International Journal of Science and Technology Research Archive

ISSN: 0799-6632 (Online)

Journal homepage: https://sciresjournals.com/ijstra/

(RESEARCH ARTICLE)

Check for updates

Epstein Barr virus nuclear antigens among University Students in Port Harcourt, Nigeria

Tochi Ifeoma Cookey [1], Juboyeibaraofori Jonah [1], Chisom Chimbundum Adim [1], Blessing Jachinma Okonko [2], Hope Chioma Innocent-Adiele [1] and Iheanyi Omezuruike Okonko [1,*]

[1] *Virus & Genomics Research Unit, Department of Microbiology, University of Port Harcourt, Choba, P.M.B. 5323, Port Harcourt, Rivers State, 500102 Nigeria.*
[2] *Virology & Immunology Research Unit, Ebonyi State University, Abakaliki, Nigeria.*

International Journal of Science and Technology Research Archive, 2023, 04(01), 113–120

Publication history: Received on 01 November 2022; revised on 12 January 2023; accepted on 15 January 2023

Article DOI: https://doi.org/10.53771/ijstra.2023.4.1.0140

Abstract

Several diseases are associated with Epstein Barr virus (EBV) and they can be life threatening especially in immunocompromised individuals. Moreover, there is no well-established EBV prevention and control strategies in Nigeria. Therefore, this study was carried out to assess the prevalence of Epstein Barr Nuclear Antigen (EBNA) IgM antibody among university students in Port Harcourt, Nigeria. A hospital based cross-sectional survey was adopted to randomly analyze 91 students attending lectures in University of Port Harcourt, Rivers State, Nigeria. Enzyme Linked Immunosorbent Assay (ELISA) was used to analyze EBNA IgM antibody in the samples obtained. Chi-square analysis was used to determine the association of the infection with socio-demographic factors. Of the 91 subjects, 3(3.2%) were seropositive for EBNA IgM antibody while 88(96.7%) were observed to be seronegative for EBNA IgM antibody. Sera EBNA IgM positivity was highest in age group 20-25 years (4.7%), single (3.2%), male (3.8%), students (3.9), sexually inactive (4.0%), oral sex (2.4%), non-Anal sex (3.4%), never using condoms (5.0%), dry kissing (11.1%), Non-smokers (4.0%), no history of blood transfusion (3.3%), no history of tissue transplant (3.2%), no history of surgery (3.2%). This study confirms the presence of Epstein Barr virus primary infection among university students in Port Harcourt, and an onward risk of Infectious mononucleosis. This comes with the responsibility of establishing surveillance programs for detection, treatment and control of EBV in Port Harcourt and Nigeria at large.

Keywords: EBNA; Prevalence; IgM; ELISA; Antibody; EBV

1 Introduction

Epstein-Barr viruses (EBV) is the causative agent of infectious mononucleosis ("mono") or glandular fever which affects adolescents and young adults causing fever, sore throat, lymphadenopathy, hepatospleenomegaly, and fatigue (Lino & Ghosh, 2021). EBV is highly prevalent worldwide, with more than 90% of the world's adults being infected with the virus (Shi et al., 2022). It is from the Herpes virus family and formally known as Human gamma herpes virus 4 or Human herpes virus 4 (HHV-4) (Rezk *et al*., 2018).

EBV is one of the most common viruses of humans and it's involved in the pathogenesis of several non-malignant and malignant diseases (Kieff & Rickinson, 2007). EBV seroprevalence has been reported to increase with age, and tends to be higher among females, non-Caucasian ethnic groups, and people living in socio-economically deprived households (Kuri et al., 2020). In immunocompetent patients, EBV establishes a life-long latent infection which is usually asymptomatic and well controlled by T cells specific for epitopes derived from various latent and lytic cycle antigens of the virus (Cirac et al., 2018). Infections of EBV usually occurs during early childhood, often times, and within a short

* Corresponding author: Iheanyi Omezuruike Okonko, Tel: +2348035380891; E-mail: iheanyi.okonko@uniport.edu.ng

period after maternal immunity stops (ACP, 2017). Adults who were seropositive, usually were infected with the virus during their childhood and so harbour the latent form of the virus (Santpere *et al*., 2014). The virus is usually present in the saliva, as well as other body fluids of asymptomatic seropositive individuals and is easily transmitted between people. (Odumade *et al*., 2011). However, immunocompromised patients may have difficulty controlling the viral infection and often develop severe and life-threatening conditions, such as severe inflammation and malignancies (Lino & Ghosh, 2021).

