vivo infection seems to chiefly involve epithelial cells. In severe disseminated CMV disease, involvement can be observed in most organ systems[5].

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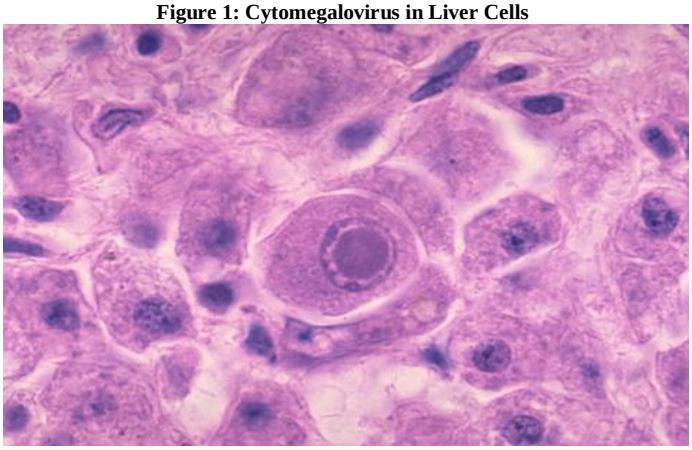
Figure 1: Cytomegalovirus in Liver Cells



2. Experimental

Our study population consisted of 185 consecutive voluntary blood donors taken over a period of three months from Dec. 2007 to Feb. 2008 at the Blood Bank of NESCOM Hospital (ISO 9001-2000 certified) Islamabad, Pakistan. Parameters like Donor's age, sex, and history of any previous disease were collected from individual blood donors.

The inclusion criteria for recruiting voluntary blood donors, as laid down by the Blood Bank of NESCOM Hospital were: age between 18 and 55 years; weight >55 kg; hemoglobin >13.5 gm/dl for males and >11.5 gm/dl for females; normal blood pressure (BP), pulse, and temperature; not belonging to intravenous drug addicts and no history of any severe current or chronic illnesses like Syphilis, Diabetes, Viral Hepatitis, Tuberculosis, Heart/Kidney disease, Rheumatic fever, Ulcer etc.

All volunteer blood donors were questioned to look for any previous disease history and to ascertain that whether they will meet the inclusion criteria or not. Eligible donors were invited to participate in the study and their willingness sought.

2.1 Sample Collection and Processing

First of all 3ml of intravenous blood was taken by venipuncture technique from every donor for the confirmation of blood groups and to check the hematological parameters like hemoglobin, WBCs count, RBCs count, Platelets count, mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC). Using 5ml syringes of Becton Dickinson (Pvt) Pakistan, for the collection of venous blood. This anticoagulated blood was used to check hematological parameters. All hematological parameters were checked by Sysmex KX-21 automated hematology analyzer by SYSMEX Corporation, Japan. Normal values of different hematological parameters are given below in table-1.

Table 1: Normal values of different hematological parameters.

Parameter	Normal Values	Units
RBC	4.5-6.0 (males) 3.8-5.8 (females)	Million/cmm
WBC	4000-10000	Pre cmm
Hemoglobin	14.0-18.0 (males) 12.0-16.0 (females)	g/dl
Platelets	140,000-425,000	Per cmm
MCV	82-98	Fl
MCH	27-31	Pg
MCHC	32-36	g/dl

After hematological analysis, detection of blood grouping of all donors was done by direct blood grouping technique also known as forward typing.

Out of more than 250 prospective blood donors only 185 have met the criteria for donation after taking complete history and checking of hematological parameters. Then donations of 185 completely healthy blood donors were taken by trained staff of Blood Bank using standardized 500ml, 17G, blood bags of ***JMS Singapore Pvt Ltd.*** These blood bags contain 70ml CPDA (Citrate, Phosphate, Dextrose and Adenine) as an anticoagulant which prevent the blood from clotting. After donation, donated blood was stored in the blood bank refrigerator that operates between 2-6°C, which was well lighted, equipped with alarm and temperature recording devices.

