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Cytomegalovirus infection among Blood Donors in Usmanu Danfodiyo University Teaching Hospital Sokoto, Nigeria.

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ABSTRACT

The aim of this study was to determine the ABO and Rhesus D Blood group and CMV Status of blood donors in Usmanu Danfodiyo University Teaching Hospital, Sokoto. Two hundred and ninety (290) consecutively –recruited blood donors, consisting of 287 males and 3 females aged 19 – 55 years with mean age 39 ± 21 years were screened for their ABO and Rh D blood group and CMV antibodies status using the Lorne Laboratories (UK) antisera and CTK Biotech (U.S.A.) Onsite CMV IgG/IgM Duo rapid test for CMV-IgG and IgM detection. The result shows that 57.9% of the donors were CMV positive for IgM only, 3.1% were positive for both IgM and IgG and 4.82% were positive for IgG only while the remaining 34.18% were negative. The prevalence of CMV positivity was higher among blood group O donors (30.7%) compared to blood group A (16.2%), B (15.5%) and AB (0.34%). CMV positivity was higher among blood donors in the 25-29 years age group (16.6%) compared to those in the 19-24 years (16.2%), 30-35 years (12.1%), 36-40 years (10.3%), 41-45 years (8.7%), 46- 50 years (1.40%) and 51-55 years age group (0.70%). This present study indicates mild endemicity of CMV infection among blood donors in Sokoto, Nigeria. There is need to routinely screen blood donors for CMV particularly for donor units intended for use in neonates, pregnant women, AIDS patients, immunosuppressed and transplant patients. The use of leucocyte- rich whole blood transfusion should be discouraged in Nigeria. Effort should be made to implement universal leucodepletion of donated units. There is also the need to educate clinical staff to ensure that CMV negative units are requested for patients in whom CMV negative units are indicated.

Keywords: Prevalence, Cytomegalovirus, Blood Donors, Usmanu Danfodio University Teaching Hospital, Sokoto, Nigeria.

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INTRODUCTION

Human cytomegalovirus (CMV) affect individuals across a diverse geographical and socio-economic groups. CMV ria a common congenital transfusion-transmissible viral infection particularly among immunocompromised patients[1]. It is an important public health problem and causes a significant morbidity and mortality in infected neonates, transplant patients, immunocompromised patients and patients with AIDS[2.]Although a transfusion transmissible infection, blood donors are not routinely screened for CMV in Nigeria. Other trnsfusion transmissible viral infections include; Hepatitis B and C, and human immunodeficiency virus (HIV) types 1 and 2 [2.] The risk of transmitting these infection through blood transfusion has been reduced significantly because blood donors are routinely screened for these agents and only donor units who test negative are transfused[3]. This is however not the case for CMV. It has been reported that most adults across the globe are seropositive for CMV [4-5.] Depending on the socioeconomic status, seropositivity for adult, population over forty years ranges from 60% to 100% possibly as result of transmission through intrauterine (or at parturition), breastfeeding, blood transfusion, sexual contact and spread from children [3,6]. It tends to be lower in developed countries than the developing countries [6].

In the majority of transmission, the infection is asymptomatic in those who developed primary infection following transfusion; only a few percent develop mononucleosis –like syndrome [3-6] weeks after transmission. A previous report among 76 CMV negative children with cancer receiving transfusion, indicated that that 1.7% were asymptomatic and community acquired [7]. The risk of transfusion transmissible CMV infection has been shown to be significantly high among multi transfused preterm children who weigh < 1200g delivered of seronegative mothers[7]. Transfusion transmissible CMV infection is potentially a clinically significant viral infection particularly among CMV negative organ transplant recipients [8].

CMV infection is associated with a variety of pathological conditions including retinitis, pneumonia, hepatitis, encephalitis etc. It is also a common cause of febrile illnesses and graft rejection in some transplant patients[9]. CMV can spread through sexual contact with infected individual; it can also be passed from mother to infant in utero as well as from cervical secretion at birth and during breast feeding in which the mother could have acquired it as acquired transfusion transmitted CMV infection during blood transfusion [10]. Transfusion transmitted –cytomegalovirus (TT-CMV) is a significant cause of morbidity and mortality in the immunocompromised host. The risk of TT-CMV from seropositive blood donor is reported to be 0.4 to 12% 10. In the developed

world, the implementation of stringent donor eligibility criteria and more sensitive methods for viral genome detection have virtually eliminated TT-CMV infection. However in developing countries, the risk as yet considerable[10]. There is paucity of data on the prevalence of CMV infection among donors. CMV negative blood is indicated in immunocompromised individuals (pregnant women, children, AIDS patients and organ transplant patients). The aim of this study is to determine the CMV status of blood donors in Sokoto. The aim of this research is to determine the prevalence of CMV infection among blood donors in Usmanu Danfodiyo University Teaching Hospital Sokoto.

