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WIDESPREAD OF CYTOMEGALOVIRUS INFECTION AMONG BLOOD DONORS IN KHARTOUM STATE

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ABSTRACT

Background: 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. In the last years transmission of CMV infection through blood transfusion is markedly reduced by transfusion of leukodepleted blood product. **Objective:** The present study aimed to determine the seroprevalence of CMV IgG & IgM antibodies among healthy blood donors in the central blood bank of Khartoum State and to investigate possible association between donors age, the type ABO blood group, hematological parameters and CMV serological status. **Methodology:** 200 participating were recruited from blood donors at central blood bank of Khartoum State (CBBKs). According to the results obtained from CBBKS records, all participating were negative for HBsAg, HIV 1/2 antibody, HCV antibody, Malaria and Syphilis. The age and geographical area of residence of the participating was obtained by blood bank records. All the participating blood samples were analyzed for anti-IgM CMV and anti-IgG CMV using the ELISA technique, ABO blood grouping using forward technique (Biomed, diagnostic, Egypt) and hematological parameters using Sysmex KX-21 automated hematology analyzer. **Result:** From 200 samples, 4.0% tested were positive for CMV IgM antibody and 84.5% 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 antibody and IgM antibody (P value > 0.05) also there was no correlation between CMV IgG and distribution of age, ABO blood group, and residence (P value > 0.05) while there is correlation in seropositivity of CMV IgM with distribution of age only (P value < 0.05). **Conclusion & Recommendation:** there is a very high seroprevalence of anti-CMV IgG among blood donors at the CBBKS. Since the CMV remains latent with the leucocytes after infection in spite of the presence of the antibodies in the seropositive individuals, screening donors for CMV and using leukodepleted blood products is recommended before transfusion for neonates, patients with AIDS and recipients for kidney transplant, etc Further studies are recommended to determine the prevalence of CMV antibodies in these patients.

KEYWORDS: cytomegalovirus; Seropositivity; ELISA; TTCMV; immunocompermised. TTIs, CBBK.

INTRODUCTION

Cytomegalovirus (CMV), a member of the human herpes family of viruses, transmissible through blood transfusions, is an important cause of concern worldwide.[1] Like most other herpes viruses, CMV remains latent in the host after primary infection and persists for life in the organism. Latent CMV is associated with white blood cells which aids its transmission by transfusion of cellular blood components.[4] Nevertheless, these viruses can be reactivated in immunosuppressed individuals and can be an important cause of morbidity and mortality. CMV can be transmitted by blood transfusion, transplacental route or by transplantation of hematopoietic stem cells and solid organs from infected donors.[5] Most studies suggest that 13-38% of immunocompromised patients will contract CMV from transfusion of unscreened and unfiltered cellular blood components.[3][6][7] Therefore, the most effective way to minimize the risk of CMV transmission in high risk recipients would be to administer CMV free blood products. The immunosuppressed population for whom CMV free blood products are requested is increasing due to advances in medical care.[8] It is a ubiquitous organism found universally in all geographic locations. However, CMV is more common in developing countries and in people belonging to lower socio-economic status.[2] The seroprevalence of CMV is 80 -100% in Africa and Latin America[3], however the prevalence in Sudan is high,

ranging from 70% up 90%, study done by Hiba et al (2016) in Khartoum (75%).

French-American-British blood bank community provides the technical regulations for blood therapy procedures, determines that CMV serological tests should be performed on all blood units or blood products that are to be transfused into CMV-seronegative patients undergoing organ transplants and newborns weight less than 1,200 g at birth, from CMV-seronegative mothers or mothers of unknown serological status. These serological tests are not required if such patients are transfused with leukocyte-depleted blood products. Unfortunately in Sudan blood banks the FAB-BBC protocol is not follow which leads to raising the rate of morbidity and mortality among neonates, immunocompremized patients and recipients of kidney transplant.

