SEROPOSITIVITY OF EPSTEIN-BARR VIRUS IMMUNOGLOBULIN G AMONG HEALTH CARE WORKERS IN PORT HARCOURT, NIGERIA

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ABSTRACT
Epstein-Barr Virus has been recognized as one of the primary causes of infectious mononucleosis and cancer, in addition to being the most common, easily transmissible infection in the world. Thus this study aimed at determining the seropositivity of Immunoglobulin G (IgG) antibodies against Epstein Barr virus (EBV) nuclear antigens (EBNA) among healthcare workers (HCWs) in Port Harcourt, Nigeria. Serum samples from 93 consenting HCWs in Obio Cottage hospital, Ozuoba primary healthcare centre, and Rivon Clinic, all in Port Harcourt, Nigeria, were analyzed using enzyme-linked immunosorbent assay (ELISA). Results showed 78.5% seropositivity of EBNA IgG antibodies. Seropositivity rate was higher in ages 29-39 years (79.2%), males (85.0%), widowed/separated (100.0%), security (100.0%), theatre attendants (100.0%), dentists (100.0%) and Radiologist (100.0%). The study showed that the seropositivity of EBNA in Port Harcourt, Nigeria is high. Thus, healthcare workers need sustained and increased education on the need to adopt EBV prevention and control protocols while carrying out their duties.

Keywords: Antibodies, EBV, EBNA, IgG, Healthcare workers

1. INTRODUCTION
Epstein-Barr virus (EBV) is also called human herpes virus 4 (HHV4). It is a double-stranded DNA virus belonging to the family Herpesviridae, subfamily Gamma Herpesvirinea and genus Lymphocriptovirus (Kieff & Rickinson, 2007; Mustapha et al., 2020). It is a virus found worldwide and infects nearly all humans by the time they reach adulthood. Consequently, most adults have serological evidence of past infection (Okonko et al., 2020).
EBV is associated with several diseases whose incidence differs drastically in different parts of the world (Kieff & Rickinson, 2007; Kafita et al., 2018). EBV infection is the primary cause of infectious mononucleosis (IM), a worldwide infection with a lifetime prevalence of 90% (Fugl & Andersen, 2019). It is also involved in the cause of other diseases like Burkitt lymphoma, Hodgkin lymphoma, nasopharyngeal carcinoma and gastric carcinoma, multiple sclerosis, lymphomatoid granulomatosis, and oral hairy leukoplakia (Shi et al., 2022). Primary infection results in transient viremia followed by a rapid immune response (Gulley, 2001). The virus later becomes latent and subclinical in the body (Okonko et al., 2020). In some cases, the virus may reactivate. This virus does not always cause symptoms, but people with weakened immune systems are more likely to develop symptoms if EBV reactivates (CDC, 2020).

The course of EBV infection is determined by the virus load and an individual's immune system state, which in turn is determined by the person's gene composition, other infection histories, and several environmental factors, which all may influence the immune capacity of

25

a person to various degrees (Houen & Trier, 2021). Diagnosing EBV infection can be challenging because the symptoms are similar to other illnesses (CDC, 2020). However, serology is reported to be the simplest way to test for EBV infection, and past infection is deduced from the presence of IgG antibodies to EBV antigens.

Most publications on the incidence and prevalence of EBV antibodies in children and adults have reported high seroprevalence rates of EBV IgG antibodies, especially in HIV-infected patients, falling within the range of 60% to about 95%. In Brazil (71%) (Figueira-Silva & Pereira, 2004), and Iran 81.4% (Sharifipour & Rad, 2020) and Germany 93.1% (Abrahamyan et al., 2020). A meta-analysis of the prevalence of EBV among Nigerian patients suffering from various diseases revealed a prevalence of 20.3% (Irekeola et al., 2022). Other studies in Abakaliki reported a seroprevalence rate of 95.6% (Okonko et al., 2020).

This study aims to determine the seropositivity of IgG antibodies against Epstein Barr virus nuclear antigens (EBNA) among healthcare workers in Port Harcourt, Nigeria. It is obvious that EBV antibodies are circulating in persons in Nigeria, and yet there is no national program to vaccinate susceptible individuals against the virus. Thus, infections with EBV will still occur among individuals, posing more risk to Health care workers and immunocompromised individuals. While significant progress has been made in understanding EBV and its associated diseases, there is still much to learn about the virus's biology and pathogenesis. Ongoing research has focused on developing new therapies and improving our understanding of the virus-host interaction in EBV-associated diseases.

2. MATERIALS AND METHODS
2.1 Study Design
This study is a cross-sectional study on the seropositivity of EBNA IgG antibodies among healthcare workers in Obio Cottage hospital, Ozuoba Primary Healthcentre, and Rivon Clinic, Port Harcourt, River State, Nigeria.