The virus nuclear antigen (EBNA) is one of the genes encoded by the EBV genome and its detection is one important method for serodiagnosis (Odumade et al., 2011). Specific IgM antibody against EBNA is generally designated as one of the indicators of a recent primary infection which are produced only transiently, or might persist at such a low concentration such that they may be missed by laboratory tests (Huang et al., 2013). Nevertheless, detection of EBNA IgM antibody, in combination with other antibody avidity tests, can be specifically used in diagnosing primary EBV infection in a population (Hess, 2004). Hess RD: Routine Epstein–Barr virus diagnostics from the laboratory perspective: still challenging after 35 years.

Thus, this study aimed at evaluating the prevalence of EBV infection among university students in Port Harcourt by determining the level of IgM antibody against Epstein Barr virus nuclear antigen.

2 Material and methods

2.1 Study Area

The study was conducted among students attending the University of Port Harcourt, Choba, and Rivers State. The University is located in Obio/Akpo Local Government Area, a metropolis of Port Harcourt, one of the major cities of the Niger delta located in Rivers State, Nigeria.

2.2 Study Design

A cross sectional study was carried out randomly on 91 consenting students attending University of Port Harcourt, Rivers State, from March 2019 through November 2019. Ethical approval was obtained from the University of Port Harcourt Research Ethics Committee and a consent form was signed by each participant. A structured questionnaire was designed and standardized.

2.3 Sample collection and processing

Three milliliters of blood was aseptically collected by venipuncture from 80 consenting patient and dispensed into appropriately labeled sample tube, screwed-capped and left at room temperature for about 40 minutes, after which it was spun at 3,000rpm for 10 minutes to separate serum from blood. Samples were clearly identified with codes in order to avoid misinterpretation of results. The sera were carefully aspirated into plain bottles and stored at -20^{o}C until analyzed.

2.4 Laboratory analysis

Laboratory analysis was carried out at the Virus Research Unit, Department of Microbiology, University of Port Harcourt, Choba, Rivers State. The samples were analyzed for EBNA specific IgM antibodies (qualitative assay of IgM antibodies) by using the commercially available ELISA kit manufactured by Dia.Pro. Diagnostic Bioprobes Srl (Milano) according to manufacturer's instructions. Washing was done automatically using an ELISA washer (ELx50, Biotek, USA). Plates were read using an ELISA plate reader (ELx808i, Biotek, USA) at an absorbance of 450 and 630 nm (Okonko & Egbogon 2022). Every stage of the ELISA process was done following the manufacturer's instructions. Test results were interpreted as a ratio of the sample OD450nm and the Cut-Off value (or S/Co) following the manufacturer's instructions.

2.5 Data Analysis

The data obtained from questionnaires and laboratory analysis were entered into Microsoft Excel, analyzed using Statistical Package for Social Sciences version 21. Pearson Chi-square was calculated at 95% confidence interval and *P*-value < 0.05 was considered significant to determine the association between the presence of the antibodies to the virus and other parameters.

3 Results

3.1 Socio-demographic characteristics of study participants

The total number of students included in this study was 91. The socio-demographic data for these samples were stratified and shown in Table 1. The age ranges from 17-33 years. The age groups 20 - 25 years constituted the largest populations making up 68.1%, followed by age group 26 - 29 years (17.5%), 17 - 19 years (8.9%), and lastly, age groups 30 - 33 years (6.7%). The males dominated the study with 85.1% whereas females were 14.2%. On the basis of educational background, 19.7% of the population had only secondary education, while 80.2% progressed to tertiary education. Also, 16.4% combined schooling and part time jobs while 83.5% were solely students.

