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Seroprevalence of cytomegalovirus infection among healthy blood donors in Bahia State, Brazil

Soroprevalência da infecção por citomegalovírus entre doadores de sangue saudáveis no estado da Bahia, Brasil

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We aimed at analyzing the seroprevalence of cytomegalovirus infection (CMV) and to assess particular aspects of the related immunological profile among blood donors in the State of Bahia, Brazil. Immunoassays were performed to detect anti-CMV IgG and IgM antibodies and the anti-CMV IgG avidity was evaluated. The methodology used was Enzyme Linked Immuno Sorbent Assay (ELISA) with results being confirmed by chemiluminescence. Reactivity to CMV was compared between genders and age groups. Among the 636 healthy blood donors tested, 428 (67.3%) were men and 208 (32.7%) were women. The overall seroprevalence of CMV was 87.9%; seroprevalence was statistically higher in women (94.7%) than in men (84.6% - $p<0.05$). No sample was positive for anti-CMV IgM antibodies. About 4.6% of the sample tested showed high titers of anti-CMV IgG; in these cases an IgG avidity assay was performed that showed: low avidity (31%), moderate avidity (21%), and high avidity (48%). The high CMV seroprevalence underscores the importance of using strategies such as leukoreduction and transfusion with CMV-seronegative blood in patients who are at high risk of developing severe CMV infection. The high titers of anti-CMV IgG antibodies and its IgG avidity profile suggest the possibility of viral reactivation or re-infection. Rev. Bras. Hematol. Hemoter.

Key words: *Cytomegalovirus infection; blood donors; prevalence.*

Introduction

Human cytomegalovirus (CMV) is ubiquitous -human herpesvirus,[5] with from 40% to 100% of the general population exhibiting prior exposure by serology. CMV is spread through close personal contact with people who excrete the virus in body fluids (e.g., saliva, urine, breast milk, semen), by vertical transmission, through organ transplant, or via blood transfusion.[1,2]

In healthy immunocompetent individuals, primary CMV infection is usually asymptomatic. After a primary infection, the virus persists in a latent state, from which it can be reactivated under certain conditions. CMV can cause severe illness in immunocompromised patients, such as patients

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undergoing organ transplantation, those with leukemia, those who are HIV positive, and infants with low birth weights.[1,3]

Latent CMV viruses are mainly associated with white blood cells, which are responsible for CMV transmission by transfusion (TT-CMV) of cellular blood components. There are strategies to prevent the spread of CMV via blood products, such as the transfusion of leukocyte-depleted blood products and screening to select CMV-seronegative blood donors.[3,4] In many countries the high prevalence of CMV among blood donors is usually a problem when assembling a CMV-negative blood inventory. Moreover, the cost of maintaining a CMV-negative blood supply can often be quite high.[4]

Thus, we aimed at assessing aspects related to the immunological profile and seroprevalence of CMV among blood donors in the State of Bahia, Brazil by analyzing volunteer donors at the very important Bahia State Blood Bank.

Patients and Method

This study was carried out between the months of July and September among blood donors at the Hematology and Hemotherapy Foundation of the State of Bahia, Brazil (Hemoba), a referral blood bank and hemotherapy service for the entire state.

Donor population

All participants were selected for blood donation after answering questionnaires, interviews by a physician and a hematocrit, all of which are routine procedures at Hemoba. The exclusion criteria were individuals that were younger than 18 years old, weight < 50 Kg, hemoglobin < 12.5 g/dL, history of jaundice, history of high-risk sexual behavior, and blood-borne infections.

After being considered suitable for blood donation, the volunteer was briefed about the aims of the study and invited to participate. All participants signed an informed consent form. The research protocol was approved by the Ethics Committee of Bahia State Health Department.

A total of 636 blood donors were recruited for this study based on a sample calculation that utilized results of a prior pilot study ($p = 0.83; q = 0.17; E = 0.03; Z_{a/2} = 1.96$).

Testing

Sera were tested at the Immunology Service of Infectious Diseases, Faculty of Pharmacy, Federal University of Bahia. The presence of IgM and IgG antibodies against CMV (Elisa; DiaSorin SA, Sallugia, Italy) were assayed in accordance with the manufacturer's instructions.

All positive results for anti-CMV IgM and extremely positive and negative values for anti-CMV IgG by ELISA were reanalyzed in duplicate by chemiluminescence (Liaison CMV IgG and IgM; DiaSorin SA, Sallugia, Italy) according to the manufacturer's instructions.

Some blood donors showed high levels of anti-CMV IgG. In these cases, we tested for IgG avidity using a chemiluminescence assay (Liaison CMV IgG avidity; DiaSorin SA, Sallugia, Italy) according to the manufacturer's instructions.

