Research Article

Detection of Epstein-Barr Virus IgM in HIV Infected Individuals in Ogbomoso, Nigeria

Oladipo Elijah Kolawole*[1,2], Oloke Julius Kola[1] and Awoyelu Hilda Elukunbi[1]

[1]Department of Pure and Applied Biology (Microbiology/Virology Unit), Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria, [2]Department of Microbiology, Adeleke University, Ede, Osun State, Nigeria.

Abstract | Epstein-Barr virus (EBV), also known as human herpes virus-4 (HHV-4), is a virus of the *Herpesviridae* family, and is one of the most common viruses in humans. It is the best known cause of infectious mononucleosis. In acquired immuno-deficient Syndrome patients, several distinct and additional EBV-associated diseases may occur and some forms of malignancies. This study was designed to determine the incidence of EBV in Human immune-deficient virus (HIV)-infected individuals in Ogbomoso, Oyo State, Nigeria. Two hundred and seventy eight HIV-infected individuals were screened for EBV antibodies over a period of four months (August to November, 2014). The sera of individuals were subjected to serological assay for IgM using enzyme-linked immunosorbent assay. The mean age of subjects was 39.5±0.48 years and the mean CD4+ was 384.27 ±17.77 cells/µl. Out of 278 subjects tested, 11 (4%) were positive for anti-EBV IgM. Anti-EBV IgM was highest in the age group 41-50 years, 5 (5.81%); females, 8 (4.85%); students (14.21%); and CD4+ 350-500 cells/µl, 4 (36.36%). The multivariate analysis using age and the CD4 count showed highest prevalence (27.27%) among the age ranging from 41 to 50 years with the CD4+ count of 350- 500cells/µl. The result from this study highlight that EBV IgM is prevalent among individuals infected with HIV in Nigeria and may warrant future investigations to ascertain the impact of co-infection on public health.

Editor | Muhammad Munir, The Pirbright Institute, UK.
Received | October 13, 2016; **Accepted** | December 20, 2016; **Published** | December 28, 2016
***Correspondence** | Oladipo Elijah Kolawole, Department of Pure and Applied Biology (Microbiology/Virology Unit), Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomoso, Oyo State, Nigeria; **Email:** koladipo2k3@yahoo.co.uk
DOI | http://dx.doi.org/10.17582/journal.bjv/2016.3.6.177.182
Citation | Kolawole, O.E, O.J. Kola and A.H. Elukunbi. 2017. Detection of Epstein-Barr virus IgM in HIV infected individuals in Ogbomoso, Nigeria. *British Journal of Virology*, 3(6): 177-182.
Keywords: EBV, HIV, IgM, CD4 cell count, Ogbomoso, Herpes, ELISA

Introduction

Epstein-Barr virus (EBV) or human herpes viruses are ubiquitous, and about 90% of adults in the world have antibodies against EBV (Rickinson et al., 2006). Acute infection is usually asymptomatic in immunocompetent children, and manifests itself as mononucleosis in 30-50% of immunocompetent adolescents and adults (Lennette, 1995). Especially in immunocompromised patients (HIV), EBV is associated with various lymph proliferative disorders and some neoplastic diseases, including Burkitt's lymphoma and nasopharyngeal carcinoma. Like other herpesviruses, EBV has a productive lytic cycle and a latent phase. B lymphocytes are infected after the viral envelope glycoprotein gp 350/220 has bound to

the CD21 cell receptor, which is also the receptor for the C3D component of complement (Fingeroth et al., 1984).

EBV carriers in the HIV-seropositive group merit concern due to the pathogenic and malignant potential of the virus (Straus, 1988). Although in most cases, EBV infection is linked to benign diseases, the virus can induce malignances (Kimura, 2006). In most individuals, a lifelong chronic infection with EBV is free from complications due to suppression from normal immune systems (Thorley-Lawson et al., 2006). However, patients with acquired immunosuppression are at a high risk for developing both benign and malignant conditions (Strowig et al., 2008). Some of the viral genes express proteins to activate and maintain the proliferation of B cells (latency III program) (Carbone et al., 2009).

Most HIV-infected people are persistently infected with EBV, and with progressive immunodeficiency numbers of EBV infected B cells in the circulation increase (van Baarle et al., 2001) and opportunistic lymphomas may develop. Although in most cases, EBV infection is sometimes linked to benign diseases and the virus can produce malignances. In most individuals, a lifelong chronic infection with EBV is free from complications due to suppression from normal immune systems (Strowig et al., 2008). However, patients with acquired immunosuppression are at a high risk for developing both benign and malignant conditions (Carbone et al., 2009).