3.2 Behavioural characteristics of the Study population

As shown in Table 1, a higher percentage, 82.4% of the population were nonsmokers, while 17.6% were smokers. A lower percentage of 18.7% had a drinking habit of greater than twelve times (>12x) in a year, 36.3% had a drinking habit of less than twelve times (<12x) in a year, while the remaining 44% of the population had a drinking habit of less than twelve times (<12x) in a life time. Forty persons (44%), carried out stressful tasks while in high school, while fifty-one person's (56%) had a stress-free learning. Also 52.7% of the population had stressful events in the University, while 48.2% had no stressful events. For sexual activity, 72.5% of the population were sexually active, while 27.5% were inactive. Forty-one of the population (45.1%) engaged in oral sex, while 50 of them (54.9%) did not. A lesser percentage of 8.8% practiced anal sex, while 91.2% do not. In terms of kissing level, 80.2% were involved in wet kissing, while 19.8% preferred dry kissing. Also, 22% have never used a condom, 52.7% seldom used condoms while 25.2% of the population had always used condoms.

3.3 Clinical characteristics of the study population

A larger proportion of 90.1% had no allergies in high school, while the remaining 9.9% experienced allergic reactions. In terms of Blood grouping, 9.9% had blood group A, 11% had blood group B, none of them had blood group AB while 79.1% had blood group O. None of the population tested had any history of tissue transplant or surgery. This data is shown in Table 1.

3.4 Overall prevalence of EBNA IgM antibody

Of the ninety-one persons, 3.2% were seropositive for EBNA IgM antibody while 96.8% were negative. The prevalence of the viral IgM antibody across the different categories of the socio-demographics of the tested population is shown in Table 1.

Table 1 Prevalence of EBNA IgM antibody and the Characteristics of the participants

Characteristics	Groups	No. Tested (%)	No. Positive (%)
Socio-Demographic			
Age (years)	17-19	8(8.7)	1(12.5)
	20-25	62(68.1)	2 (3.2)
	26-29	16(17.6)	0 (0.0)
	30-33	5(4.2)	0 (0.0)
Gender	Male	78(85.7)	3 (3.8)
	Female	13(14.3)	0 (0.0)
Marital status	Married	0 (0.0)	0 (0.0)
	Single	91(100.0)	3 (3.2)
Educational status	High school	18(19.8)	0 (0.0)
	University	73(80.2)	3 (4.1)
Occupational status	Students	76(83.5)	3 (3.9)

	Workers	15(16.5)	0 (0.0)
Behavioural			
Smoking habit	Smokers	16(17.6)	0 (0.0)
	Non-smokers	75(82.4)	3 (4.0)
Drinking habit	<12x in a lifetime	40(44.0)	2 (5.0)
	<12x per year	33(36.3)	1 (3.0)
	>12x per year	17(18.7)	0 (0.0)
Stressful event in high school	Yes	40(44.0)	1 (2.5)
	No	51(56.0)	2 (3.9)
Stressful event in the university	Yes	48 (52.7)	2 (4.1)
	No	43 (48.3)	1 (2.3)
Sexually active	Yes	66 (72.5)	2 (3.0)
	No	25 (27.5)	1 (4.0)
Oral sex	Yes	41 (45.1)	1 (2.4)
	No	50 (54.9)	2 (2.0)
Anal sex	Yes	8 (8.8)	0 (0.0)
	No	83 (91.2)	3 (3.4)
Use of condoms	Never	20 (22.0)	1 (5.0)
	Seldom	48 (52.7)	1 (2.0)
	Always	23 (25.3)	1 (4.3)
Kissing level	Wet kissing	73 (80.2)	1 (1.3)
	Dry kissing	18 (19.8)	2 (11.1)
Clinical characteristics			
Experienced allergies in High school	Yes	9 (9.9)	1 (11.1)
	No	82 (90.1)	2 (2.4)
Blood Group	Yes	1 (1.1)	0 (0.0)
	No	90 (98.9)	3 (3.3)
Blood group (N=91)	A	9 (9.9)	1 (11.1)
	B	10 (11.0)	0 (0.0)
Blood transfusion	AB	0 (0.00)	0 (0.0)
	O	72 (79.1)	2 (2.7)
Tissue transplant	Yes	0 (0.0)	0 (0.0)
	No	91 (100.0)	3 (3.2)
Undergone surgery	Yes	0(0.0)	0 (0.0)
	No	91 (100.0)	3 (3.2)
Total		91 (100.0)	3 (3.2)

