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ORIGINAL RESEARCH ARTICLE

Serological Evidence of Epstein-Barr Virus Capsid Antigen (EB-CA) Among Pregnant Women from Cottage Hospital, Ilorin

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ABSTRACT
Epstein-Barr Virus (EBV), a member of the Herpesvirus family, has been observed to be increasing among pregnant women, leading to congenital defects. This study aimed to determine the prevalence of EBV infection among pregnant women attending Cottage Hospital in Ilorin. A total of 45 pregnant women were tested, and questionnaires were administered to volunteer subjects after obtaining their informed consent. Approximately 5 ml of blood was aseptically collected via venous puncture from the antecubital fossa and dispensed into EDTA containers. Plasma was separated after centrifugation of the blood, and the obtained plasma was screened for anti-EBV IgG antibodies using ELISA techniques. Results revealed that 43 subjects (95.69%) tested positive for EBV, while 2 (4.4%) tested negative. Among different age groups, women aged 19-20, 26-30, and 36-40 showed a prevalence rate of 100%, with the lowest prevalence of 88.8% recorded among the 31-35 age group. Regarding the pregnancy stage, 100% was seen among first-trimester participants, while the lowest prevalence of 92.3% was noted during the third trimester. The majority of respondents were married, with those from polygamous families and single individuals showing a prevalence of 100%. Participants with primary and secondary education levels displayed the highest prevalence, while those with tertiary education had the lowest. Muslims had the highest prevalence, whereas Christians had the lowest. Moreover, regarding occupation, the unemployed, artisans, and students had the highest prevalence, while civil servants and businesswomen had the lowest prevalence. In conclusion, the results from this study highlight a notable prevalence of EBV (IgG) antibodies among pregnant women. These outcomes emphasize the necessity of raising awareness in the community about the implications of EBV infection and the Significance of maintaining proper personal hygiene to mitigate the chances of infection and spread.

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INTRODUCTION
Epstein-Barr virus (EBV) was the first isolated human tumor virus, identified in 1964 by Epstein's group in a cell derived from Burkitt's lymphoma (Epstein *et al.*, 1964). EBV is a member of the herpesvirus family, which includes herpes simplex 1, herpes simplex 2, varicella-zoster virus (alphavirus subfamily), cytomegalovirus, human herpes 6 and 7 (betaherpesvirus subfamily), human herpesvirus 8 and Epstein-Barr virus (Gammaherpesvirus subfamily), serving as a prototype of the Lymphocryptovirus genus. The enveloped EBV virus consists of a tegument and an icosahedral nucleocapsid surrounding a DNA core (Schafer *et al.*, 2015). Both human herpesvirus 8 (associated with Kaposi's sarcoma, primary effusion lymphoma, and Castleman's disease) and EBV have been implicated in various human cancers, including Burkitt's lymphoma, nasopharyngeal carcinoma, Hodgkin's and non-Hodgkin's lymphoma. While herpesviruses are prevalent in nature, EBV infection is unique to humans. Evidence links EBV to autoimmune disorders such as multiple sclerosis, rheumatoid arthritis, systemic lupus, and dermatomyositis. Despite the common presence of the EBV virus, most carriers remain asymptomatic (Ai *et al.* 2012).

EBV infects more than 90% of adults globally (Schafer *et al.*, 2015). Upon infection, the individual becomes a lifelong carrier of the virus. Swallowing an infected individual's saliva can spread EBV. During acute infection, EBV predominantly infects and replicates in the oropharynx's stratified squamous epithelium. The B cells'

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latent infection comes next. The oropharyngeal lymphoid organ is believed to be where EBV infects B lymphocytes. In healthy carriers, the virus continues to circulate in memory B cells. The virus's capacity to render normal resting B lymphoblastoid cell lines immortal proves that EBV is a B lymphotropic virus.

Furthermore, viruses are released more frequently into saliva in the initial stages of illness. However, they can persistently be shed from the oropharynx into saliva for prolonged periods, spanning years. Once the virus has established itself in the B lymphoid compartment, it can reactivate from a dormant state in any mucosal site, such as the cervix, where B cells are present (Schafer *et al.*, 2015).

Primary Epstein-Barr virus (EBV) infection in early childhood is typically asymptomatic in the majority of developing countries. In more developed regions, primary infection onset, leading to infectious mononucleosis, may be delayed until late adolescence. Antibody detection is less informative in immunocompromised individuals due to impaired immune function, leading to variable antibody characteristics and maintenance dynamics over time-based on disease progression (De Paschale*et al.*, 2012). Antibodies against the viral capsid antigen (VCA) and EBV nuclear antigen-1 (EBNA-1) serve as markers for viral reactivation and infection history, respectively, commonly used in combination to investigate latent and lytic infection stages. According to Sallah *et al.* (2017), inter-individual variability in IgG responses to EBNA-1 and VCA is a heritable trait ranging from 32% to 48%. This study aims to assess the prevalence of EBV antibodies among pregnant women at Cottage Hospital, Ilorin, Kwara State.

