

Studies on the Prevalence of Human Cytomegalovirus and Malaria Parasite Among Blood Donors in a Tertiary Health Institution in Nigeria

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Abstract

The study evaluated the prevalence of human cytomegalovirus (CMV) and malaria parasite among eligible blood donors at Madonna University Teaching Hospital, Nigeria, Elele River State. A total of two hundred subjects made up of one hundred and eight (54%) females and ninety-two (46%) males participated in the study. Blood samples were collected from the subjects and screened for CMV IgG and IgM using lateral flow chromatographic immunoassay and malaria parasite using standard microscopy. This study reported 0% prevalence of human cytomegalovirus infection among the subjects evaluated. Among the 200 subjects studied, 115(57.5%) were positive while 85(41.5%) were negative for malaria parasite. Among the positive subjects, 70(60.87%) were females while 45(39.13%) were males. Among the negative subjects, 38(44.71%) were females while 47(55.29%) males. With the overall prevalence of 57.5% for malaria parasite among blood donors, compulsory malaria parasite screening should form part of the transfusion transmissible infections screened in this locality to avert the deleterious effect of malaria on recipients. Proactive strategies of educating people on prevention and control measures should be instituted.

Keywords: Human Cytomegalovirus, Malaria Parasite, Blood Donors, Prevalence, Tertiary Health Institution, Transfusion-Transmitted Infections, Nigeria, Public Health, Screening, Coinfection.

Introduction

The administration of Blood is a lifesaving procedure and has greatly increased over the years. In every country, surgery, trauma, severe anaemia and complications of pregnancy are among clinical conditions that demand blood transfusion (WHO, 2011). Adequate blood supply can be achieved via blood donations, either as voluntary unpaid donation or recruited paid donation. Adequate screening of donated blood and blood components represent critical processes that must be followed to ensure that blood units are safe for human consumption.

Cytomegalovirus (CMV) is a DNA virus that belongs to a beta sub-group of herpes virus family. It is also known as β human herpes virus type 5. It is transmissible through blood transfusions, is an important cause of concern worldwide [1]. Other

routes of transmission are contact with infected body secretions, sexual contact, breast feeding, transplacental route or by transplantation of hematopoietic stems and solid organs from infected donors [2]. 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 [3, 4]. Like most other herpes viruses, CMV remains latent in the host after primary infection and persists for life in the organism. Latent infection is characterized by a state, where the viral genome is sustained within the cell with limited viral gene expression and no viral progeny is produced.

Nevertheless, these viruses can be reactivated in immunosuppressed individuals and can be an important cause of morbidity and mortality. Most studies suggest that 13-38% of immu-

nocompromised patients will contract CMV from transfusion of unscreened and unfiltered cellular blood components [4-6]. Human cytomegalovirus infection shows low pathogenicity in healthy immunocompetent individuals and is usually asymptomatic or only causes mild symptoms, which may manifest as mononucleosis-like syndrome. In these individuals, CMV infection can result in leukopenia, malaise, encephalitis, hepatitis, pneumonia, gastroenteritis, retinitis and even death. Therefore, the most effective way to minimize the risk of CMV transmission in high-risk recipients would be to administer CMV free blood products. 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. This test is not able to determine the exact time of infection. 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 [7, 8].

The transmission of malaria parasites by blood transfusion has been one of the first reported incidents of transfusion-transmitted infections [9]. Transmission of Malaria by blood transfusion is a significant public health problem especially in the malaria endemic regions of the world [10]. Malaria is a protozoan disease transmitted by the bite of infected female anopheles mosquitoes. It is the most important of the parasitic disease of humans with transmission in 108 countries containing 3 billion people and causing nearly 1 million deaths each year (Idro et al., 2010). Transfusion-transmitted malaria (TTM) was first described in 1911 [9]. Although the international policies recommended that blood for transfusion should be screened for transfusion-transmitted infections.