3.5 Prevalence of EBNA IgM antibody in relation to sociodemographic characteristics

The prevalence of EBNA IgM antibody in relation to age is shown in Table 1. Two out of the three age groups were reactive. Higher prevalence of EBNA IgM antibodies was observed in age group 17 - 19 years (12.5%), followed by 20-25 years (3.2%). None was obtained in 30 - 33 years. Seropositivity rate was only obtained in the males (3.8%). All the females that participated in this study were seronegative. All the participants enrolled in this study were single. The prevalence of EBNA IgM antibody in this population was 3.2%. The prevalence of EBV IgM antibody in University students was 4.1%, while none was obtained for High school students. No prevalence rate was obtained for workers while 3.9% was obtained for students. This data is represented in Table 1.

3.6 Prevalence of EBNA IgM antibody in relation to Behavioural Characteristics

As for sexuality, seropositivity rate was 4% for non-sexually active students and 3% for the sexually active (Table 1). The highest prevalence of EBNA IgM antibody was obtained amongst those who have no history of condom usage (5.0%). Students who constantly use condom and those who seldomly use condoms have a seropositivity rate of 4.3% and 2% respectively (Table 1). Prevalence of EBNA IgM antibody was higher among students who practice oral sex (2.4%) than those who do not (2.0%). Seropositivity of 3.4% was obtained in students who do not engage in anal sex while no value was obtained in those who do. Prevalence of EBNA IgM antibody in people who engage in dry kissing (11.1%) was higher than those who practiced wet kissing (1.3%) (Table 1). Students who don't smoke had an EBNA IgM seroprevalence rate of 4.0%, while no value was obtained for those who smoke (Table 1).

3.7 Prevalence of EBNA IgM antibody in relation to Clinical Characteristics

Individuals with Blood group B and AB had no prevalence rate for EBNA IgM antibody while those with blood group A and O had seropositivity rates of 11.0% and 2.7%, respectively**.** This can be seen in Table 1. Lower prevalence rate (2.4%) was obtained in students who had no allergies in high school while higher prevalence of 11.1% was obtained from those who experienced allergic reactions (Table 1). Prevalence of EBNA IgM antibody among those who experienced stressful events during their high school days was 2.5%, while 3.9% was for those who were not involved in stressful events (Table 1). The prevalence of EBNA IgM antibody in individuals who were engaged in stressful events in the University was 4.1%, which is higher than those who had no stressful events (2.3%) (Table 1). As shown in Table 1, 3.2% seropositivity rate of EBNA IgM antibody was obtained among students who have not had surgery while for those who have had surgery, no value was obtained. In the population tested, none of the students have had any history of tissue transplant. Nonetheless, the test results showed a 3.2% prevalence among them (Table 1). Table 1 also showed Prevalence of EBNA IgM antibody in relation to those who had done blood transfusion. None of the individuals tested have transfused or received blood. The prevalence of EBNA IgM antibody among those who have not done blood transfusion was 3.3% (Table 1).

4 Discussion

Infection with Epstein Barr virus can cause severe inflammation, associated disorders and malignancies especially in persons with defects in their immune system. However, in healthy adolescents and young adults, the infection may be self-limiting with mild illnesses associated with acute inflammation (Lino & Ghosh, 2021). The virus primarily transmits through oral secretions and persists as a latent infection in human B-cells. However, it can be transmitted through organ transplantations and blood transfusions (Smatti *et al*., 2018).