MATERIALS AND METHODS

Study location

The study was conducted at Cottage Hospital, Ilorin, Kwara State, Nigeria. This healthcare facility is under the state government's jurisdiction and is located next to the Yebumot Hotel on Adewole Road in Ilorin, Kwara State.

Study population

A total of 45 participants, exclusively comprising pregnant women at Cottage Hospital in Ilorin, Kwara State, were enrolled in the study. The pregnant individuals included in the research ranged in age from 19 to 40. Informed consent was obtained from the participants before the commencement of sample collection.

Sample collection

5 ml of blood samples were obtained from each participant through venipuncture into sterile plain bottles. Subsequently, the collected samples were transported under cold chain conditions to the University of Ilorin, Department of Microbiology. Upon arrival, the samples underwent centrifugation at 3000 revolutions per minute (rpm) for 5 minutes. After centrifugation, the sera were carefully separated and transferred into appropriately labeled cryovials, which were then stored at -20°C until needed for further analysis.

Ethical approval

The ethical clearance for this study was obtained from the Kwara State Ministry of Health in Ilorin.

Materials

ELISA kit and its components, along with other necessary chemicals (Horseradish peroxidase (HRP), phosphate-buffered saline (PBS), sulfuric acid (H2SO4), 3,3',5,5'-Tetramethylbenzidine (TMB), bovine serum albumin (BSA) were procured from a supplier in Ilorin, Kwara State. All materials were utilized following standard laboratory procedures and manufacturer guidelines. The study upheld calibrated equipment, appropriate storage conditions, and compliance with quality control measures to ensure precise and dependable outcomes.

Storage and Stability of the testing kit

The sealed test kit and micro-assay plate were stored at 2°C to 8°C. Unused strips were promptly resealed in a resealable bag with desiccant and returned to storage within the same temperature range. The HRP conjugate, Calibrator, High Positive Control, Low Positive Control, and Negative Control were kept at 2°C to 8°C. Serum diluent type 1, 20X wash buffer type 1, and chromogen/substrate type 1 were similarly stored between 2°C and 8°C. Moreover, 1X (diluted) wash buffer was maintained at room temperature (21°C to 25°C) for up to 5 days or up to one week if stored at 2°C to 8°C.

Principle of the assay

The Enzyme-Linked Immunosorbent Assay (ELISA) relies on the capacity of biological materials, particularly antigens, to bind to plastic surfaces, like polystyrene (solid phase) (Zhou *et al.*, 2012). As antigens attached to the solid substrate come into contact with the patient's serum, antibodies specific to those in the serum will bind to the antigens on the solid substrate, forming complexes between antigens and antibodies.

Subsequent to this binding, excess antibodies are removed through a washing step. After washing, a conjugate of goat anti-human IgG with horseradish peroxidase is applied, forming associations with the antibody-antigen complexes. The surplus conjugate is subsequently eliminated through additional washing, and tetramethylbenzidine (TMB) is added. A blue hue emerges if the patient's serum possesses a particular antibody against the antigen. The enzymatic process is arrested by adding 1N H_2SO_4, transforming well contents into yellow (Lecordier*et al.*, 2000).

The resulting color, indicative of the antibody concentration in the serum, can be measured using an appropriate spectrophotometer or an ELISA microwell plate reader. The sensitivity, specificity, and

reproducibility of ELISAs are comparable to other serological antibody tests, such as immunofluorescence, complement fixation, hemagglutination, and radioimmunoassay (Chiswick*et al.,* 2011).

Assay procedure

The process and utilization of microwell plates outlined below adhere to the established procedures for conducting an enzyme-linked immunosorbent assay (ELISA), as demonstrated by Mahmoud and AlQahtani (2016) and Shah and Maghsoudlou (2016) with slight modifications briefly explained.

The microwell frame was loaded with the specified number of strips. Each run allowed for the determination of six control/cutoff calibrators, including a negative control, three calibrators, and one each of high positive and low positive controls. An assay with a reagent blank was carried out simultaneously, and the correct control/calibrator arrangement was validated following software and reader specifications. Any unused strips were returned to a sealable bag with desiccant, sealed, and promptly refrigerated.