It is well known that EBV is a common opportunistic infection agent in the immunocompromised, including human immunodeficiency virus-infected individuals. Nonetheless, infections of EBV in HIV infected individuals have received little attention, the study is therefore designed to know its incidence in Ogbomoso, southwestern Nigeria.

Materials and Methods

Study Centre

The study centre was located in Ogbomoso, Oyo State, situated in the tropical belt of Southern Western part of Nigeria. Its geographical coordinates are 80 8' 0" North, 40 16' 0" East. It is situated about 600m above sea level with a mean annual temperature of about 26.2°C.

Study Population

The study subjects were consented HIV positive individuals living in Ogbomoso, Nigeria.

Enrolment of Subjects

Consenting HIV positive individuals were enrolled from different HIV/AIDS support groups in Ogbomoso. Enrolment took place between July and December 2014. Thereafter, consenting subjects were enrolled for the study. Demographic and other relevant information were retrieved from the study subjects using a structured questionnaire. Afterwards, blood sample was collected from each of the 278 consenting subjects out of 300 enrolled for the study. The study protocol was in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All study subjects gave written informed consent. Any subject who did not give consent was excluded.

Sample Collection

Samples were collected in a tube without anticoagulant for serology and with coagulants for the CD4 count analysis. A tourniquet was firmly tied to the upper arm of the subjects while sitting and the skin was sterilized with 70% alcohol. Sterile needle was inserted into conspicuous antecubital vein and the plunger of the sterile syringe was withdrawn and pressure applied to the puncture site with a cotton wool to stop bleeding. Blood sample was spun on a bench centrifuge at 3,000 rpm for 10 minutes to obtain serum. Serum was separated immediately. Aliquots of serum were made per sample in labelled sterile cryovials which were stored at −20°C until ready for analysis.

CD4 Count and Serology

CD4 count was determined using standard laboratory procedure with the use of Counter 2 (Version 2.4, Partec Germany). All the 278 samples were subjected to Epstein-Barr virus specific Enzyme Linked Immunosorbent Assay (ELISA) using the Epstein-Barr virus IgM detection ELISA kit (WKEA Med Supplies Corp, Changchun, China). The assay was performed according to manufacturer's instructions. The optical density was read using the Emax endpoint ELISA microplate reader (Molecular Devices, California, USA) and the result was interpreted according to the manufacturer's instructions.

Statistical Analysis

The data obtained from the study were subjected to

descriptive statistical analysis. Variance analysis of serological pattern of EBV antibody was analyzed. All interval estimates are 95% confidence intervals. SPSS program for Windows (Version 21.0; SPSS, Inc., Chicago, IL) was used.

Results

Incidence of Anti-EBV IgM among Different Age Groups of the Study Subjects

Figure 1 shows incidence of EBV antibody in the study subject, out of 278 subjects whose sera were tested using ELISA, 11 (3.96%) were positive for EBV IgM and 267 (96.04%) were negative. Highest prevalence of anti-EBV IgM was found in subjects whose ages were between 41-50 years (5.81%) followed 31-40 years (4.95%).

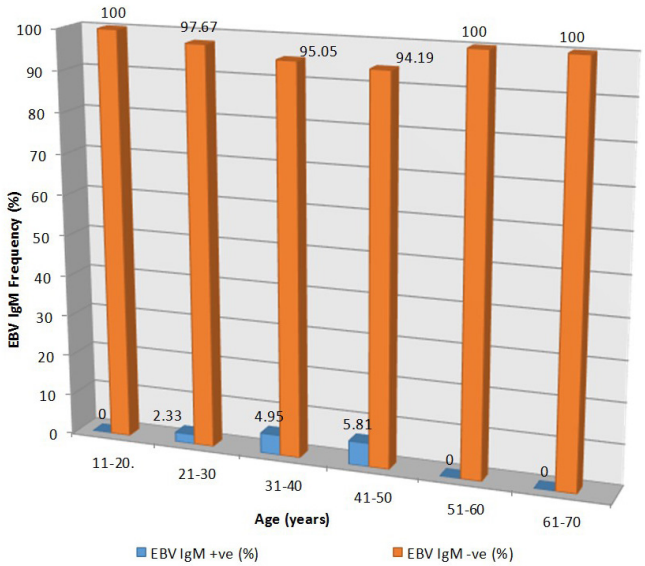

Figure 1: *Sero-prevalence of EBV IgM by age distribution*

Sex Distribution of Anti-EBV IgM among the Study Subjects

The sex distribution of the study subjects for EBV IgM is shown in Figure 2, out of 113 males, 3 (2.65%) were positive for anti-EBV IgM, while out of 165 females, 8 (4.85%) were positive for anti-EBV IgM.