MATERIALS AND METHOD

Description of the Study Area

Usmanu Danfodiyo University Teaching Hospital, Sokoto is located in Sokoto State in the extreme North West of Nigeria, between longitude4^{0}8'and 6^0 54' and between longitude 120N and 13^0 581N. Sokoto is located in the Sudan savannah of North-Western Nigeria. It covers a land area of about 60.33Km^2. It has a mean annual rainfall of 500-1300mm. The rainy season is from June to October during which showers are a daily occurrence. There are two major seasons, wet and dry which are distinct and are characterized by high and low malarial transmission respectively. The state share border with the Republic of Niger to the North, Kebbi State to the West and South East and Zamfara State to the East. Based on 2007 National Population Commission census, the state has a population of 3.6million[11].

Study Population

The study population consisted of consecutively- recruited blood donors that came to donate blood at the Haematology /Blood Group Serology Laboratory of Usmanu Danfodiyo University Teaching Hospital, Sokoto.

Study Design

This was a prospective study which aimed to determine the prevalence of CMV antibodies among blood donors in Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto.

Sample Size

The study population consisted of 290 blood donors who presented to the Usmanu Danfodiyo University Teaching Hospital, Sokoto, comprising of 287 males and 3 females aging between 18 and 60 years.

Inclusion Criteria:

Inclusion criteria included age (≥ 18 years), meeting the haemoglobin requirement to donate blood, residence in Sokoto, without history of recent blood transfusion in the last 4 months, non-menstruating (female) and willingness to offer a written informed consent to participate in the study.

Exclusion Criteria:

Donors who were < 18 years, anaemic, non-resident in Sokoto, who has had a blood transfusion in the last 4 months, menstruating females and non-consenting blood donors were excluded from participating in this study.

Sample Collection

About five milliliters (5ml) of venous blood was collected from each of the study subjects, two milliliters (2ml) of which was placed into Ethylene Diamine Tetra Acetic Acid (EDTA) tube. The remaining was placed in to plain container (for CMV antibodies screening and for blood grouping).

METHODOLOGY

ABO Blood Grouping

Principle. Antigens present on the surface of RBC (A, B and D), reacts with corresponding antibodies present in anti-sera (anti-A, anti-B and anti-D) forming a visible agglutination [12].

Procedure

1. A drop of anti-A, anti-B, anti-D and the donor serum (negative control) were placed in to separate test tube.
2. An equal volume of 3% of donor's blood was then added to each tube.
3. The mixtures were mixed by tapping the bottom of the tube.
4. Agglutination was then examined macroscopically after 5 min at room temperature.

CMV Anti-Bodies Screening (IgM/IgG)

The Onsite CMV IgG/IgM Duo rapid test is a dual lateral flow chromatographic immunoassay panel device. The test cassette consist of CMV-IgG detection panel and CMV-IgM detection panel. The IgM and IgG has a burgundy colored conjugates pad containing mouse anti-human IgM conjugates with colloid gold, a nitrocellulose membrane strip containing a test band (T-band) and a control band (C-band), The T-band is pre coated with recombinant CMV antigen, and the C- band is pre coated with goat anti-mouse IgG. When an adequate volume of test specimen and buffer are dispensed in to the sample well(S) on the cassette, the specimen migrates by capillary action across the cassette. The IgM or IgG antibodies if present will bind to the antigen conjugates forming a

burgundy colour at both the T-panel and C-panel. Absence of any T- band but with C-band suggests a negative result.

Procedure

1. A drop of donor serum was dispensed in to the sample well on each panel of the cassette.
2. A drop of sample diluent (Buffer) was then added.
3. The result was then read in 15 minutes time.

Control

The positive and negative control provided with the kits was tested first before analyzing the donor samples.