2.2 Study Area
This study was carried out in Port-Harcourt, Rivers State. Rivers state lies between Latitude 4''30˚N and 5"45˚N approximately longitude 6"30˚E of the Greenwich meridian, with a total area of 11,077km^2 with a mean annual rainfall which ranges from 4,700 mm on the coast to about 1,700 mm (National Bureau Statistics, 2012). Obio-Akpor Local government area of Rivers State, Nigeria, is located between latitudes of 4°45'N and 4°60'N and longitudes of 6°50'E and 8°00'E. It covers 100 square meters, that are 260 kilometres.

2.3 Study Population
The targeted population constituted healthcare workers in Obio Cottage hospital, Ozuoba primary health centre, and Rivon Clinic, Port Harcourt. The persons included in this research work comprised healthcare workers who served in health centres in Port Harcourt, on which they consented to participate in the study. Those excluded from this study were non-consenting health workers.

2.4. Sample Collection, Transport, Preparation & Storage
From the study population, 93 blood samples were collected from healthcare workers. Four millimetres of blood samples were aseptically collected from each patient into sterile EDTA tubes. The collected blood samples were transported with an ice pack to the Virus & Genomics

Research Unit. In the laboratory, the blood samples were centrifuged, after which serum samples were collected using a micropipette. After collection, the serums were stored at -20°C till the analysis.

2.5 Serological Analysis

Serum samples were analyzed using Enzyme-Linked Immunosorbent Assay (ELISA manufactured by DIA.PRO Diagnostic Bioprobes Srl, Milano – Italy) for the qualitative determination of IgG antibodies to Epstein Barr Virus Nuclear Antigen (EBNA) in human plasma and sera according to manufacturer's instruction.

3. RESULT

3.1 Patients Characterization

In this study, 93(100.0%) healthcare workers (HCWs) were tested for EBNA IgG antibodies. Their age range was between 18-61 years—the socio-demographic data of the health workers stratified as shown in Table 1.

3.2 ELISA Results Analysis

Of 93(100%) persons tested for EBV antibody, 73(78.5%) were positive, and 20 (21.5%) test ed negative (Table 1). This study shows that the prevalence of EBNA IgG is higher among in dividuals within the age range 29-39 years (79.2%) than in other age groups. Nevertheless, th ere was no significant relationship ($p > 0.05$) between the age groups and the prevalence of E BNA antibodies (Table 1). It also showed a higher prevalence in males (85.0%) than in femal es (76.7%). Nevertheless, there was no significant relationship ($p > 0.05$) between the gender and prevalence of EBNA IgG antibodies (Table 1).

A higher prevalence was seen in those who were widowed or separated (100.0) than in the ma rried (80.3%) and singles (70.8%). Nevertheless, there was no significant relationship ($p > 0.0$ 5) between marital status and the prevalence of EBNA IgG antibodies (Table 1).

From the result obtained, the percentage of positive individuals concerning their profession sh owed a higher prevalence among security, theatre attendants, dentists, and radiologists (100.0 %) than other professions (Table 1). Nevertheless, there was no significant relationship ($p > 0$ .05) between the profession and the prevalence of EBNA IgG antibodies (Table 1).

Table 1: EBNA IgG and socio-demographic characteristics of Healthcare Workers (HCWs)

Characteristics	Categories	No. Tested	No. Positive	Percentage
Age (years)	18-28	19	14	73.7
	29-39	53	42	79.2
	40 & above	21	16	76.2
Gender	Females	73	56	76.7
	Males	20	17	85.0
Marital status	Married	61	49	80.3
	Singles	24	17	70.8
	Widowed/Separated	8	8	100.0
Professions	Medical doctor	10	9	90.0

27

	Nurse	17	13	76.5
	Administrative Staff	19	16	84.2
	Security	2	2	100.0
	Lab. Scientist	12	7	58.3
	Pharmacist	9	7	77.8
	Physiotherapist	3	2	66.7
	Theatre attendants	2	2	100.0
	Cleaning staffs	15	10	66.7
	Dentist	1	1	100.0
	Students	2	1	50.0
	Radiologist	1	1	100.0
Total		**93**	**73**	**78.5**

4. DISCUSSION

The detection of IgG antibodies in sera has been a valuable and standard method in determining the incidence and prevalence of EBV infection and the level of immunity in a population (Sharifipour & Rad, 2020). In this study, the seropositivity of EBNA IgG antibodies among Healthcare Workers (HCW) in Port Harcourt was 78.5%. This result is in contrast with the study of Kuri et al. (2020)) having an overall prevalence of 85.3%. This study also agrees with the study of Westmoreland et al. (2016), who reported 89.0%. Also, several investigators have reported a low prevalence rate of EBNA IgG in other countries such as Ghana 20.0% (Adjei et al., 2008), and Nigeria, where 20.3% was reported from a systematic review and meta-analysis of EBV infection (Irekeola et al., 2022). These results are consistent with the results of studies of EBV seroprevalence in different populations worldwide (Farid & Al-Biltagi, 2018; Beader et al., 2018; Zając et al., 2020). Unfortunately, the scientific literature does not provide data on the prevalence of EBV IgG antibodies among the professional group in Nigeria and other countries, which would allow us to assess and compare the obtained research findings (Zając et al., 2020). The changes in prevalence may be due to socio-demographic factors, hygiene practices, and, basically, the insensitivity to the health condition of the individuals in the environment.