The result of this study revealed an Epstein Barr virus seroprevalence rate of 3.2% among young adults attending a university in Port Harcourt. This compared with the prevalence of 4% and 6.6% reported in a similar study in Ogbomosho and Zaria respectively (Kolawole *et al*., 2017; Bishop & Adegoke, 2016) but much lower than the prevalence of 20% reported in Ghana (Adjei *et al*., 2008) and 56.1% in India (Patel *et al.*, 2021). The fluctuations in seroprevalence rates may be as a result of differences in geographic locations or differing ELISA kits used for laboratory tests. The result of this study implies that 3.2% of the participants had recent contact with the virus since EBNA-IgM is used as an indicator of recent primary EBV infection and is secreted transitionally (Patel *et al.*, 2021).

The age distribution of EBV IgM antibody in this study showed that it was highest at age group 17-19 years (12.5%), then decreased in age-group 20-25 years (3.2%) and came to zero at the older age-groups. The decline in seropositivity rates of EBNA IgM antibody as age increases, in this study, is similar to a previous work carried out by Cui et al. (2018) and Anejo-okpi *et al*. (2019) This result relates to the fact that primary EBV infection occurs early in life and persists asymptomatically as one matures especially among the immunocompetent (Bishop & Adegoke, 2016). It could also be as a result of high social interaction, contacts and a high sexual activity within this age group since the virus is widely

disseminated as it is spread by intimate contact between asymptomatic EBV-infected persons who shed the virus and susceptible persons (Sureshbabu, 2022).

The prevalence of EBNA IgM antibody in this study was higher in the males (3.8%) than in the females (0%). This corroborates with the findings of of Adjei *et al.* (2008) and Chakraborty et al. (2010) and contradicts the reports of Kolawole et al. (2016), Schaftenaar et al. (2014) and Anejo-okpi *et al*. (2019) where the prevalence was dominant in the female population rather than the males. The higher seroprevalence of EBV in males could be due to the homosexual relationship (Abdollahi *et al*., 2014).

Students and not workers had a higher seropositivity rate for EBV IgM in this study among which the University students had the highest when compared with those in high school. This implies that students with tertiary education might be more exposed to the infection. Though there is limited information about the involvement of an individual's educational level in respect to the prevalence of EBV (Okonko & Egbogon, 2022), this study contradicted a previous work carried out by Levine *et al*. (2012) which opined that lower education was found to be associated with EBV seroprevalence.

Aside age and gender, several other factors and behavioral characteristics such as smoking, drinking habits, stressful work, kissing level and sexual activities have been implicated in EBNA seropositivity (Anejo-Okopi *et al*., 2019). In this study, a higher prevalence of IgM antibodies against EBNA was found among non-smokers and those with drinking habits less than 12x in a lifetime. Also, those who experienced stressful events in the university and those who were not involved in stressful events in secondary school, had higher prevalence of EBNA IgM antibody when compared to their counterparts.

Analyses of other risk factors confirms their significance and association to EBNA infection at different levels. The sexually active students that participated in this study were observed to have a lower prevalence of IgM antibodies than students who were not active. This result is unlike that of Higgins *et al*., (2007) where sexually active students had a higher prevalence rate. Though sexual activity has been reported to be a strong risk factor for EBV positivity, the result in this study has shown that childhood, rather than sexual factors are the reason for the increased seropositivity agreeing with a previous study (Higgins *et al*., 2007, Adjei et al., 2008). Higher prevalence was also obtained in students who have been involved in oral sex and those who have not been in involved in anal sex. Those that never use condom had the highest prevalence of IgM antibody against EBNA infection, compared to those who seldom or always use condom, implying that the infection could be acquired through the sexual route (Okonko & Egbogon, 2022).