For the preparation of diluted test sera, Cutoff Calibrator, and control sera (diluted at a ratio of 1:21, e.g., 10µl + 200µl), serum diluents were dispensed into the test tube first, followed by the addition of the patient's serum. Subsequently, 100µl of the suitably diluted calibrator, controls, and patient sera were dispensed into individual wells. In the reagent blank well, 100µl of serum diluents was added, and the correct reagent blank well configuration was verified based on software and reader requirements. Each well underwent incubation at room temperature (21°C to 25°C) for 25 minutes (Mahmoud and AlQahtani 2016)

The liquid was removed from each well, and 250-300µL of diluted wash buffer was introduced into each well. This process was repeated four times, totaling five washes. The plate was then blotted on paper toweling to remove any remaining liquid. After the final wash, each well was inspected thoroughly to confirm the absence of wash buffer and bubbles. Subsequently, 100µl of conjugate, including the reagent blank well, was added to each well with careful attention to prevent bubble formation. Each well was then incubated at room temperature (21°C to 25°C) for 25 minutes.

Following this incubation, 100µl of the chromogenic substrate was dispensed into each well, including the reagent blank well, maintaining a consistent rate of addition across the plate. The plate was then incubated at room temperature (21°C to 25°C) for 10-15 minutes. In the same order of substrate addition, the reaction was halted by adding 100µl of stop solution (1N H_2SO_4), which also included the reagent blank well. To ensure thorough mixing of well contents, the plate was gently tapped along the periphery and held for one hour after adding the stop solution before readings were taken. The resulting yellow coloration was measured using an ELISA plate reader with a 450nm filter. The plate was aseptically disposed of after obtaining the readings.

Data Analysis

The data were analysed using SPSS v25 software package. The Chi-square test of association was used for group comparison; a p-value less than 0.05 was considered statistically significant.

RESULTS

Of the total 45 samples tested, 43 were positive, resulting in a prevalence of 95.6%, with 2 samples testing negative, accounting for 4.4%, as depicted in Figure 1. Data on the pregnant women enrolled in the study were collected, including age, marital status, parity, gestational age, family type, education level, religion, and occupation. The prevalence of EBV IgG antibodies concerning socio-demographic factors is presented in Table 1.

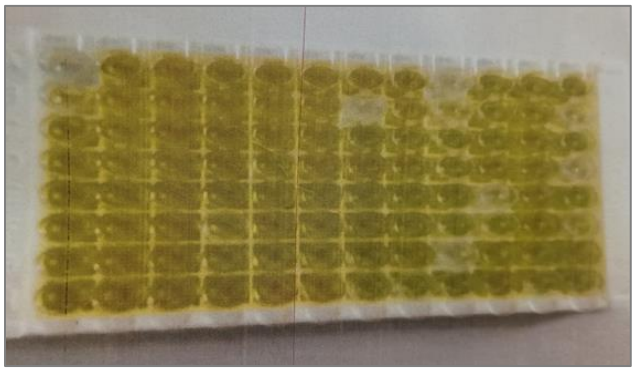

Plate 1: Microwell plate showing positive EBV

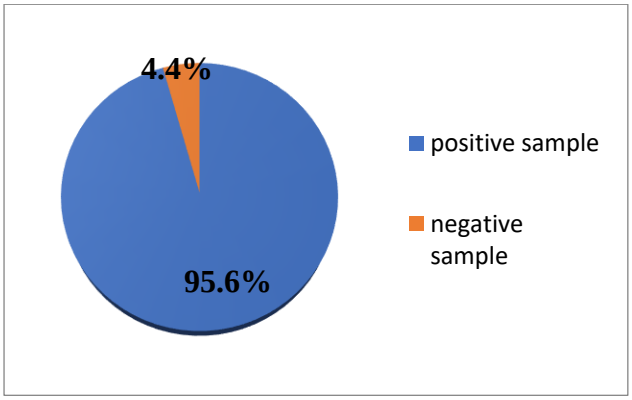

Figure 1: Prevalence of Epstein Barr virus among respondents

The highest prevalence of 100% was recorded among the age groups 19-20 and 26-30, while the lowest prevalence of 88.8% was observed in the age group 31-35. Additionally, the age group 21-25 had a prevalence of 94.4%, as depicted in Table 1.

Furthermore, in relation to their educational level, the highest prevalence of 100% was recorded among subjects who attained a primary level of education and a secondary level of education, while those with a tertiary level of education had the lowest prevalence of 90.9%. In terms of

religion, Muslims had the highest prevalence of 96%, while Christians had a prevalence of 95%.

Further analysis of the results based on occupational level shows that the highest prevalence of 100% was recorded among those engaged in student, unemployed, and Artisans, while the lowest prevalence of 90.9% was recorded among civil servants and business individuals, with a prevalence of 95.4% as shown in Table 1.

