IgG Avidity Index in the Differential Diagnosis of Primary Cytomegalovirus Infection from Non-Primary Infection in HIV/AIDS Patients in Bida, Nigeria.

Omosigho O.P., Odedina E.O. Emumwen E.G.
Medical Microbiology Department, Federal Medical Centre Bida. Niger State, Nigeria.

Emumwen E.F

All correspondence to: Omosigho Omoruyi Pius Medical Microbiology Department,
Federal Medical Centre Bida. Niger State .Nigeria Email –omosighoop@gmail.com.

ABSTRACT

Cytomegalovirus (CMV) is a common opportunistic infection in immunocompromised individuals particularly HIV/AIDS. Avidity is the strength with which the IgG attached to antigen; it measures the length of time after primary infection. CMV IgG avidity assay seems to be one of the most accessible tools to differentiate between primary and non-primary CMV infection. This study was conducted to evaluate the significance of the utility of the relationship of IgG Avidity Index and IgM levels for differential diagnosis of primary Cytomegalovirus
infection from non-primary infection in HIV/AIDS patients in Federal Medical Centre Bida. Blood samples were collected from HIV positive patients on Highly Active Anti-Retroviral Therapy for IgM Enzyme Linked Immunosorbent Assay, all positive IgM samples were tested for IgG Avidity. Out of the three hundred and eighty five (385) HIV positive subjects, an overall prevalence of 19.8% IgM was found among patients with HIV in Bida. Out of the 77 IgM positive samples tested for IgG Avidity index, 12(15.6%) had high avidity while 65(84.4%) had low avidity. Low avidity indices indicate low maturation of IgG antibodies in blood caused by recent or acute primary CMV infection. There was a significant difference (P=0.001) between the utility of IgG avidity index and IgM in the differential diagnosis of primary Cytomegalovirus infection. Primary CMV infections by IgM ELISA alone was significantly overestimated as only 65(84.4%) out of the 77 CMV IgM positive are actually primary infection while the rest 12 (15.6%) are non-primary infections. The utility of IgG avidity ELISA significantly reduced the reported prevalence of primary CMV infection with IgM ELISA method in this study. IgG avidity index is a sensitive, specific and less expensive method for identification of recent CMV infection in HIV positive patients compared to more sophisticated and expensive molecular methods.

Keywords: IgG Avidity, Cytomegalovirus, HIV/AIDS, Primary infection, Bida, Nigeria.

INTRODUCTION

Cytomegalovirus a member of the human Herpes family of viruses, transmissible through blood component, transfusion is an important cause for concern worldwide this is because the majority of adults have serological evidence of previous infection. (Zhang et al., 1995). The prevalence of seropositivity of CMV in the adult population over 40 years of age worldwide are reported to be 60-100%, due to transmission through sexual contact, spread from children and breastfeeding (Helkeret al., 2004).

Like most other Heperviruses, CMV is latent in their host after primary infection and persists for life (Drobyskinet al., 1993). Nevertheless, reactivation in immunosuppressive individuals leads to end organ disease such as retinitis, encephalitis and interstitial pneumonitis (Manezet al., 1993). Acute infection of CMV leads to serious morbidity and mortality (Hillyeret al., 1990). CMV co-infection is a major cause of morbidity and mortality in individuals with depressed cell mediated immunity associated with Acquired Immunodeficiency Syndrome AIDS (Macheret al., 1983).

CMV is one of the most successful of human pathogens, since it can be transmitted both vertically and horizontally, usually with little effect on the host. CMV is shed in body fluids (e.g., saliva, urine, semen, breast milk). Transmission usually occurs from person to person, including through the
intrauterine route, but it can also be spread during transfusion or organ transplantation.

Globally, between 60 and 90% of the general population is infected with CMV, with generally higher rates in developing countries (Rowshaniet al., 2005).

The functional binding affinity of anti-CMV IgG antibodies increases progressively over time after immunity by infection; it is otherwise referred to as maturation of the humoral immune response. Low IgG avidity indices may indicate primary infection whereas high avidity indices indicate non-primary infection (Bonalumiet al., 2011).