Biochemical studies have shown that pathogen and erythrocyte membrane interactions can indicate antigenic similarity, adherence via different receptors, or antibody response modulation (Sandler & Mallory, 1995; Al Tale *et al.,* 2019). Hence, there is possible association of blood group antigens with Epstein Barr viral infection. A study carried out in Iraq by Al Tale *et al*. (2019) among thalassemia patients who received blood transfusion documented the highest rate of EBV infection in patients with blood group AB unlike the result of this study where the highest seroprevalence of IgM antibody against EBNA was observed in sera with blood group A. Although blood transfusion has been implicated in the transmission of EBV infection especially in hyperendemic countries, the result of this study showed a higher prevalence among those who had not had blood transfusion.

5 Conclusion

This study confirms the presence of Epstein Barr virus primary infection among university students in Port Harcourt, and an onward risk of Infectious mononucleosis. This comes with the responsibility of establishing surveillance programs for detection, treatment and control of EBV in Port Harcourt and Nigeria at large.

Compliance with ethical standards

Acknowledgments

The authors would like to acknowledge the support obtained from the students of University of Port Harcourt during the enrollment and collection of samples used in this study. The authors are grateful to the participants for their willingness to be part of the study.

Disclosure of conflict of interest

Authors have declared that no competing interests exist.

Statement of ethical approval

All authors hereby declare that all experiments have been examined and approved by the University of Port Harcourt Research Ethics committee and have, therefore, been performed following the ethical standards laid down in the 1964 Declaration of Helsinki.

Statement of informed consent

All authors declare that informed consent was obtained from all individual participants included in the study.

References

[1] Abdollahi, A., Shoar, S., Rasoulinejad, M., & Sheikhbahaei, S. (2014). Seroprevalence of Epstein-Barr virus among HIV positive patients moreover and its association with CD4 positive lymphocyte count. Acta Medica Iranica, 916-921.

[2] Adjei, A. A., Armah, H. B., Gbagbo, F., Ampofo, W. K., Boamah, I., Adu-Gyamfi, C., & Mensah, G. (2008). Correlates of HIV, HBV, HCV and syphilis infections among prison inmates and officers in Ghana: A national multicenter study. BMC infectious diseases, 8(1), 1-12.

[3] Al Taie, A. A., Ibrahim, M. A., Fadhil, K. B. (2019). Epstein-Barr virus infection in thalassemia patients related to blood group in Mosul, Iraq. Annals of Tropical Medicine & Public Health, 22(8): 238-246.

[4] Anejo-Okopi, J., Julius, O. O., Adamu, N., Ogbonna, J. & Odugbo, I. G. (2019). Epstein - Barr virus Capsid Antigen (EBV-VCA) IgM Antibodies among HIV Infected Individuals in Jos, Nigeria. Trends Journal of Sciences Research

[5] Bishop, H. G. & Adegoke, O. (2016). Seroprevalence of Epstein-Barr Virus IgM Antibodies among HIV-Patients and Apparently Healthy Blood Donors Attending Ahmadu Bello University Teaching Hospital, Shika-Zaria, Nigeria. International Journal of Scientific Research in Knowledge. 4. 105-111.

[6] Chakraborty, N., Bhattacharyya, S., De, C., Mukherjee, A., Bhattacharya, D., Santra, S. & Chakrabarti, S. (2010). Incidence of multiple Herpesvirus infection in HIV seropositive patients, a big concern for Eastern Indian scenario. Virology journal, 7(1), 1-7.

[7] Cirac, A., Stützle, S., Dieckmeyer, M., Adhikary, D., Moosmann, A., Körber, N. & Mautner, J. (2018). Epstein–Barr virus strain heterogeneity impairs human T-cell immunity. Cancer Immunology, Immunotherapy, 67(4), 663-674.

[8] Cui, J., Yan, W., Xu, S., Wang, Q., Zhang, W., Liu, W. & Ni, A. (2018). Anti-Epstein–Barr virus antibodies in Beijing during 2013–2017: what we have found in the different patients. PLoS One, 13(3), e0193171.