One of the most common available serologic tests to detect CMV IgG and CMV IgM antibodies is based on enzyme-linked immunosorbent assay (ELISA). IgG positive result is indicative of a person infected by CMV during his or her life.[9] CMV IgM presence could be interpreted as new infection, acute infection or re-activation of CMV. It has been reported that CMV infection rate increases with blood donor age. Transfusion transmissible infections (TTIs) are a very serious complication of blood transfusion. These infections continue to pose a great challenge to transfusion medicine, especially in Africa, due to a high transfusion demand.[5]

To analyze the impact of CMV transmission in blood transfusion, it is necessary to determine the prevalence of prior exposure to CMV among blood donors. This study aimed to determine the seroprevalence of anti-CMV IgG and IgM antibodies among blood donors in CBBKS. This study also aimed to investigate possible association between the donor's age, type ABO blood group, haematological parameters and CMV serological status.

MATERIAL AND METHOD

A cross-sectional study was conducted on 200 blood samples from donors attended to CBBK, taken over a period of three months from June 2016 to Aug 2016 at the Central Blood Bank in Khartoum state, Sudan. Parameters like Donor's age, sex, area and history of any previous disease were collected from individual blood donors.

The inclusion criteria for recruiting blood donors, as laid down by the Blood Bank were: age between 18 and 40 years; weight >55 kg; hemoglobin >13.5 gm/dl for males, 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. They were negative for HBsAg, HIV 1/2 antibody, HCV antibody, Malaria and Syphilis. Eligible donors were invited to participate in the study and their willingness sought, also This study was approved by Sudan ministry of health.

First of all 3ml of intravenous blood was taken by vein puncture technique from every donor for the confirmation of blood groups and also to check the hematological parameters like hemoglobin, WBCs count, RBCs count, Platelets count later on after detection of CMV-IgM seropositivity. Using 5ml syringes, for the collection of venous blood. The anticoagulant vacotainer was used is EDTA (ethylene, diamin, tetra, acetic acid) to check hematological parameters. All hematological parameters were checked by Sysmex KX-21 automated hematology analyzer by SYSMEX Corporation. Detection of blood grouping of all donors was done by forward typing (Biomed, Diagnostic, Egypt).

All completely healthy blood donors were then tested for CMV IgG and IgM antibodies by ELISA (Fortress Diagnostic Ltd, United Kindom). Enzyme Linked Immunosorbant Assay (human Elysis UNO, Germay) was performed manually using a 96 well micro titration plate. The principle of ELISA depends on using the pre-coated microtiter wells with purified CMV antigens that react with diluted sample sera after they are added to the wells. If they contain CMV IgG & IgM specific antibodies, these will bind the Ag in the wells. The unbound antibodies are removed by washing after incubation time which is 30 minutes, and then enzyme conjugate added to bind Ag-Abs complex, the excess amount is washed after incubation (30 min). After that the substrate solution immediately added to the wells, and then incubated for 10 minutes. The reaction is then stopped by adding high acidity. The result of color is read photometrically, and then the reading of samples is compared with the control and calibrated.

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.

The data was analyzed using SPSS program. The chi-square test was used to investigate possible association between the donor's age, blood group, hematological parameters and presence of CMV antibodies. Values of $P \leq 0.05$ were considered statistically significant.

RESULTS

All of the 200 healthy individuals selected for blood donation were male and none of them was female, the Age of individual range between (20-40 years) mean age of the individuals was 29 years.

Out of 200 blood donors 84.5% (n=169) were positive for CMV IgG antibodies, while 4.0% (n=8) were positive for CMV IgM antibodies. No statistical significant value

on seroprevalence for CMV infection (P value > 0.05) see (Table 1).

Table: 1 Seroprevalence of CMV infection among blood donors

Variables	Number	Percent
IgM(n=200)		
Positive	8	4%
Negative	192	96%
IgG(n=200)		
Positive	169	84.5%
Negative	31	15.5%

*P value (>0.05).

Distribution of blood donors according to residence, blood group and age

Each blood donor was tested for his blood group using forward grouping technique. Out of a total of 200 completely healthy blood donors, 60.0% had blood group (O+ve), 22.0% blood group (A+ve), 15.0% blood group (B+ve) and 1.5% had (AB+ve) blood group, 98.5% of the blood donors were Rh +ve, while only 1.5% were Rh –ve which were involved only in group (O). All details are show belong in (table 2).