Transfusion-transmitted malaria can have serious consequences, as infection with *Plasmodium falciparum* may prove rapidly fatal [9]. The presence of Malaria in the blood may lead to fatalities when such blood is transfused especially into children under 5 years, pregnant women, serious blood loss in accident victims, and immuno-suppressive patients [10]. In developed countries, tests have been developed to detect *Plasmodium* antibodies and these are used for selected blood donors in various blood centres. They also use tests to detect *Plasmodium* antigens and nucleic acid amplification testing (NAT) for parasitic DNA (Reesink et al., 2010). Prevalence of transfusion-transmitted malaria varies from 0.6% to above 50% in Africa (Owusu-Ofori et al., 2013). Malaria is highly endemic in Nigeria. In fact, Nigeria has more reported cases of deaths due to malaria than any other country in the world (CDC, 2012).

In 2016, there were an estimated 216 million cases of malaria in 91 countries, an increase of 5 million cases over 2015. Thirty countries in Sub Saharan Africa account for 90% of global malaria deaths. Almost 1 out of 5 deaths of children under 5 in Africa are due to malaria [7]. Nigeria is currently a malaria endemic country with its entire population at risk of contracting malaria. Malaria is a major public health problem in Nigeria where it accounts for more cases and deaths than any other country in the world. There is an estimated 100 million malaria cases with over 300,000 deaths per year in Nigeria. This compares with 215,000 deaths per year in Nigeria from HIV/AIDS. Although, most attention has been paid to viral infections, a number of hospitals

do not carry out malaria parasite test even though it causes death in a large population. The risk of transfusion transmitted malaria (TTM) is not only present in highly endemic countries but also occur in malaria free countries. Current measures to exclude potential infected donors mainly rely on donor interviews but the effectiveness of this is being debated [11]. The purpose of this study was to conduct a meta-analysis in order to define the rate of CMV seropositivity and malaria parasite infection among the blood donors and suggest a better way to limit transmission to recipients. Screening of blood donors for malaria as recommended by World Health Organisation is currently not included in the protocols of many Nigerian blood banks. There is however paucity of information on the prevalence of malaria parasite in donated blood at moment; thus creating a gap for effective campaign for malaria screening of donor's blood before transfusion. Considering the fact that Madonna University Teaching Hospital is situated in the riverine area with much stagnant water around and the humid environment that encourages mosquito breeding, there was a need for this research.

Materials and Methods

Research Design

This research is completely randomized experimental study to determine the prevalence of human cytomegalovirus and malaria parasite among blood donors in a tertiary health institution in Nigeria.

Study Area

This study was carried out in Madonna University Teaching Hospital Elele, River State, Nigeria. It is a private tertiary health institution located in the southern region of Nigeria between two cities; Owerri and Port Harcourt and is easily accessible. Elele town is surrounded by other towns and villages which include; Isiokpo, Ndonii, Omagwa, Ahoda, Omoku and others. The predominant occupation of the inhabitants is farming and petty trading

Ethical Approval

The study was approved by the ethics committee of Madonna University Teaching Hospital. Informed consent was obtained from all participants before the commencement of the work.

Selection Criteria for Subjects

The subjects were apparently healthy male and female commercial donors aged between 18-55 years. Subjects must have not taken anti-malarial drugs for the past two months. Those who refused consent were excluded.

Samples Collection Techniques

Blood samples were obtained from the subjects using standard venipuncture technique previously described by Eledo, et al [5]. Approximately 3mls of blood was collected from each subject and dispensed into sterile EDTA (Ethylenediamine Tetra Acetic Acid) bottles containing 1.5mg/ml of blood of the anhydrous salt, mixed thoroughly to avoid clotting and then labelled

Methodology

Method: Giemsa Staining Technique

Principle:

Giemsa stain is a differential stain and contains a mixture of Azure, Methylene blue, and Eosin dye. It is specific for the phos-

phate groups of DNA and attaches itself to where there are high amounts of adenine-thymine bonding. Azure and eosin are acidic dye which variably stains the basic components of the cells like the cytoplasm, granules etc. Methylene blue acts as the basic dye, which stains the acidic components, especially the nucleus of the cell. Methanol acts as a fixative as well as the cellular stain. The fixative does not allow any further change in the cells and makes them adhere to the glass slide