Statistical analyses

SPSS for Windows version 9.0 was used for data analyses. Seropositivity rates were calculated and compared according to the age group and gender. Differences were evaluated using the Chi-square test with the Yates correction. A p-value of < 0.05 was considered statistically significant.

Results

Of the 636 healthy blood donors tested, 428 (67.3%) were men and 208 (32.7%) were women. Their mean age was 31.3 years old (median age: 29.0, range: 18 to 66-years-old). The age group distribution according to gender can be seen in Table 1.

The overall seroprevalence of CMV observed among these healthy blood donors was 87.9%. None were reactive for CMV IgM antibodies as analyzed by ELISA or chemiluminescence. Positivity for anti-CMV IgG was observed in 362 (84.6%) of the 428 men and 197 (94.7%) of the 208 women. The difference in the CMV seropositivity rate between genders was statistically significant ($p<0.05$) (Figure 1).

Table 1. Age group frequency in years among blood donors according to gender

Age Group	Frequency	Male	Female
18-28	304	212 (69.7%)	92 (30.3%)
28-38	189	114 (60.3%)	75 (39.7%)
38-48	96	72 (75.0%)	24 (25.0%)
48-58	40	25 (62.5%)	15 (37.5%)
> 58	7	5 (71.4%)	2 (28.6%)
Total	636	428 (67.3%)	208 (32.7%)

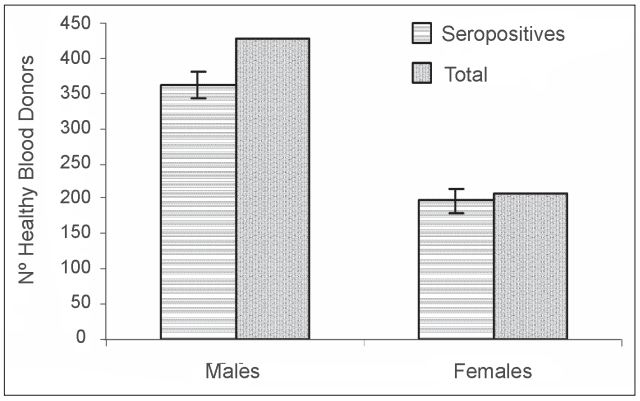

Figure 1. Seropositivity for CMV among healthy blood donors according to gender

Table 2. CMV Seropositivity according to age group and gender

Age	Male			Female		
	Pos.	Neg.	All	Pos.	Neg.	All
18-28	169 (79.7%)	43 (20.3%)	212	87 (94.6%)	05 (5.4%)	92
28-38	101 (88.6%)	13 (11.4%)	114	73 (97.3%)	02 (2.7%)	75
38-48	65 (90.3%)	07 (9.7%)	72	22 (91.7%)	02 (8.3%)	24
48-58	23 (92.0%)	02 (8.0%)	25	14 (93.3%)	01 (6.7%)	15
> 58	04 (80.0%)	01 (20.0%)	05	01 (50.0)%	01 (50.0%)	02

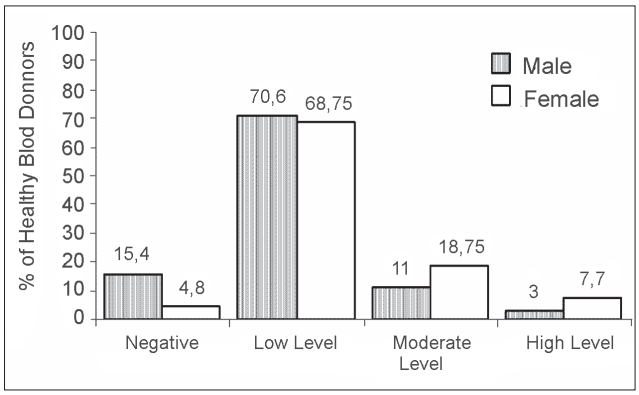

Figure 2. Percentage (%) of IgG anti-CMV level groups according to gender. (Negative = <0.45 IU/mL; Low Level = 0.45-5.00 IU/mL; Moderate Level = 5.00-15.00 IU/mL; High Level = > 15.00 IU/mL)

Table 3. Distribution of IgG anti-CMV level according to age group

IgG level (IU/mL)	Age Groups					Total
	18 - 28	28 - 38	38 - 48	48 - 58	> 58	
Negative	48 (15.8%)	15 (7.9%)	09 (9.4%)	02 (5.0%)	02 (28.6%)	76
0.45 - 5.00	207 (68.1%)	138 (73.0%)	73 (76.0%)	24 (60.0%)	03 (42.8%)	445
5.00-15.00	35 (11.5%)	28 (14.8%)	12 (12.5%)	09 (22.5%)	02 (28.6%)	86
>15.00	14 (4.6%)	08 (4.3%)	02 (2.1%)	05 (12.5%)	00 (0.0%)	29
Total	304	189	96	40	07	636

Table 2 shows CMV seropositivity according to gender and age group. Among the men, CMV seropositivity had the highest rates in 28 to 58 year olds (ranging from 88.6% to 92.0%). Women had more seropositive results for CMV than men, with the highest seroprevalence being observed for 18 to 58 year olds (ranging from 91.7% to 97.3%). There were no significant differences ($p > 0.05$) in CMV IgG status between different age groups of the same gender. On the other hand, when we analyzed the same age groups for different genders, a statistically significant difference was observed ($p < 0.05$) in the 18 to 28-year-old and 28 to 38-year-old age groups.