Before other analysis, screening of all sera was done. Following tests were performed on donor's blood:

- Detection of Hepatitis B Surface Antigen (HBsAg)
- Detection of Antibodies to the Hepatitis C Virus (Anti-HCV)
- Detection of Antibodies to the Human Immunodeficiency Virus (Anti-HIV-1, -2)
- Screening for Malarial Parasite.
- Screening test for Syphilis

HBsAg, Anti-HCV and Anti-HIV-1,-2 were tested by 3rd generation MEIA according to manufacturer's instruction in the automated version (MEIA-Microparticle Enzyme Immunoassay, AxSYM; ABBOTT Laboratories) while Syphilis and Malaria by Immunochromatographic Techniques by ABBOTT Laboratories and Dia-Med AG Diagnostics, Switzerland, respectively.

After checking of hematological parameters, blood groups and screening of the donors, selected completely healthy blood donors to participate in the study. All completely healthy blood donors were then tested for CMV IgG and IgM antibodies by ELISA method. Enzyme Linked Immunosorbant Assay (ELISA) was performed manually using a 96 well micro titration plate using BioCheck, Inc CMV IgM and IgG Enzyme Immunoassay, USA test kits. This is a sandwich enzyme immunoassay for the detection of IgG and IgM antibodies to CMV in serum.

Results were calculated qualitatively. The ratio between the average O.D. value of the sample and that of the cut-off was calculated. The sample was considered, positive, if the ratio was >1.0 and doubtful, if the ratio was between 0.91-0.99 and negative, if the ratio was <0.9.

2.1 Statistical analysis

The data gathered was correlated with laboratory results using two statistical tests to make desired inferences about the seroprevalence of this viral infection in local population using Chi-square and Percentage Test. The calculated P value for IgG which was P=0.2 and considered the value of α=5.0% that is equal to 0.05 and P value for IgM was 1.8 and α was same as 0.05.

3. Results

All of the 185 healthy individuals selected for blood donation were male and none of them was female. These individuals were passed through a strict process of donor selection which involved blood testing and interview of each person. The mean age of the individuals was 28.2 years (median 27.4 years, range 19-50 years).

3.1 Blood Screening For Infectious Diseases

All the blood samples, taken from blood bags after donation were screened using AxSYM Immunoassays System from Abbott for different markers for infectious diseases and 175 blood donors were found negative for all HBsAg, Anti-HCV Antibodies, Anti-HIV ½ antibodies. These samples were also screened for the presence of Malarial and Syphilis Pathogens and were found negative. 10 individuals were excluded from the study because of the presence of either of the pathogens and 175 completely healthy blood donors were free of any kind of sign and symptoms of any disease.

Out of the 185 voluntary blood donors, four gave positive results for anti-HCV Antibodies, also four were positive for HBsAg, one was found positive for anti HIV ½ antibodies and one was positive for syphilis. None of them was found positive for malarial parasites. All of these donations were excluded from the study (Table 2). Hence the results presented are that of 175 donors.

Table 2: Blood Screening for Infectious Diseases

Test	Positive Individuals	Negative Individuals
HBsAg	4	181
Anti HCV Antibodies	4	181
Syphilis	1	184
Malarial Pathogen	0	185
HIV ½ Antibodies	1	184
Total	10	175

3.2 Blood Grouping

Each blood donor was tested for his blood group using forward grouping technique. Out of a total of 175 completely healthy blood donors, 36.0% had blood group O, 24.0% blood group A, 36.0% blood group B and 4.0 % had AB blood group. Ninety-six percent of the blood donors were Rh +ve, while only 4% were Rh -ve (Table: 4). Maximum number of blood donors belongs to blood groups "B" which were 63 making 36.0 % of total blood donor population also similar number were from blood group "O" that were also 36.0 % while 42 blood donors having blood group "A" that were 24.0% of total blood donors and only seven were having blood group "AB" making 4.0 % of total blood donor population.

Table: 3 Total Blood Donors and their Blood Groups

Blood Groups	RH Positive	RH Negative	Total	% age
O	59	04	63	36.0
A	41	01	42	24.0
B	61	02	63	36.0
AB	07	00	07	4.0
TOTAL	168	7	175	100

3.3 Hematological Parameters of Donors

Blood donors were tested for various hematology parameters such as Hemoglobin (HB), RBC count, MCV, MCH and MCHC, WBC count and platelets to check the health status. As given in the Table 5 mean blood hemoglobin was 14.6 g/dl, mean RBC Count was 4.85 million/cmm, mean WBC Count was 7200/cmm and mean platelet count was 258,000/cmm as tested by the Sysmex Automatic Hematology Analyzer. Other calculated parameters such as MCV, MCH and MCHC were also within acceptable limits as shown in the Table 4.