Statistical Analysis

Statistical analyses was carried out using the SPSS statistical software (Version 18). Comparisons between the blood donors was done using the Student's t-test and Mann-Whitney test for parametric and non-parametric data respectively. In all statistical comparisons, a p-value of < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

In this research work, a total of 290 donors comprising of 98.97% males and 1.03% females of aged 19-55 years, were screened for anti-CMV IgM and IgG. Among the blood donors screened, 65.82% were positive while the remaining 34.18% were negative for CMV infection. Table 1 show that 57.90% were positive for IgM only, 3.10% were positive for both IgM and IgG and 4.82% were positive for IgG only. The prevalence of CMV among blood donors based on their blood group indicated a CMV prevalence of 16.20% among blood group A, 15.5% among group B, 0.34% among group AB and 33.79% among blood group O donors. CMV negative status was evident in 7.60%, 9.31%, 0.34% and 20.34% of blood group A, B, AB and O donors respectively as shown in table 2. The distribution of donors based on the ABO and Rhesus D blood groups indicated that blood group O had the highest prevalence (51.03%) while the lowest prevalence (0.35%) was observed among blood group AB donors. The prevalence among of blood group A and B were 23.80% and 24.82% respectively. The Rhesus D blood grouping of the study population indicated that 95.20% were Rhesus D positive while the remaining 4.8% was Rhesus D negative as shown in table 3. The prevalence of CMV antibodies among the different blood group showed that the highest prevalence of CMV infection was among blood group O donors (30.70%) while lowest prevalence was among blood group AB (0.34%) donors as shown in table 4. The distribution of CMV infection was compared based on the age group of blood donors. The highest

prevalence was observed among donors in the 25-29 years (16.60%) compared to 16.20%, 12.06%, 10.34%, 8.70%,1.40% and 0.70% respectively in the 19-24, 30-35, 36-40, 41-45, 46-50 and 51-55 years age groups as shown in table 5.

Table 1: Anti-CMV IgM and anti IgG status among blood donors in UDUTH.

CMV Status	Number	Anti-CMV IgM(%)	Number	Anti-CMV IgG(%)		IgM and IgG(%)	Total (%)
Positive	168	57.9	9	3.10	14	4.82	65.82
Negative	122	42.1	281	96.9	276	95.18	34.18
Total	290	100	290	100	290	100	100

Table 2 Distribution of Blood Group Antigens in the study population.

ABO Blood Group						Rhesus Blood Group		
Blood Groups	A	B	AB	0	Total	Rh^+	$R\bar{h}$	Total
Number	69	72	01	148	290	276	14	290
Percentage(%)	23.80	24.82	0.35	51.03	100	95.20	4.80	100

Table 3: Prevalence of various blood groups (ABO& Rh) in the study population.

Blood group	A^+	A^-	B^+	B^-	AB^+	AB^-	O^+	O^-	Total
Blood Donor	640	5	66	6	1	0	141	3	290
Percentage(%)	22.10	1.72	22.80	2.10	0.34	0	50.0	1.03	100

Table 4: Prevalence of CMV among blood group types.

Blood groups	Seropositive CMV anti bodies (%)	Seronegative CMV antibodies (%)	Total
A	47(16.21)	22 (7.59)	69 (23.79)
B	45 (15.52)	27 (9.31)	72 (24.83)
AB	1 (0.35)	0 (0.00)	1 (0.35)
O	98 (33.79)	50 (17.24)	148 (51.04)
Total	191 (65.82)	99 (34.18)	290 (100.00)

Table 5: Distribution of CMV seropositive among different age group

Age group (years)	No tested	Positive CMV-Antibodies (%)
19-24	59	47 (16.20)
25-29	76	48 (16.55)
30-35	61	35 (12.06)
36-40	46	30 (10.34)
41-45	38	25 (8.62)
46-50	6	4(1.37)
51-55	4	2 (0.68)
Total	290	191 (65.82)

Transfusion transmitted Infections (TTI's) are a major challenge to the transfusion services particularly in developing countries. The presence of CMV in blood of asymptomatic donors is the major risk factor for transmitting CMV through blood transfusion. In this present study, we investigated the prevalence of CMV infection among our cohort of 290 consecutively-recruited

blood donors in Usmanu Danfodiyo University Teaching Hospital in Sokoto North Western Nigeria.