Table 3 shows the distributions of anti-CMV IgG levels according to age group. Samples with anti-CMV IgG levels less than 0.45 IU/mL were considered negative. It was observed that between CMV-seropositive blood donors (anti-CMV IgG more than 0.45 IU/mL), there were different levels of IgG antibodies that we allocated to three groups: 0.45-5.00, 5.00-15.00, and >15.00 IU/mL. For all age groups, the most prevalent anti-CMV IgG antibody group was 0.45-5.00 IU/mL. The seroprevalence of the 0.45-5.00 IU/mL anti-CMV IgG group was significantly higher ($p < 0.05$) than the other two anti-CMV IgG groups.

Figure 2 shows the percentages (%) of each anti-CMV IgG group according to gender. Approximately 70% of healthy blood donors tested were CMV seropositive with low levels of serum antibodies for CMV. The difference of anti-CMV IgG groups between genders was not significant ($p>0.05$). On the other hand, the prevalence of anti-CMV IgG antibodies at low levels was significantly higher ($p < 0.05$) than other anti-CMV IgG groups among participants of the same gender. It was observed that 29 (4.6%) blood donors, that is, about 7.7% of women and 3.0% of men had anti-CMV IgG > 15.00 IU/mL. Nine of these 29 blood donors with high levels of anti-CMV IgG had low avidity anti-CMV IgG, six had moderate avidity, and 14 had high avidity.

Discussion

Several studies have suggested that CMV is an important transfusion-transmitted (TT) pathogen. In contrast to asymptomatic CMV infection in healthy people, TT-CMV can cause diseases with significant risk of morbidity and mortality in immunocompromised individuals.[3-5]

The participants in this study were healthy blood donors at Hemoba. Similar to other studies, we observed more male (428- 67.3%) than female (208- 32.7%) blood donors.[6,7]

None of the blood units tested was positive for anti-CMV IgM antibodies, hence indicating the absence of primary CMV infection among our study sample. On the other hand, 87.9% of the blood donors were positive for anti-CMV IgG antibodies, indicating past exposure to infection. Others

studies have also shown a high worldwide seroprevalence for CMV among blood donors, such as in Ghana[8] (93.2%), India[7] (95.0%), Turkey[9] (97.2%), Nigeria[10] (92.0%), Tunisia[11] (97.14%), and the USA[12] (35.5%).

Figure 1 shows that CMV seroprevalence was significantly ($p < 0.05$) higher in women than in men (94.7% versus 84.6%, respectively). Gargouri and colleagues[11] also found that prevalence of CMV in Tunisian blood donors was higher in women than in men (98.57% *versus* 95.71%, respectively). The most common mode of CMV transmission for adults is via exposure to toddlers. Infected infants and children, in particular those under 30 months old, actively excrete the virus in their saliva and urine. Thus, one hypothesis to explain the higher female CMV seroprevalence would be that women have more contact with children.[13]

There was no significant difference ($p > 0.05$) in the anti-CMV IgG levels in participants from different age groups of the same gender. On the other hand, when we analyzed the same age groups of different genders, we observed a significant difference ($p<0.05$) between the 18-28 and 28-38 age groups (Table 2). The decrease in the seroprevalence of CMV in the > 58 years old age group is likely to be due to a smaller number of blood donors, since similar studies reported a significantly increased seropositivity with the increasing age of blood donors.[6]

The observed seroprevalence of CMV indicates that the endemicity of infection is probably related to socioeconomic and environmental factors.[14] The high seroprevalence of CMV among Brazilian blood donors is a high risk factor for TT-CMV, which is associated with morbidity and mortality in at-risk populations, such as CMV-seronegative neonates, HIV positive individuals, and transplant recipients.[5]

The American Association of Blood Banks recommends the transfusion of blood that is "CMV-safe" for all individuals that are at-risk of developing severe CMV infection.6 There are two main strategies to transfuse blood that is "CMV-safe": leukocyte depletion and the use of seronegative blood products.[4,15]

CMV exists mainly in peripheral blood leukocytes.[3] Blood filters act by reducing the number of leukocytes transfused through blood products, with their efficacy depending mainly on the reliability of the filters and the number of leukocytes remaining at the time of transfusion. A significant reduction in risk of CMV transmission was observed with the transfusion of leukodepleted blood components.[4]