Table: 2 distribution of total Blood Donors based on their residence, blood group and age group

Variables	Number	Percent
Residence (n=200)		
Khartoum	58	29%
Omdurman	45	22.5%
Bahri	67	33.5%
ShargAlneel	30	15%
Blood groups(n=200)		
(A+ve)	44	22%
(B+ve)	30	15%
(O+ve)	120	60%
(AB+ve)	3	1.5%
(O-ve)	3	1.5%
IgM(n=200)		
Positive	8	4%
Negative	192	96%
IgG(n=200)		
Positive	169	84.5%
Negative	31	15.5%
Age group (Years)(n=200)		
(≤30)	122	61%
(>30)	78	39%

CMV IgG Seropositivity

Out of 200 blood donors one hundred sixty nine individuals were seropositive for IgG antibodies giving an overall percentage of 84.5% indicating old infection to the blood donors (Table 1).

Association of Seropositivity IgG with Age

53.5% of the blood donors in the age between 20 to 30 years (n=107) were seropositive for CMV IgG as against 31.0% in 31 to 40 year age group (n=62). There was no statistically significant difference seen in the CMV IgG status (P value > 0.05) in different age groups (Table 3).

Association of Seropositivity IgG with Blood Groups

In the IgG seropositive donors out of total 200 blood donors one hundred two belong to blood group "O" making 51.0%, it is show big value than the other groups, may that due to that the group "O" is the most spreading group in population, statistically there was no significant value associated with blood groups (P value > 0.05), see (table 3).

Association of Seropositivity IgG with residence

Out of total 200 blood donors fifty one belong to Khartoum (25.5%), while in Omdurman is thirty nine (19%), fifty five belong to bahri (27.5%) and the less one is in Sharg Alneel which is twenty four making (12%). There was no statistical significant value (P value > 0.05) see (table 3) below.

Table 3: IgG status of blood donors in different variable

Variables		IgG		Chi square p value
		Positive	Negative	
Residence (n=200)	Khartoum	51	7	0.695*
		25.50%	3.50%	
	Omdurman	39	6	
		19.50%	3.00%	
	Bahri	55	12	
		27.50%	6.00%	
	ShargAlneel	24	6	
		12.00%	3.00%	
Blood groups(n=200)	(A+ve)	36	8	0.660*
		18.00%	4.00%	
	(B+ve)	27	3	
		13.50%	1.50%	
	(O+ve)	102	18	
		51.00%	9.00%	
	(AB+ve)	2	1	
		1.00%	0.50%	
	(O-ve)	2	1	
		1.00%	0.50%	
Age group (Years)(n=200)	(<=30)	107	15	0.117*
		53.50%	7.50%	
	(>30)	62	16	
		31.00%	8.00%	

- *.P value > 0.05 that's considered as statistically insignificant.
- **.P value < 0.05 that's considered as statistically significant.

CMV IgM Seropositivity

In case of CMV IgM out of 200 blood donors only eight individuals were seropositive for IgM antibodies giving an overall percentage of 4.0% indicating primary or acute infection to the blood donors (Table 1).

Hematological Parameters of Donors with CMV- IgM Seropositive

IgM seropositive blood donors were tested for various hematology parameters such as Hemoglobin (HB), RBC count, WBC count, neutrophil, lymphocyte, mix and platelets to check the health status. As given in the (Table 4) mean RBC Count was 5.11 million/cmm, mean hemoglobin was 14.6 g/dl, mean WBC Count was 5075/cmm and mean platelet count was 285,38/cmm as tested by the Sysmex Automatic Hematology Analyzer. Other calculated parameters such as neutrophil, lymphocyte and mix were also within acceptable limits as shown in the (Table 4).

Table 4: Mean values of different hematological parameters of CMV-IgM seropositive.

Descriptive Statistics	Minimum	Maximum	Mean	Std. Deviation
RBCs (Million/cmm)	4	6	5.11	0.38
HB (g/dl)	13.7	15.6	14.625	0.63864
WBCs (Per cmm)	4500	6000	5075	570.088
Lymphocyte (×10ˣ9)	1	2	1.41	0.181
Neutrophil (×10ˣ9)	2	4	2.68	0.483
Mix (×10ˣ9)	1	2	1.38	0.373
PLT (Per cmm)	200	360	285.38	55.649

Association of Seropositivity IgM with Age

In case of CMV IgM there is 1.0% (n=2) in age group 20 to 30 years and 3.0% (n=6) blood donations in age group 31 to 40 years, were positive for CMV IgM showing an active infection in the donors. There was statistically significant difference in the CMV IgM status (P value < 0.05) in different age groups (Table 5).