The CMV-IgG avidity ELISA has also been successfully used to diagnose CMV in children. Vilibic-Cavleket al in 2011 evaluated the value of IgG avidity in diagnosis of CMV infection in newborns and infants. Their analysis of 40 serum samples from under 1 year old infants with suspected CMV infection. 13 (32.5%) of the subjects were seropositive to anti-CMV IgM, 3 (7.5%) had equivocal IgM antibodies result, and 24 (60.0%) patients had IgG antibodies only. With IgG avidity ELISA, CMV infections (with low avidity indices) were reported in 61.5% IgM positive and 54.2% IgM negative infants. IgG avidity distribution across age of infants demonstrated recent primary CMV infection in 58.8% patients younger than 3 months compared with 91.7% and 81.8% in 3–6 and 6–12 months old infants respectively. The study showed that IgG avidity ELISA results in children older than 3 months of age was significantly useful for CMV diagnosis regardless of their IgM result. (Vilibic-Cavleket al., 2011).

Primary CMV infection is likely to occur when there is seroconversion from IgM negativity to IgM positivity in combination with low IgG avidity index. The anti-cytomegalovirus IgG avidity ELISA is presently the most reliable investigation used to identify primary CMV infection in pregnant women. The IgG avidity test is highly specific (100%) and sensitive (94.3%). The degree of antibody avidity slowly and progressively increases reflecting the maturation of humoral immuneresponse over time (Lazzarottoet al., 2002, Egger et al., 2000). Low avidity indices indicate low maturation of IgG antibodies in blood caused by recent or acute primary CMV infection (Lazzarottoet al., 1997). Low avidity indices are encountered 18–20 weeks after the onset of symptoms in apparently healthy individuals (Egger et al., 2000).

The aim of this work was to establish the significance of IgG Avidity Index in the differential diagnosis of primary Cytomegalovirus infection from non-primary infection in patients attending Anti-Retroviral Therapy Clinic in FMC Bida Niger State.
Materials and Methods

This study was a cross sectional study of patients with HIV infection attending the Anti-Retroviral Therapy (ART) Clinic of Federal Medical Centre Bida, Niger State. The study was carried out in the Anti-Retroviral Therapy (ART) Clinic of Federal Medical Centre Bida, located in Bida Local Government Area of Niger State, North Central Nigeria.

Approval for this study was obtained from the Ethical Review Committee of FMC, Bida. The sample collection was explained to the subjects using the information sheet prepared by the researcher. Each subject was required to give a written informed consent before being eligible to participate in the study. The minimum sample size for this study was determined using the Fischer formula. (Fisher et al., 1998)

Subjects:

Five milliliters of whole blood was drawn from the median cubital vein of every volunteer into a plain tube, allowed to clot; specimen was processed into serum by room temperature centrifugation at 3000 revolution per minutes for 10 minutes and stored at -200c. The frozen serum were thawed at room temperatures for 45 mins. Enzyme Linked Immonosorbent Assay (ELISA) IgM DIA Source (KAPRCVG01) CMV IgM kit was used following manufacturer instruction. All samples that were IgM positive were tested for IgG Avidity to differentiate primary from non-primary CVM infection.

IgG Avidity ELISA

Principle of the Test

Measurement of CMV -specific IgG avidity has proved to be a powerful tool for distinguishing primary from non-primary CMV infection. Defined as the strength with which the IgG attaches to antigen. IgG avidity measures the length of time following primary infection. Thus IgG produced within the first few months following primary infection exhibits low avidity, whereas IgG produced several months or years exhibits high avidity. However detection of CMV -specific IgG of high avidity is actually more informative from clinical standpoint as the presence of high avidity IgG essentially excludes the possibility that infection occurred within the previous four months.
Description of Methods

Cytomegalovirus IgG avidity ELISA utilizes urea, an agent that disrupts hydrogen bonds, to differentiate low avidity from high avidity antibodies. Following attachment to immobilized antigen, low avidity IgG readily dissociates from the antigen in the presence of urea whereas high avidity IgG does not. Thus, avidity was assessed by performing duplicate sets of the routine ELISA for CMV-specific IgG. The only difference was that after the initial incubation, one set was washed with buffer containing urea and the other set was washed with buffer lacking urea.

The CMV-specific IgG signal (optical density) of the set washed with urea buffer was divided by the CMV-specific IgG signal of the set washed with non-urea buffer providing the avidity index (AI). Avidity Index values lesser or equal to 0.50 indicates low avidity, values of 0.51-0.59 indicate intermediate avidity and values equal or greater than 0.60 indicate high avidity.