Incidence of Anti-EBV IgM using CD4+ Counts of the Study Subjects

A total of 278 study subjects with different CD4+ counts were tested for EBV antibody as shown in Figure 4. The incidence of EBV antibody in persons with CD4+ counts of <200, 200-350, 350-500 and >500 (cells/µl) is found in Figure 3. CD4+ count of 350-500 cells/µl had the highest incidence (10.81%) and CD4+ count of >500 cells/µl (1.49%) had the lowest.

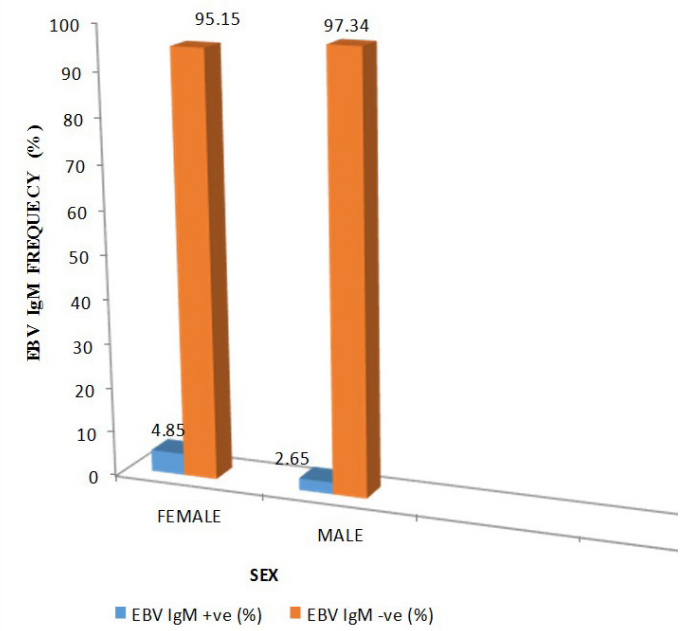

Figure 2: *Sex distribution of EBV IgM*

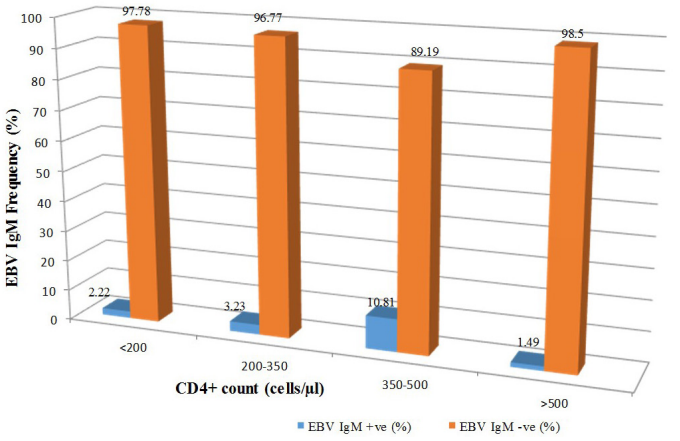

Figure 3: *Incidence of EBV based on CD+ count*

Table 1: *Mean value of seropositive and seronegative of the study subjects*

Factor	Seropositive	Seronegative
Age (years)	37.82±2.056	39.56±0.540
CD4+ Count	454.64±94.769	380.76±8.828

Values: *mean scores ± standard error*

Variance Analysis for Serological Incidence Pattern of EBV Antibody among HIV-Infected Individuals

There is a significant difference, that is, relationship in the mean value of seropositive groups as shown in Table 1. The CD4 + count has a mean value of 454.64 which is significantly different from that of sex with a mean value of 1.73 and also significantly different from that of age which is 37.82. The mean values for seronegative groups are Sex is 1.57; CD4+ count is 380.76; Age is 39.56 which are of no significant value.

Smith & Franklin Academic Publishing Corporation

Multivariate Prevalence Analysis Using CD4 and Age Range

Figure 4 shows the multivariate analysis using age and the CD4 count. The highest incidence (27.27%) is seen in age range (41-50) years with the CD4+ count of 350- 500 cells/µl.