Table 4: Mean values of different hematological parameters.

Parameters	Units	Mean Value
Hemoglobin	g/dl	14.6
RBC Count	Million/cmm	4.85
WBC Count	per cmm	7200
Platelet Count	per cmm	258,000
MCV	Fl	86.6
MCH	Pg	37.9
MCHC	g/dl	32.2

3.4 CMV IgG Seropositivity

All of the blood donations from 175 individuals were tested for IgG and IgM antibodies by ELISA method. Out of 175 blood donors 169 individuals were seropositive for IgG antibodies giving an overall percentage of 96.5 % indicating old infection to the blood donors.

90% of the blood donors in the age group of aged ≤20 years (n=10) were seropositive for CMV IgG as against 96.3% in 21-30 year age group (n=110), 98% in 31-40-year (n=52) and 100% in 41-50-year (n=03) age group. There was no statistically significant difference seen in the CMV IgG status in different age groups (Table 5).

Table 5: IgG status of blood donors in different age groups

Age (years)	Total Donors	Seropositive	Seronegative	% age of Seropositive donors
< 20	10	9	1	90.0
21-30	110	106	4	96.3
31-40	52	51	1	98.0
41-50	3	3	0	100.0

3.5 CMV IgM Seropositivity

In case of CMV IgM out of 175 blood donors only 6 individuals were seropositive for IgM antibodies giving an overall percentage of 3.4 % indicating primary or acute infection to the blood donors.

In case of CMV IgM there was no seropositive individual between the ages of 41-50 years and also in those with less than 20 years. However 3.6% (n=4) in age group 21-30 years and 3.8% (n=2) blood donations in age group 31-40 years, were positive for CMV IgM showing an active infection in the donors. There was no statistically significant difference in the CMV IgG status (Table 5) in different age groups as seen in case of CMV IgM (Table 6).

Table 6: IgM status of blood donors in different age groups

Age (Years)	Total Donors	Sero-positive	%age of Seropositive Donors	Sero- negative	%age of Seronegative Donors
< 20	10	0	0.0	10	100.0
21-30	110	4	3.6	106	96.4
31-40	52	2	3.8	50	96.2
41-50	3	0	0.0	3	100.0

3.6 Correlation of Seropositivity with Blood Groups

All donors having Rh –ve Blood Groups were positive for IgG antibodies and none of IgM positive donor was Rh –ve even they are 4 % of the total blood donor population. Out of total IgM Six positive donors,16.7% belong to blood group "O" and similarly 16.7% from Blood group "A" while maximum numbers of IgM positive blood donors have blood group "B" which is four making 66.6 % of total IgM positive blood donor population and none of IgM seropositive individual belong to group "AB". On the other hand out of total 169 IgG seropositive donors 60 belong to blood group "O" making 35.6%, 41 having blood group "A" making 24.2% while maximum number of IgG positive blood donors were having blood group "B" which are 61 out of 169 making 36.0 % of total IgG positive blood donors and IgG positive donors having blood group "AB" were 7 making 4.2% of total 169 IgG positive blood donors.

Statistically no significant correlation between CMV IgG or IgM status and blood groups of the individuals was detected (Table 7).

Table 7: Seropositivity of IgG and IgM in different Blood Groups

Blood Groups	Total Donors	IgG Positive	% age	IgM positive	% age
O	63	60	35.6	1	16.7
A	42	41	24.2	1	16.7
B	63	61	36.0	4	66.6
AB	7	7	4.2	0	0.0
Total	175	169	100	6	100

4. Discussion

As results shown in our study that 96.5% of the blood donors were positive for CMV IgG antibodies, indicating past exposure of infection while 3.4% were positive for CMV IgM antibodies indicating primary infection in blood donors.