Procedure

- Using the blood samples, thick films smears were made on clean grease-free slides and allowed to air-dry
- The smears were then flooded with giemsa in 1 in 10 dilutions and allowed to stain for 10 minutes.
- The stained slide was washed off after 10 minutes and the slide kept in upright position for air-drying.
- After, the smears were examined using oil immersion objective (100 x objectives) with immersion oil, malaria parasite is counted per high power field and the density was graded as follows;
 - 1-10 parasites / 100 field: low density (+)
 - 11-100 parasites / 100 field: medium density (++)
 - 10 parasites / every field: high density (+++)

Cytomegalovirus

Kit Supplied by: CTK Biotech Incorporated USA

Lot Number: F 1211P7A00

Principle

The Cytomegalovirus IgG/IgM Rapid Test is a lateral flow chromatographic immunoassay. When an adequate volume of test specimen and sample diluents is dispensed into the sample well of the cassette, the specimen migrates by capillary action across the test strip. Anti-CMV IgG, if present in the specimen, will bind to the CMV conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti-human IgG forming a burgundy-colored G will bind to the CMV conjugates. The immunocomplex is then captured on the membrane by pre-coated anti-human IgM forming a burgundy-colored M line, indicating an anti-CMV IgM positive result. Absence of any test lines (G or M) suggests a negative test result. The test contains an internal control (C line) which should exhibit a burgundy-colored line of the immunocomplex of control line develops, the test result is invalid and the specimen must be released with another device.

Procedure

- The test strips were stored at room temperature of 15-30°C.

- The strip test and the already withdrawn plasma from EDTA bottle containing blood was brought to the table.
- The test is opened and the device is removed and placed on a clean flat place
- The specimen was labeled with the patient's ID number.
- The capillary tube was filled with plasma
- It was held vertically and dispensed in the centre of the device making sure there was no air bubbles.
- The timer was set. The result was read at 10 minutes.

Interpretation of Result:

For Positive Result:

- In addition to the presence of C line, if only the G line develops, the test result indicates the presence of anti-CMV/IgG. The result is anti-CMV IgG positive or reactive.
- In addition to the presence of the C line, if only the M line develops, the test result indicates the presence of anti-CMV IgM. The result is anti-CMV IgM positive or reactive.
- In addition to the presence of C line, if only the G and M lines develop, the test indicates the presence of anti-CMV IgM and IgG. The result is anti-CMV IgG and IgM positive or reactive.
- For Negative Result: One distinct pink to red will appear on the C line which indicates that anti-CMV antibodies are not obtained in the specimen and that is negative or non-reactive.

Statistical Analysis

Data analysis was performed using the SPSS version 20 and presented using Chi square test to show the prevalence of cytomegalovirus and malaria parasite among blood donors.

Result and Discussion

The comparison of the prevalence of Malaria Parasite between male and female Blood Donors in MUTH was shown in table 4.1. The result showed that 60.87% of the females were positive while 39.13% of the males were positive.

Figure 4.2 was a bar chart showing the overall prevalence of malaria parasite among blood donors. The prevalence of malaria parasite among the blood donors was 57.50%.

The overall prevalence of Cytomegalovirus among blood donors in MUTH was presented in table 4.3. The result showed that there was 0% prevalence of human cytomegalovirus among the study population.

Table 1: Comparison of Prevalence of malaria parasite between male and female blood donors in MUTH.