[9] Higgins, C. D., Swerdlow, A. J., Macsween, K. F., Harrison, N., Williams, H., McAulay, K. & Crawford, D. H. (2007). A study of risk factors for acquisition of Epstein-Barr virus and its subtypes. The Journal of infectious diseases, 195(4), 474-482.

[10] Huang, Y., Wei, C., Zheng, K. & Zhao, D. (2013). The impact of serological features in Chinese children with primary or past Epstein–Barr virus infections. Virology journal, 10(1), 1-8.

[11] Kieff, E. & Rickinson, A. B. (2007). Epstein-Barr virus and its replication, p. 2603-2654. In D. M. Knipe, P. M. Howley, D. E. Griffin, R. A. Lamb, M. M. Martin, B. Roizman, and S. E. Straus (ed.), Fields virology, 5th ed., vol. II. Lippincott Williams & Wilkins, Philadelphia, PA.

[12] Kolawole, O. E., Kola, O. J. & Elukunbi, A. H. (2017). Detection of Epstein-Barr virus IgM in HIV infected individuals in Ogbomoso, Nigeria. British Journal of Virology, 3(6), 177-182.

[13] Kuri, A., Jacobs, B. M., Vickaryous, N., Pakpoor, J., Middeldorp, J., Giovannoni, G. & Dobson, R. (2020). Epidemiology of Epstein-Barr virus infection and infectious mononucleosis in the United Kingdom. BMC Public Health, 20(1), 1-9.

[14] Levine, H., Balicer, R. D., Rozhavski, V., Halperin, T., Shreberk, M., Davidovitch, N. & Ankol, O. E. (2012). Seroepidemiology of Epstein– Barr virus and cytomegalovirus among Israeli male young adults. Annals of Epidemiology, 22(11), 783-788.

[15] Lino, C. N. R. & Ghosh, S. (2021). Epstein–Barr Virus in Inborn Immunodeficiency—More Than Infection. Cancers, 13, 4752.

[16] Okonko, I. O. & Egbogon, J. (2022). Plasma Immunoglobulin-G (IgG) Against Epstein Barr Virus Nuclear Antigens among University Students in Port Harcourt, Nigeria. Biotechnology, 21: 110-119

[17] Patel, S. S., Singh, S., Sahu, C., Ghoshal, U., & Verma, H. (2021). A three-year Seroepidemiological and molecular study of Epstein–Barr virus infection among different age groups with hematological malignancies in a Tertiary care centre of North India (2017-2019). Journal of Family Medicine and Primary Care, 10(1), 373.

[18] Rezk, S. A., Zhao, X. & Weiss, L. M. (2018). Epstein-Barr virus (EBV)-associated lymphoid proliferations, a 2018 update. Human Pathology. 79, 18–41.

[19] Sandler, S. G. & Mallory, D. (1995). Biological functions of the blood groups in health and disease. Haematologia (Budap)., 27(1), 1-13.

[20] Schaftenaar, E., Verjans, G. M., Getu, S., McIntyre, J. A., Struthers, H. E., Osterhaus, A. D. & Peters, R. P. (2014). High seroprevalence of human herpesviruses in HIV-infected individuals attending primary healthcare facilities in rural South Africa. PLoS One, 9(6), e99243.

[21] Shi, T., Huang, L. & Tian, J. (2022). Prevalence of Epstein-Barr Viral DNA among children at a single hospital in Suzhou, China. Jornal de Pediatria, 98, 142-146.

[22] Smatti, M. K., Al-Sadeq, D. W., Ali, N. H., Pintus, G., Abou-Saleh, H. & Nasrallah, G. K. (2018). Epstein–Barr virus epidemiology, serology, and genetic variability of LMP-1 oncogene among healthy population: an update. Frontiers in oncology, 8, 211.

[23] Sureshbabu, M. (2022). Pediatric Mononucleosis and Epstein - Barr virus Infection. https://emedicine.medscape.com/article/963894-guidelines. Accessed January 10, 2023.