This high seroprevalence in Pakistan and India is in contrast to Western literature which describes seroprevalence in voluntary blood donors ranging from 38% to 75% in different parts of the world[6]. Although several studies on the prevalence of CMV antibody in different parts of the world have been done, the results are not comparable in detail because methodologies used are different. A study by Krech, (1973) is an important one in this respect since complement-fixing antibody assay (using the same control serum) was used on sera from donors of comparable ages. His results showed that seropositivity of CMV varies between 40% in industrialized countries and 100% in developing countries. Interestingly, the prevalence of antibody in Japan and Hong Kong is over 90%, although they cannot be considered 'developing' nations[7]. The high seroprevalence in adults in India, indicates the endemicity of infection. This could be related to socioeconomic, environmental and climatic factors[8].

In our study, no correlation was observed between seropositivity of CMV and blood groups. This is similar to the findings of other investigators[9]. In our study, the seroprevalence of CMV among the blood donors varied with age ranging from 90% in the 19-40 year age group to 100% in the 41-50 year age group. The increase in the percentage seropositivity in the 41-50 year age group is most likely due to the fact that data in this age band are based, relatively on a smaller numbers. There is no statistically significant difference in seropositivity of CMV based on distribution of age. This data is similar with the study of Galea and Urbaniak, (1993) that also showed a significantly increased seropositivity with increasing age of blood donors[9].

The variation of Seroprevalence of CMV-lgG antibodies between different countries may be due to the differences is socioeconomic levels in the study areas. Weber and Doerr (1994) mentioned that people who come from lower socioeconomic areas show a higher HCMV-lgG seroprevalence than do people from an upper or middle income levels.

The American Association of Blood Banks has recommended transfusion from donors who are seronegative for CMV or the use of deglycerolized frozen RBCs whenever transfusion is contemplated in a seronegative pre-term (<1,200 g) child born to a mother with negative or unknown immune status with regard to CMV infection[11]. These guidelines have helped in eliminating transfusion-induced CMV infection syndrome in pre-term infants in the West America. Other preventive strategies, such as leukoreduction filtration, saline-washed RBCs, frozen deglycerolized RBCs, etc., are being increasingly recommended to minimize the transmission of CMV through transfusion[12].

The incidence of TT CMV infection by unscreened blood products to seronegative recipients is 10-70% with an average 30%. On the other hand; the incidence reduced to 1-3% in seronegative donor/receipient bone marrow transplant and other seronegative high risk patients[13,14,15].

A study by Pal *et al*., (1972) in Chandigarh, India showed 100% seropositivity for CMV in the population aged above 20 years[16], while Madhavan *et al*, (1974), in Pondicherry, India, showed that 84-96% of adults had the antibody against CMV[17]. A study in Vellore, India, showed a seroprevalence of 92% in normal individuals [18].

Therefore, by the information obtained from the previous studies and results obtained in the present work, it is found that prevention of TT-CMV infection is recommended for CMV seronegative immunocompromised patients and this can be achieved by using CMV-seronegative blood and blood products or white blood cells depleted blood products. Also large scale screening of blood donors for CMV using different assays is recommended in Pakistan.

These may be more appropriate and cost-effective in the Pakistani scenario for the prevention of transmission of CMV through infected blood to immunosuppressed individuals. More studies in the Pakistani context need to be done to elucidate the transmission of transfusion associated CMV before proper guidelines on routine screening for CMV in voluntary blood donors can be formulated.

In summary, Cytomegalovirus (CMV) is known to be a significant cause of morbidity and mortality following blood transfusion in children and immunocompromised adults. In Pakistan, it is not mandatory to screen donated blood for CMV in blood banks. Very few studies

have been conducted in Pakistan to estimate the seroprevalence of this infection in voluntary blood donors. This study was conducted to estimate the seroprevalence of CMV among voluntary blood donors in local population of Islamabad, Pakistan. In this study, 3.4% donors tested were positive for CMV IgM antibody indicating primary infection while 96.5% were positive for CMV IgG antibody indicating old infection. There was no statistically significant difference in seropositivity of CMV IgG based on distribution of age. All donors tested were negative for HBsAg, HIV antibody, HCV antibody and syphilis.

As about 96.5% of blood donors were seropositive for CMV, it should be mandatory in blood banks to screen blood donors for CMV. Also there is need to maintain record of CMV negative blood bags and also of CMV negative blood donors and these should only be issued to immune-compromised patients and to neonates. Other preventive strategies, such as leukoreduction etc. could be more appropriate and cost-effective for the prevention of transmission of CMV through infected blood to immunosuppressed individuals.

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