This study indicated that 191 (65.82%) blood donors were positive for CMV antibodies. A total of 168 (57.90%) were positive for IgM, 9 (3.10%) were positive for both IgM and IgG and 14 (4.82%) were positive for IgG only. Our observed prevalence of 57.90% positivity for CMV IgM antibody is higher than previously reported by Akinbami and colleagues [13] in Nigeria (19.5%) and Adjei and colleagues [6] among Ghanaian blood donors (3.1%). Similarly, a previous report among 400 participants who were recruited from blood donors at the National Blood Transfusion Service at the Kenyatta National Hospital (KNH) immunology laboratories in Kenya observed anti- CMV IgG and IgM positivity of 97.0% and 3.6% respectively. Females had a higher prevalence of CMV antibodies [14]. A retrospective study was done on 5600 serum samples stored frozen in a repository for CMV antibodies using the ELISA technique. Four cases (0.071%) out of 5600 samples were positive for anti-IgM CMV 15. A prospective study was conducted among 431 voluntary blood donors who were screened for CMV IgG and IgM by EIA in India. A total of 379 (87.9 %) voluntary blood donors were seropositive for CMV IgG and 7 (1.6%) were CMV IgM positive [16].

The high seroprevalence rate observed among adult blood donors in this study is comparable to the rates (97% and 96%) reported in Tunisia and India, respectively [17-18]. Our observed prevalence is lower than prevalence rates of 92% obtained in separate study previously carried out in Jos Nigeria [19]. Similarly, study carried out in Ghana and India showed prevalence rates of 93.2% and 95% respectively [20]. The differences in the prevalence of CMV infection among blood donors from different parts of the world may be to differences in methodologies, socioeconomic, environmental or climatic factors. Countries with high CMV seroprevalence may have difficulties maintaining adequate supplies of CMV-seronegative products [21]. A previous report in India that investigated the prevalence of CMV among 200 voluntary blood donors indicated a zero percent prevalence for CMV IgM antibody and 95% prevalence for CMV IgG antibody [22]. Similarly a World Health Organization (WHO) reported CMV prevalence ranging from 40% to 100% in highly industrialized and developing nations respectively [20.] Report from western literature indicates that the seroprevalence of CMV in voluntary blood donors ranges from 38% to 75% [23]. In the developed world, it is best practice to transfuse high risk individuals with donor units that have been screened and found to be seronegative for CMV frozen red blood cells that has been deglycerolized[24]. These evidenced-based best practice has significantly reduced the risk of transfusion-transmitted CMV infection particularly among immuno compromised patients in the developed world.

It is recommended that in a tertiary care hospital, blood units to be transfused to neonates, organ transplant recipients, pregnant women, those suffering from malignancies and other immunocompromised patients should be screened for anti-IgM CMV or preventive strategies like universal leucodepletion be implemented to decrease the risk of transmission of CMV in these groups of patients [25-26]. Active infection can result in serious morbidity and mortality, and many consider CMV to be one of the most serious pathogens affecting immunosuppressed individuals and an important contribution to intrauterine infection [27-29]. It is a huge challenge for blood banks to provide CMV seronegative blood products for high risk patients particularly with the high prevalence of CMV among blood donors in most countries. Some of the effective ways to prevent CMV transmission in high-risk patients is to transfuse them with units that have been screened and found to be seronegative or leucodepleted blood products [30-31]. This strategy has significantly reduced the rate of TT-CMV [32-34]. It is good practice to maintain a stock of CMV-seropositive and CMV-seronegative units. This is to allow for the selection of CMV negative units for patients in whom it is indicated (neonate, pregnant women, patients with acquired immune deficiency syndrome (AIDS), patient on myelosuppressive cancer therapy and organ transplant recipients). Although maintenance of dual inventories may be expensive, it can become an alternate methods for the provision of "CMV safe" blood products including the use of leucoreduced blood products as ways to prevent TT-CMV infection after marrow transplant [35]. Leukoreduction using white blood cell (WBC) filters has been widely implemented in blood facilities in the developed economies to help reduce the side effects of residual WBCs in blood components such as febrile reactions or alloimmunization against WBC antigen. Leukoreduction under good manufacturing practices could also abrogate the transmission of WBC-associated virus such as CMV, Epstein-Barr virus, or human T cell leukemia virus. Thus, leukoreduced blood components have been advocated as an alternative transfusion practice for patients at risk for CMV when seronegative blood is unavailable, although whether leukoreduced blood is as safe as seronegative blood in terms of TT-CMV risk remains a matter of debate. However, a more recent study has demonstrated that TT-CMV does occur even after leucoreduction, that CMV-seronegative blood products may thus be superior to leucoreduced blood products, and cautioned against the premature abandonment of the use of CMV-negative inventories particularly for the populations at high risk of and vulnerable to CMV disease for an era of "universal" leucoreduction [36].