The seropositivity of EBNA IgG antibodies among the study population with age was seen to be higher in 30-39 years (79.2%) than 18-28 (73.7%) and $\geq$40 years (76.2%). This result disagrees with Okonko and Egbogon (2022), who reported a higher prevalence in those within 17-25 years in Port Harcourt, Nigeria. Some studies, however, in other populations have demonstrated a decline in age-adjusted seroprevalence of EBV (Fourcade et al., 2017). Moreover, the consistently higher EBV IgG seroprevalence among all age groups may reflect a genuine increase in seroprevalence in the study area, differences in population sampling, or different sensitivity and specificity of the assays used (Kuri et al., 2020).

In this study, males had higher EBNA IgG prevalence (85.0%) than females (76.7%). This result disagreed with Okonko et al. (2022), who reported a higher prevalence in females than males in Port Harcourt, Nigeria. It negates the findings by Okonko et al. (2020), who reported a higher prevalence in females than males in Abakaliki, Nigeria. The increased prevalence

among males over their female counterparts may be due to increased sexual practices among men and their randy lifestyle.

Epstein-Barr nuclear antigen (EBNA) IgG antibodies in HCWs were more prevalent in widowed and separated persons (100.0%) than singles (80.3%) and married (70.8%). This finding agrees with the study by Joseph Anejo-Okopi et al. (2019) in Jos, Nigeria, where a higher prevalence was seen in widows than in married and singles. It also agrees with Okonko et al. (2022), who reported a higher prevalence in singles in Port Harcourt, Nigeria. However, this may be a result of promiscuity.

In this study, EBNA IgG antibodies were more prevalent among security, theatre attendants, dentists, and radiologists (100.0%) than in other professions. However, this finding contrasts with Zając et al. (2020), who reported a higher prevalence among paediatrics, translatology, and primary health care nurses. It deviated from that of Okonko et al. (2022), who reported a higher prevalence among unemployed than employed participants in Port Harcourt, Nigeria.

5. CONCLUSION

The study has shown high seropositivity of IgG antibodies against Epstein Barr nuclear antigens (EBNA) among healthcare workers (HCWs) in Port Harcourt, Nigeria. It can be seen that EBNA can affect HCWs of different ages, marital statuses, and occupational levels in different health centres. Therefore, the need for a continuous public health campaign to create and leave a long-lasting awareness on the prevention of the disease, ways of contacting the infection, and treatment/management of EBV infections are also essential to limit the spread of the virus. Early and accurate laboratory diagnosis of EBV infection is critical for effectively managing the infection.

REFERENCES

Abrahamyan, A., Eberspacher, B., Hoshi, M. M., Aly, L., Felix, Luessi, Groppa, S., Klotz, L., Meuth, S. G., Schroeder, C., Gruter, T., Tackenberg, B., Paul, F., Then-Bergh, F., Kumpfel, T., Weber, F., Stangel, M., Bayas, Antonios., Wildemann, B., Heesen, C., Zettl, U., Warnke, C., Anthony, G., Hessler, N., Wiendl, H., Bittner, S., Hemmer, B., Gold. R., Salmen, A., Ruprecht, K. (2020). Complete Epstein-Barr virus seropositivity in a large cohort of patients with early multiple sclerosis. *Journal of Neurology, Neurosurgery and Psychiatry*,0, 1-6.

Adjei, A. A., Armah, H. B., Gbagbo, F., Boamah, I., Adu-Gyamfi, C. and Asare, I. (2008). Seroprevalence of HHV-8, CMV, and EBV among the general population in Ghana, West Africa. *BMC Infectious Disease*, 8, 111.

Beader, N., Kolarić, B., Slačanac, D., Tabain, I., & Vilibić-Čavlek, T. (2018). Seroepidemiological Study of Epstein-Barr Virus in Different Population Groups in Croatia. *The Israel Medical Association journal: IMAJ*, *20*(2), 86–90.

CDC, (2020). Epstein-Barr virus and infectious mononucleosis.