Assay Procedure IgG ELISA

1. The required number of cavities was placed in the frame and a protocol sheet was prepared.
2. Samples were diluted 1:101, distributing ten microliter of sample into one milliliter of sample diluents.
3. One hundred microliters of each diluted sample and calibrators (0, 1, 2 and 3) were pipetted to the appropriate wells. One well was left for the substrate blank.
4. The micro plate was incubated for 45 mins at 370C.
5. The micro plate was aspirated and each well was washed four times for 30 seconds with washing solution manually using a dispenser. Blotted and dried by inverting plate on absorbent material.
6. One hundred microliters of each Enzyme –Labeled 2nd Antibody was added into each well.
7. The micro plate was incubated for 45 mins at 370C.
8. Each well was aspirated and washed four times for 30 seconds with washing solution manually using a dispenser. Blotted and dried by inverting plate on absorbent material.
9. One hundred microliters of TMB Chromatogen solution was added to each of the well using a dispenser.
10. Incubated for 15 mins at room temperature. Exposure to direct sunlight was avoided.
11. One hundred microliters of stop solution was added to each well using a dispenser.
12. The absorbance of the solution in the wells was read using a microplate reader set to 450 nm. If wavelength correction was necessary, the instrument was set to dual wavelength measurement at 450nm with background wavelength correction set at 600 or 620nm.

Note:

(i) Avidity was assessed by performing duplicate sets of the routine ELISA for CMV-specific IgG.

(ii) One set was washed with buffer containing urea and the other set was washed with buffer lacking urea

Statistical Analysis

Generated data from the study was entered into and analysed with SPSS version 20.0. Data was summarized in frequency tables. Relationships and interactions among variables were determined using Chi-square crosstabs. Statistical significance tests were based on the 95% Confidence Interval (CI), P value of less than 0.05.

RESULTS

Three hundred and eighty five (385) blood samples were collected among patients with HIV/AIDS on Highly Active Anti-Retroviral Therapy attending Anti-Retroviral Therapy clinic in Federal Medical Centre Bida.

The prevalence of CMV IgM study report among HIV subjects in Bida is presented in Table 1. Out of the total three hundred and eighty five (385) HIV seropositive subjects, 77(19.8%) were positive for anti-CMV IgM antibodies.

IgG Avidity Index and IgM relationship in the differential diagnosis of primary Cytomegalovirus infection from non-primary infection in HIV/AIDS subjects is presented in Table 2. Out of the 77 IgM positive samples tested for IgG Avidity index, 12(15.6%) were high avid while 65(84.4%) were low avid. There was a significant difference (p= 0.001) between the prevalence of primary infection from non-primary infection with the use of IgG avidity index ELISA.

Discussion
The specific CMV IgM antibodies prevalence was found to be 77(19.8%), this prevalence reported in Bida was one of the highest reported among HIV patients in recent times compared to other reports in other developing countries. The prevalence of 9.52% was reported by Basawaraju et al., (2011) from HIV seropositive patients in Khammam India, while Ojide et al., (2013) in Benin City reported 7.0%, Musa et al., (2003) in Kano reported 13.0% and Akinbamì et al.,(2010) in Lagos reported 6.6%. All reported a lower prevalence compared to our findings in Bida.

Out of the 77 IgM positive sample this study reported 65(84.4%) low avidity and 12(15.6%) high avidity among the subjects, this agreed well with the report among pregnant women in Kenya (Mainngi and Nyamache, 2014) who reported 11 (20.37%) high avidity and 44(79.63) low avidity out of the 54 IgM positive women.

This study found a statistical difference (P=0.001) between IgG Avidity index and IgM levels for differential diagnosis of CMV primary from non-primary infection among HIV subjects in Bida. However, Chakravaritet al., (2009) observed that CMV active infection might be a marker of extremely severe immunosuppression, which may ultimately lead to fatal outcome in HIV patients and the presence of IgM antibodies may be due to primary infection, reactivation or re-infection by CMV.

Our study have convincingly demonstrates that measurement of CMV IgG avidity is both sensitive and specific method for identifying patients with recent primary CMV infection which is also a less expensive techniques compared to polymerase chain reaction (PCR) for confirmation. Low CMV IgG avidity is an accurate indicator of primary infection of CMV within the preceding 3-4 months while high avidity excludes primary infection (Prince and Nixon, 2014).

The utility of IgM avidity ELISA significantly reduced the prevalence of primary CMV infection with IgM ELISA in this study, in other words primary CMV infections by IgM ELISA alone was significantly overestimated as only 84.4% CMV IgM are actually primary infection while the rest 15.4% are non-primary infections.

In conclusion, CMV IgG avidity assay is an accessible method in the differential diagnosis of primary Cytomegalovirus from non-primary CMV infection in HIV patients, this technique is not expensive compared to the highly sophiscated Polymerase Chain Reaction it is highly recommended for use in the confirmation of primary CMV infection.

REFERENCES:


13. Manez, R., Kusne, S., Martin, M., Linden, P., Irish, W., Torre-Cisneros, J.