Other strategies to prevent TT-CMV involve screening blood donors and only using CMV-seronegative blood products for transfusion recipients that are at high risk for CMV infection. The main difficulty is to provide CMV-seronegative products from donor populations where CMV prevalence is high.[3,6] On the other hand, the great reduction in TT-CMV after CMV-seronegative blood products shows the importance of having a CMV-seronegative blood inventory available to all individuals that are at-risk of developing severe CMV infection.[8] In Table 2, we observed that the most seronegative group of blood donors (20.3%) was the group of 18- to 28-year-old men. Since the high prevalence of CMV is considered the main difficulty in maintaining a CMV-seronegative inventory, routine screening of those in the most seronegative category could be the best strategy.

After primary infection, CMV establishes a lifelong latency, called a non-productive infectious state, in its host. Reactivation from latency occurs periodically throughout life in seropositive individuals which provides the stimulus for lifelong antibody positivity.[3] During CMV latency, an individual can be re-infected with different strains of CMV; the immune response to re-infection needs better clarification.[2]

Some studies show different levels of anti-CMV IgG antibodies in the seropositive population.[11,16] The IgG titer can vary up to 50-fold between individuals. To evaluate this question, we categorized the blood donors according to the serum concentration of anti-CMV IgG antibodies. The most prevalent anti-CMV IgG level for all age groups was 0.45-5.00 IU/mL (Table 3). The prevalence of IgG anti-CMV at low levels was significantly higher ($p < 0.05$) than for other anti-CMV IgG groups. The difference of the same anti-CMV IgG group between the genders was not statistically significant ($p>0.05$) (Figure 2).

Gargouri and colleagues[11] showed anti-CMV IgG titers greater than 12 IU/ml in 56.43% of CMV-positive donors in Tunisia. The anti-CMV IgG titer was greater than 15 IU/mL in 4.6% of our blood donors. An avidity assay was performed to better characterize the "high level" group (anti-CMV IgG >15 IU/mL). This assay was carried out to confirm whether the CMV infection was recently acquired or had been acquired a long time previously.[16-17] We observed low avidity (31%), moderate avidity (21%), and high avidity (48%). Thus, a hypothesis to explain the high titers of anti-CMV IgG would possibly be a recent viral reactivation, which has already been suggested by other studies.[18-19] On the other hand, it is more likely that a recent re-infection by a different CMV strain was responsible for a combination of "high titers of IgG + low avidity". However, to prove these hypotheses, more specific tests are needed, such as assessing the CMV genome in these patients.[20]

In conclusion, it is worth emphasizing the high CMV seroprevalence found, which shows the importance of using strategies such as leukoreduction and transfusion of CMV seronegative blood in patients at high risk of developing severe CMV infection. High seroprevalence is an obstacle to building an inventory of CMV seronegative blood in the blood bank; however, we observed that focused screening conducted on a particular category of blood donors (in our study, men in the 18 to 28 years old age range) would be the

most efficient way of achieving this goal. There are individuals with high titers of anti-CMV IgG antibodies, suggesting the possibility of reactivation or recent re-infection; however, more effort is needed to better characterize the dynamics of CMV infection in these cases.

Resumo

Nós objetivamos analisar a soroprevalência para infecção por citomegalovírus (CMV) e avaliar aspectos particulares do perfil imunológico relacionado em doadores de sangue no estado da Bahia. Foram realizados imunoensaios de detecção de IgG e IgM anti-CMV, bem como avaliação da avidez dos anticorpos IgG anti-CMV. A metodologia utilizada foi o Teste imunoenzimático ELISA, confirmado por quimioluminescência. A reatividade das amostras para a infecção por CMV foi comparada entre gêneros e grupos etários. Entre os 636 doadores testados, 428 (67,3%) eram do sexo masculino e 208 (32,7%) do sexo feminino. A soroprevalência geral para CMV observada foi de 87,9%, sendo maior estatisticamente entre as mulheres (94,7%) do que entre os homens (84,6%) (p<0,05). Nenhuma amostra foi positiva para IgM anti-CMV. Cerca de 4,6% das amostras testadas apresentaram IgG anti-CMV em altos títulos, nestes casos foi realizado o imunoensaio de avidez do IgG anti-CMV que evidenciou: baixa avidez (31%), moderada avidez (21%) e alta avidez (48%). A alta soroprevalência encontrada ressalta a importância do uso de estratégias como a leucorredução e a transfusão com hemocomponente CMV-negativo em pacientes com alto risco de desenvolverem infecção severa por CMV. Os altos títulos de IgG anti-CMV e o perfil da avidez dessa IgG sugerem a possibilidade da reativação ou reinfecção. Rev. Bras. Hematol. Hemoter.

Palavras-chave*: Infecção por citomegalovirus; Doador de sangue; prevalência.*

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