In this study, majority of the donors were males (287 males and 3 females), of the three women blood donors, 1 (0.34%) was found CMV seropositive. Previous report among 400 participants were recruited from blood donors at the NBTS at the Kenyatta National Hospital (KNH)

immunology laboratories and the NBTC in Kenya observed anti- CMV IgG and IgM positivity was 97.0% and 3.6% respectively. Females had a higher prevalence of CMV antibodies [14].

This study indicated that CMV infection was more prevalent among blood group O Rhesus D positive donors (51.03%) and least among group AB Rhesus D positive donors (0.35%). The gene frequencies with respect to ABO blood group system in this study was found to follow the pattern (O> B> A> AB) indicating a preponderance of phenotype B over A. Our finding is consistent with a previous report by Erhabor and colleagues among blood donors in Gusau Zamfara State [37], by Akhigbe and colleagues 38 among students of Ladoke Akintola University of Technology in Ogbomoso and by Pennap and colleagues 39 among students of Nasarawa State University. Similarly our finding is also consistent with previous result obtained among Guinean population in which the prevalence of A, B and O phenotype in the population was 14.70, 15.48, 69.83 respectively 40. Our finding is however at variance with previous reports in other parts of Nigeria; Erhabor and colleagues [41], Jeremiah [42], Worlledge and colleagues [43], Falusi and colleagues [44], Omotade and colleagues [45], Oluwadare and Shonekan 46 and in other previous reports in other parts of Nigeria [47-48] which observed a preponderance of phenotype A over B.

The distribution of CMV based on the age groups of blood donors indicated that the highest prevalence of CMV infection was observed among blood donors in the [25-29] years while the lowest prevalence were seen among blood donors in the 51-55 years age group. This finding is consistent with a previous report [19] in Jos, Nigeria which observed a peak CMV prevalence among their cohort of blood donors in the [25-29] years age group. Our finding is however at variance with a previous report which indicated that the prevalence of CMV infection increases with increasing age of blood donors [49.] Common sources of CMV infection include; sexual intercourse with an infected person, blood transfusion of infected unit, breastfeeding (from infected mother to suckling child), solid organ transplant and close non-sexual contact involving body fluids. The risk that CMV infection will lead to a clinical disease is however low except in immunocompromised subjects; preterm low birth weight neonates, some oncology patients and haematopoietic stem cell (HSC) transplant recipients can cause severe illness with substantial morbidity and mortality. The severity of clinical CMV infection correlates with the severity of cellular immunosuppression and with the CMV serological status of the patient and the blood, organ, or stem cells donor.

The American Association of Blood Banks has recommended transfusion from donors who are seronegative for CMV, or the use of deglycerolized frozen red blood cells (RBCs) for transfusion in a seronegative preterm (<1200 g) child born to a mother with negative or unknown immune status regarding CMV infection [50]. These guidelines have helped to eliminate transfusion-induced

CMV infection syndrome in preterm infants in the West. Other preventive strategies such as leukoreduction filtration, saline-washed RBCs, frozen deglycerolized RBCs are increasingly recommended to minimize the risk of transmission of CMV through blood transfusion [50]. These methods may be more appropriate and cost-effective in developing countries for the prevention of CMV transmission through infected blood to immunosuppressed individuals.

CONCLUSION AND RECOMMENDATIONS

This present study indicates mild endemicity of CMV infection among blood donors in Sokoto, Nigeria. There is need to routinely screen blood donors for CMV infection particularly for units intended for use in neonates, pregnant women, AIDS patients, immunosuppressed and transplant patients. The use of leucocyte rich whole blood transfusion should be discouraged in Nigeria. Effort should be made to implement universal leucodepletion of donated units. There is also the need to educate clinical staff to ensure that CMV negative units are requested for patients in who it is indicated.

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