Association of Seropositivity IgM with Blood Groups

In the IgM seropositive donors out of total 200 blood donors there was four making 2.0% belong to blood group "O+ve" which is maximum one, the other groups show less value than group "O" (Table 5).

Association of Seropositivity IgM with residence

Out of total 200 blood donors there was one belong to Khartoum, Omdurman and Sharg Alneel making (0.5%), while the maximum number was found in Bahri which

are five (2.5%). Statistically there was no significant value (P value > 0.05) (see table 5).

Table 5: IgM status of blood donors in different variable

Variables		IgM		Chi square p value
		Positive	Negative	
Residence(n=200)	Khartoum	1	57	0.351*
		0.50%	28.50%	
	Omdurman	1	44	
		0.50%	22.00%	
	Bahri	5	62	
		2.50%	31.00%	
	ShargAlneel	1	29	
		0.50%	14.50%	
Blood groups(n=200)	(A+ve)	2	42	0.133*
		1.00%	21.00%	
	(B+ve)	1	29	
		0.50%	14.50%	
	(O+ve)	4	116	
		2.00%	58.00%	
	(AB+ve)	0	3	
		0.00%	1.50%	
	(O-ve)	1	2	
		0.50%	1.00%	
IgG(n=200)	Positive	0	169	0.00001**
		0.00%	84.50%	
	Negative	8	23	
		4.00%	11.50%	
Age group (Years)(n=200)	(≤30)	2	120	0.003**
		1.00%	60.00%	
	(>30)	6	72	
		3.00%	36.00%	

- *.P value > 0.05 that's considered as statistically insignificant.
- **.P value < 0.05 that's considered as statistically significant.

DISCUSSION

As results shown in our study that 84.5% of the blood donors were positive for CMV IgG antibodies, indicating past exposure of infection while 4.0% were positive for CMV IgM antibodies indicating primary infection in blood donors.

This high seroprevalence in Sudan and is in agreement to Western literature which describes seroprevalence in voluntary blood donors ranging from 38% to 75% in different parts of the world.[10] 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. In our study, our results were in agreement with those previously reported among blood donors by Hiba et al (2016) in Khartoum (75%), Pennap et al (2016) in Nigeria (74%).

The variation of Seroprevalence of CMV-lgG antibodies between different countries may be due to the differences in socioeconomic levels in the study areas. People who come from lower socioeconomic areas show a higher HCMV-lgG seroprevalence than do people from an upper or middle income levels[11], when we compare our result with those previous result we found that the prevalence of this infection was increased during last year, perhaps that due to decrease in socioeconomic in Sudan and degradation of health situation which lead to this consequences.

In our study, the seroprevalence of CMV among the blood donors varied with age ranging from 30% in the 30-40 year age group to 70% in the 20-30 year age group, i e the range is decrease following by increasing of age, this similar to previous study by Udomah FA et al (2016). There is no statistically significant difference in seropositivity of CMV IgG based on distribution of age, (P value >0.05), while there is significant value in seropositivity of CMV IgM based on distribution of age only (P value<0.05), However no previous study or report examined the effect of the major blood groups on CMV infection and transmission., so this study revealed no effect of the ABO, Rhesus blood groups and the residence on the target group of CMV IgG or IgM prevalence, that indicate no direct association of these factors to CMV infection and transmission.

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[12], unfortunately These protocol are not mandatory or recommended in Sudan. These guidelines have helped in eliminating transfusion-induced CMV infection syndrome in pre-term infants in the West America.[13] 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.[13]

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.[14][15][16]

CONCLUSION

In summary, based on the result in our study and reports of previous studies in Sudan among blood donors and other target groups, the Cytomegalovirus (CMV) shown to be highly endemic and common in Sudan and is also known to be a significant cause of morbidity and mortality following blood transfusion in children and immunocompromised adults. Some factor have potential effect on HCMV infection.

There is need to routinely screen blood donors for CMV infection particularly for blood donors in Sudan. These may be more appropriate and cost-effective in the Sudan scenario for the prevention of transmission of CMV through infected blood to immunosuppressed individuals, The use of leukoreduction filtration, saline-washed RBCs, frozen deglycerolized RBCs, etc for transfusion should be conducted in Sudan, also more studies in the Sudan 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.

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