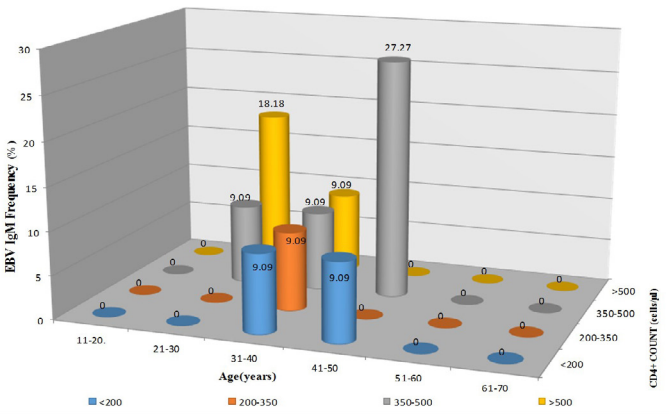

Figure 4: *Incidence of EBV IgM multivariate analysis using age and CD+ count*

Discussion

Epstein Barr virus is one of the leading cause of death and opportunistic infection that play an important role in HIV-positive individuals. EBV likewise is contributing to the pathogenesis of most of lymphoproliferative diseases, such as oral hairy leukoplakia, in HIV positive patients (Abdollahi et al., 2014). The appearance of this virus in HIV positive individuals, according to Arotiba et al. (2005), indicate the deterioration of HIV infection. In this study, 278 samples from HIV infected individuals were tested and the sero-prevalence of EBV was 4%. The highest incidence of anti-EBV IgM (5.81%) was in the age range (41-50) years. This indicated that EBV antibody prevalence increases with age which is in accordance with Schaftenaar et al. (2014). However, it is in contrast with studies carried out by Chakraborty et al. (2010) and Abdollahi et al. (2014) that age range 21-40 and 30 ≤ 40 have the highest EBV antibody incidence, respectively. The difference can be attributed to difference in antibody type, geographical location, sexual activity as well as low living standard of the study subjects as compared to the age range mentioned in earlier reports.

Considering the sex distribution prevalence, the highest prevalence of anti-EBV was found among the females (4.85%) in accordance with the findings of Hjalgrim et al. (2007) and Schaftenaar et al. (2014). This disagrees with the reports of Adjei et al. (2008) and Chakraborty et al. (2010) where the incidence was dominant in the male population rather than the female. This difference is in the notion that women amount more vigorous antibody and cell-mediated immune response following infection than in men. Also, it might be as a result of high record of sexual activeness in females than their male counterpart in the study area.

The highest incidence of anti-EBV IgM is in the CD4+ range 350-500 cells/µl (10.81%). This result is indicative of primary infection rather than secondary infection in the study subjects. However, antibodies of the IgM type are not necessarily produced in all patients with primary infections (Hess, 2004). Elevated titres of IgM is frequently found in conditions characterized by ongoing EBV replication. Significantly, CD4 counts of HIV positive patients with oral lesions (like oral hairy leukoplakia) during EBV infection are lower than those without oral manifestations (Arotiba et al., 2005; Hess, 2004). The multivariate analysis between age and CD4+ count shows the highest incidence among the age range (41-50) years and CD4+ 350-500 cells/µl. The overall percentage of incidence from the study is 27.27%.

Conclusion

Following the incidence of anti-EBV IgM antibody among the study subject, there is consequently high risk for Herpes simplex virus related diseases among HIV-infected individuals which calls for increased awareness among healthcare workers for early clinical signs of these conditions to initiate prompt antiviral treatment. Even though the route of transmission of EBV is through saliva, however, the use of protection such as condoms should be encouraged in order to prevent bacterial and viral spread carried in semen.

Acknowledgements

We are thankful to all subjects who participated in the study and staff in the research laboratory of Prof. J.K. Oloke at Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria who made a great contribution to the study.

Conflict of Interests

The authors have no competing interests to declare.

Authors' Contributions

OJK, OEK and AEH conceptualized the study, participated in its design and coordination, participated in the statistical analysis, and revised the manuscript. OEK led and performed the statistical analysis and drafted the manuscript. OJK, OEK, AEH participated in the study and contributed reagents / materials / analysis tools. AEH participated in the statistical analysis and revised the manuscript. All the authors read and approved the final manuscript.