Farid, E., & Al-Biltagi, M. (2018). Trend and seroprevalence of Epstein-Barr virus in Bahrain: 2001-2015. *Eastern Mediterranean health journal = La revue de sante de la Mediterranee orientale = al-Majallah al-sihhiyah li-sharq al-mutawassit*, *23*(12), 821–829.

Figueira-Silva, C. M. & Pereira (2004). Prevalence of Epstein-Barr virus antibodies in healthy children and adolescents in Vitoria, State of Espirito Santo, Brazil. *Revista da Sociedade Brasileira de Medicina Tropical*, 37(5), 409-412.

Fourcade, G., Germi, R., Guerber, F., Lupo, J., Baccard, M., Seigneurin, A., Semenova, T., Morand, P. & Epaulard, O. (2017). Evolution of EBV seroprevalence and primary infection age in a french hospital and a city laboratory network, 2000-2016. *PLoS One*, 12(4), e0175574.

Fugl, A. & Andersen, C. L. (2019). Epstein-Barr virus and its association with disease- a review of relevance to general practice. *BMC Fam Prac*.,20(1), 62.

Gulley, M. L. (2001). Molecular diagnosis of Epstein-Barr virus-related diseases. *Journal of Molecular Diagnostics*, 3,1.

Houen, G. & Trier, N. H. (2021). Epstein-Barr virus and systemic autoimmune diseases. *Frontiers in Immunology*,7(11), 587380.

Irekeola, A. A., Syafirah, E. N. E. A. R., Wada, Yusuf, Mohamud, R., Lazim, N. M., Yean, C. Y. & Shueb, R. H. (2022). *Indian Journal of Medical Microbiology*, 40(3), 420-426.

Kieff, E. & Rickinson, A. B. (2007). *Epstein-Barr virus and its replication*. In: Field's Virology, Lippincott Williams and Wilkins, Philadelphia, pp. 2603–2654.

Kafita, D., Kaile, T., Malyangu, E.,Tembo, R., Zulu, E., Chisanga, C., Kalonda, A., Samutela, M., Polepole, P. & Kwenda, G. (2018). *Pan African Medical Journal*, 29, 181.

Kuri, A., Jacobs, B. M., Vickaryous, N., Pakpoor, J., Middledorp, J., Giovannoni, G. & Dobson, R. (2020). Epidemiology of Epstein Barr virus infection and infectious mononucleosis in the United Kingdom. BMC Public Health, 20(1), 912.

Okonko I. O., Adim, C. C., Innocent-Adiele, H. C. & Cookey, T. I. (2022). Seroprevalence of Epstein-Barr virus IgG antibody among patients presenting at a tertiary hospital in Port Harcourt, Nigeria. *International Journal of Multidisciplinary Research Updates,* pp. 4, 001–007.

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.

Okonko, I. O., Makinde, T. S., Okonko, B. J., & Ogbu, O. (2020). Immunological and epidemiological evaluation of EBV infections among HIV-1 infected individuals in Abakaliki, Nigeria, supports the potential use of neutrophils as a marker of EBV in HIV disease progression and as useful markers of immune activation. *Journal of immunoassay & immunochemistry*, 41(2), 158–170.

Patel, D.,

Sharifipour, S. & Rad, K. D. (2020). Seroprevalence of Epstein-Barr virus among children and adults in Tehran, Iran. *New Microbes and New Infections*, p. 34, 100641.

Shi, T., Huang, L. & Tian J. (2022). Prevalence of Epstein-Barr virus DNA among children at a single hospital in Suzhou, China. *Journal de Pediatria*, 98(2), 142-146.

Westmoreland, K. D., Stanley, C. C., Montgomery, N. D., Kaimila, B., Kasonkaji, E., El-Mallaawany, N. K., Waasswa, P., Mtete, I., Butia, M., Itimu, S., Chasela, M., Mtunda, M., Chikasema, M., Makwakwa, V., Kampani, C., Dhugel, B. M., Sanders, M. K., Krysiac, R., Tomoka, T., Liomba, N. G., Dittmer, D. P., Fedoriw, Y. & Gopal, S. (2017). Hodgkin Lymphoma, HIV, and Epstein-Barr virus in Malawi: Longitudinal results from the Kamuzu Central Hospital Lymphoma study. *Pediatric Blood and Cancer*, 64(5), e26302.

Zając, P. W., Czarkowska-Pączek, B., & Wyczałkowska-Tomasik, A. (2020). Prevalence and molecular epidemiology of CMV and EBV among nurses working in paediatrics, translatology, and primary health care. *Journal of occupational health*, *62*(1), e12112. https://doi.org/10.1002/1348-9585.12112Farrell, P. J. (2015). Epstein–Barr virus strain variation. *Epstein Barr Virus Volume 1: One Herpes Virus: Many Diseases*, 45-69.