Gender	Positive cases		Negative		Total based on gender	Percentage based on gender	Chi square	p-value
	Number	Percentage	Number	Percentage				
Female	70	60.87	38	44.71	108	54.00	2.425	0.120
Male	45	39.13	47	55.29	92	46.00	2.723	0.099
Total	115	100	85	100	200	100	NA	NA
Chi square	NA	4.840	NA	1.000	NA	0.640	NA	NA
p-value	NA	0.028	NA	0.317	NA	0.424	NA	NA

Key: NA= Not Applicable

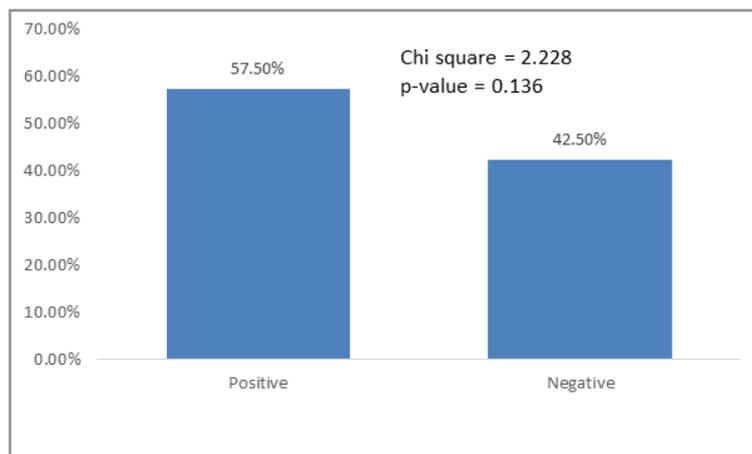


Figure 1: Overall malaria prevalence among blood donor in MUTH

Table 2: Prevalence of cytomegalovirus among blood donors in MUTH.

Gender	Positive cases		Negative		Total based on gender	Percentage based on gender	Chi square	p-value
	Number	Percentage	Number	Percentage				
Female	0	0.00	108	100.00	108	54.00	NA	NA
Male	0	0.00	92	100.00	92	46.00	NA	NA
Total	0	0.00	200	100.00	200	100	NA	NA
Chi square	NA	NA	NA	NA	NA	0.640	NA	NA
p-value	NA	NA	NA	NA	NA	0.424	NA	NA

Key: NA= Not Applicable

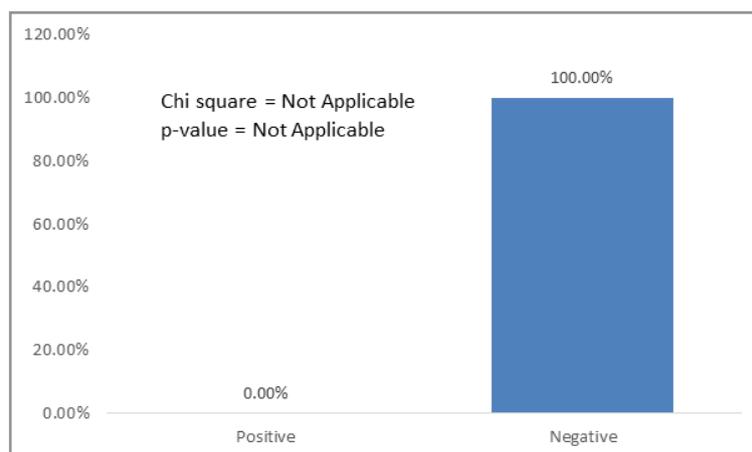


Figure 2: Overall cytomegalovirus among blood donors in MUTH.

Discussion

This work was carried out to investigate the prevalence of malaria parasite and cytomegalovirus among blood donors in Madonna University Teaching Hospital Elele River State (MUTH). The result from the study demonstrated the endemicity and occurrence of non-significant level of malaria parasite among blood donors in MUTH. Malaria have been accepted as a high risk in donated blood, prevalence level of these infections in them is employed globally as yardstick for evaluating the trend of such infection within the general population at high risk of getting infected with the parasite. Out of 200 blood samples examined in this study, 70(60.87%) of female and 42(39.13%) were affected from the study population, 108 female and 92 male.

A breakdown of the infections is presented in table 1. The age group recruited for the study was between 1 to 60 years. Highest

rate of infection of 50% was observed among the age group of 20 - 67 years (Figure 1).