References

- Abdollahi, A., S. Shoar, M. Rasoulinejad and S. Sheikhbahaei. Sero-prevalence of epstein-barr virus among HIV positive patients moreover and its association with CD4 positive lymphocyte count. Acta Medica Iranica, 2014; 52(12), 916-921.
- Adjei, A., H.B. Armah, F. Gbagbo, I. Boamah, C. Adu-Gyamfi and I. Asare. Seroprevalence of HHV-8, CMV and EBV among the general population in Ghana, West Africa. BMC Infectious Diseases, 2008; 8, 111. https://doi.org/10.1186/1471-2334-8-111
- Arotiba, J.T., R.A. Adebola, Iliyasu, M. Babashani, W.A. Shokunbi, M.M.A. Ladipo, B. Akhiwu and O.D. Osude. Oral manifestations of HIV/ AIDS infection in Nigerian patients. Nigerian Journal of Surgical Research, 2005; 7, 176-181.
- Carbone, A., E. Cesarman, M. Spina, A. Gloghini and T.F. Schulz. HIV-associated lymphomas and gamma-herpesviruses. Virus Research, 2009; 143, 209-221. https://doi.org/10.1182/blood-2008-09-180315
- Chakraborty, N., S. Bhattacharyya, C.A. de Mukherjee, D. Bhattacharya, S. Santra, R.N. Sarkar, D. Banerjee, S.K. Guha, U.K. Datta and S. Chakrabarti. Incidence of multiple herpesvirus infection in HIV seropositive patients, a big concern for Eastern Indian scenario. Virology Journal, 2010; 7, 147. https://doi.org/10.1186/1743-422X-7-147
- Fingeroth, J.D., J.J. Weis, T.F. Tedder, J.L. Strominger, P.A. Biro and D.T. Fearon. Epstein–Barr virus receptor of human B lymphocytes is the C3d receptor CR2. Proceedings of the National Academy of Science, 1984; 81, 4510–4514. https://doi.org/10.1073/pnas.81.14.4510
- Hess, R.D. Routine Epstein-Barr virus diagnostics from the laboratory perspective: still challenging after 35 years. Journal of Clinical Microbiology, 2004; 42(8), 3381-3387. https://doi.org/10.1128/JCM.42.8.3381-3387.2004
- Hjalgrim, H., J. Friborg and M. Melbye. 2007. The epidemiology of EBV and its association with malignant disease. In: Human herpesviruses: Biology therapy and immunoprophylaxis (eds. A Arvin, G Campadelli-Fiume, E Mocarski), Cambridge University Press, Cambridge, pp. 929–959. https://doi.org/10.1017/CBO9780511545313.054
- Kimura, H. Pathogenesis of chronic active Epstein-Barr virus infection: is this an infectious disease, lymphoproliferative disorder, or immunodeficiency? Reviews in Medical Virology, 2006; 16, 251–261. https://doi.org/10.1002/rmv.505
- Lennette, E.T. 1995. Epstein-Barr virus (EBV). In Diagnostic procedures for viral, rickettsial, and chlamydial infections (eds. EH Lennette, DA Lennette, ET Lennette), 7th ed. American Public Health Association, Washington, D.C., pp. 299-312.
- Rickinson, A.B., and E. Kieff. 2006. Epstein-Barr virus. In: Virology (eds. DM Knipe, PM Howly) 5th ed., Vol. 2, Wolters Kluwer / Lippincott Williams & Wilkins, Philadelphia, pp. 2655-2700.
- Schaftenaar, E., G.M. Verjans, S. Getu, C.J.A. McIntyre, H.E. Struthers, A.D.M.E. Osterhaus and R.P.H. Peters. High seroprevalence of human herpesviruses in HIV-infected individuals attending primary healthcare facilities in rural South Africa. PLoS One, 2014; 9(6), e99243. https://doi.org/10.1371/journal.pone.0099243
- Straus, S.E. The chronic mononucleosis syndrome. Journal of Infectious Disease, 1988; 157(3), 405–412. https://doi.org/10.1093/infdis/157.3.405
- Strowig, T., F. Brilot, F. Arrey, G. Bougras, D. Thomas, W.A. Muller and C. Mun. Tonsilar NK cells restrict B cell transformation by the Epstein-Barr virus via IFN-gamma. PLoS Pathogen, 2008; 4, e27. https://doi.org/10.1371/journal.ppat.0040027
- Thorley-Lawson, D.A., E.M. Miyashita and G. Khan. Epstein-Barr virus and the B cell: that's all it takes. Trends in Microbiology, 2006; 4, 204–208. https://doi.org/10.1016/S0966-

842X(96)90020-7

- van Baarle, D., E. Hovenkamp and M.F.C. Callan. Dysfunctional Epstein-Barr virus (EBV)-specific CD8+ T lymphocytes and increased EBV load in HIV-1 infected individuals progressing to AIDS-related non-Hogkin lymphoma. Blood, 2001; 98, 146–155. https://doi.org/10.1182/blood.V98.1.146