Similar observation was recorded by Uneke et al., (2006). Larger population of donors were in this age- group which may be as a result of adventure or pecuniary gain. Plasmodium falciparum was the only Plasmodium species identified in the present study. The finding in the present study is similar to that of (pondei et al., 2012; Abah & Temple, 2015). This lays additional emphasis to the WHO's earlier report that Plasmodium falciparum is the most common of the four human malaria parasites across much of Sub-Saharan Africa (WHO, 2003). Present investigation revealed that out of a total of 200 blood donors screened for Plasmodium parasites 56(28.00%) haboured the parasites. The prevalence of 28.00% is relatively high and should be a cause for concern as recipients of these bloods are mostly pregnant

women, children under 5 years, accident victims and other immuno-suppressive patients. Transfusion transmitted malaria can have serious consequences, as infection with *Plasmodium falciparum* may prove rapidly fatal [9]. Again, this high prevalence calls for concern when compared with report from previous research by [12] who reported 10.2% and 12.5% by (Pondei et al., 2012) in the Niger Delta area as the prevalence in the present study is more than twice the value reported in each of their work. How be it, it agrees with (Oladeinde et al., 2014) who reported 27.5%. But lower than [13] 40.5% in south Eastern Nigeria and [14], 51.5% in Abakiliki. These variations may be as a result of what has been established earlier, that Prevalence of transfusion-transmitted malaria varies from 0.2 cases per million in non-endemic countries to 50 or more cases in per million in endemic areas. Also the prevalence of malaria parasitemia among blood donors had been reported to be as high as 55% in the highly endemic northern Nigeria [7]. However, WHO had observed that younger people in malaria endemic areas are more susceptible to malaria infections than the older people (WHO, 2003).

In cytomegalovirus, 200 voluntary blood donors were recruited in this study. Of these, males were 46.00% while females were 54.00%. CMV IgG seronegativity among male donors was 100.00% and 100.00% among female donors. There was no statistically significant difference in the prevalence of the viral infection between the sexes. Also, no correlation observed between the viral prevalence of infection and either history of blood transfusion or occupation. The 100.00% CMV IgG and IgM prevalence among eligible blood donors in this study is relatively normal 0.00%. This suggests that many people in the study area have not been exposed to the virus. It also means that all the eligible blood donors of MUTH can comfortably donate blood with respect to safety of CMV infection. The seronegative rate reported in this study is very normal than prevalence rates observed in previous studies carried out in other parts of Nigeria. It was 92% in Jos (Alao et al., 2008), 95.8% in Benin [3] and 96% in Lagos (Akinbami et al., 2009) prevalence. Lower rates were observed in Ogbomoso 25.8% [6]. Several studies on the prevalence of CMV IgG antibody in different parts of the world have also shown different rates. For example, Ghana 93.2% [15], Iran 94.82% and 92% [15-30], Turkey 97.2% (Mutlu et al., 2008) and Brazil 96.4% [5]. These differences might be as a result of different screening methods, environmental and climatic factors and socioeconomic status of the study populations [30-41]. In this study there was no statistically significant association between the viral IgG and IgM antibody prevalence, implying that the donors has no relationship with CMV infection. However it is in contrast with the work of Arun et al., (2012) where the seroprevalence rate was higher in males (76.03%) than females (23.97%). The overall prevalence for CMV IgG was 74% [42-46]. The prevalence of CMV IgG antibody based on sex was 73.9% in males and 74.2% in the females. Pennap et al, 2016 [47, 48].

Conclusion

The prevalence of malaria parasite infection among blood donors in MUTH is high from the present study [49-53]. Therefore, it is recommended that screening for malaria parasitemia be included in the routine investigation of potential blood donors in Nigeria [54-60]. This is because breaking the transmission chain from blood donors to recipients is another step at achieving that

desired goal of curbing malaria infections and reducing deaths due to malaria to near zero worldwide [61-65]. Alternatively, a curative dose of anti-malaria prophylaxis to all patients transfused with the infected blood should be advocated [66-70]. Also, the result of this study has shown that routine screening of blood donors for CMV infection in such a resource limited environment might not be feasible [71-75].

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