Table 2 below shows the prevalence of EBV IgG antibodies in relation to family and gynecological parameters. Regarding marital status, subjects who are un-married have the highest prevalence with 100%, while those who are married have a prevalence of 95.4%, as shown in Table 2

The family structure of the subjects indicates that polygamous and single families have the highest prevalence at 100%, while monogamous families have a prevalence of 96.9%. Regarding the parity of the subjects, those with more than two children have the highest prevalence at 100%, while subjects with two or fewer children have a prevalence of 90.5%, as shown in Table 2. This study also presents the prevalence of subjects based on the stage of pregnancy in Table 2. The highest prevalence of 100% was observed in those in their first trimester, while those in their third trimester had the lowest prevalence at 92.3%. A prevalence of 96.7% was recorded in those in their second trimester.

Table 3 shows the distribution of EBV based on health-related criteria and risk factors such as circumcision, blood transfusion, tattoo, incision, surgery, presence of rash, and fever. In relation to circumcision shows a prevalence of 92.8% among those circumcised and a prevalence of 96.7% among un-circumcised

In relation to blood transfusion history, a prevalence of 100% was recorded for those who have ever received blood, while a prevalence of 95.3% was noted for those who have never received blood. Regarding the presence of tattoos, there was no prevalence rate among subjects with tattoos, while those without tattoos had a prevalence of 100%.

Furthermore, analysis of the results by incisions shows that the highest prevalence of 95.5% was recorded among those who had incisions, while a prevalence of zero was observed among those without incisions. Regarding surgeries, the highest prevalence of 100% was noted among those who have undergone surgery, while 95.3% was recorded among those who have never had surgery.

In the analysis based on the presence of a rash, a zero prevalence was recorded among those with a rash and 95.5% among those without a rash. Regarding fever, individuals with a fever had the highest prevalence of 100%, while those without a fever had a prevalence of 94.8%.

Test of association between EBV diagnosis and social demographic factors using Chi-Square

Table 4 shows that the asymptomatic Significance for the chi-squared test of association between age group and EBV diagnosis is 0.895 which is greater than 0.05. This explains that there is no significant association between age group and EBV diagnosis among participants.

Table 5 shows that the asymptomatic Significance for chi squared test of association between occupation and EBV diagnosis is 0.0902, greater than 0.05. This explains that there is no significant association between occupation and EBV diagnosis among participants.

Table 6 shows that the asymptomatic Significance for chi squared test of association between religion and EBV diagnosis is 0.000, less than 0.05. This explains that there is a significant association between religion and EBV diagnosis among participants.

Table 7 shows that the asymptomatic Significance for the test of association between education level and EBV diagnosis is 0.335 which is greater than 0.05. this explains that there is no significant association between education level and EBV diagnosis among patients.

DISCUSSION

The results obtained from this study reveal a notably high seroprevalence rate of Epstein-Barr Virus (EBV) IgG antibodies among pregnant women in Ilorin, Kwara State. The observed 95.6% seropositivity rate of EBV IgG antibodies aligns with findings from various studies indicating the presence of Immunoglobulin G (IgG) due to previous exposure to the virus. This rate also corresponds with Schafer *et al.*'s assertion that over 90% of the global population possesses EBV IgG antibodies (Schafer *et al.*, 2015).

The high prevalence of maternal EBV infection in developing countries, as demonstrated by our study, may be indicative of factors such as suboptimal hygienic practices, socio-economic status, the societal strata of women in developing countries, as well as various risk factors and health-related criteria (Hjalgrim*et al.*, 2007).

The hygienic practices implemented in advanced nations have reduced the likelihood of virus exposure until adolescence, a stage at which transmission becomes more probable through close oral contact. In contrast, infections are often acquired in developing countries in early life. Hjalgrim*et al.* (2007a) identified pre-chewing food for infants as a contributing factor, a common practice in developing countries where mothers chew food before feeding it to their children. In developing nations, the heightened risk of transmitting the Epstein-Barr virus (EBV) can be attributed to exchanging personal belongings, such as toothbrushes and cooking utensils, with infected individuals.

In light of this finding, a notably high prevalence was observed across all age groups, particularly among those aged 19-30, 26-30, and 36-40, each exhibiting a prevalence rate of 100%, surpassing that of the other age brackets. This heightened prevalence may be attributed to factors

such as poor hygiene, a lower level of educational background, a carefree attitude, and an increase in sexual desire. According to Junker (2005), an escalation in sexual activity during the childbearing age, potentially driven by peer pressure, individual desires, or incidents of sexual assault, could contribute to the elevated risk of infection.

Furthermore, the recorded prevalence could be explained by the probability that a significant portion of females in the examined demographic had previously been exposed to and successfully overcome the infection before reaching reproductive age.

The marital status of subjects reveals a prevalence of 95.4% among those who are married, whereas unmarried subjects exhibit a prevalence of 100%. This difference could be attributed to the likelihood that unmarried individuals engage in unprotected sex and may have multiple sexual partners, increasing the risk of infection through sexually transmitted routes. This observation aligns with the findings asserted by Anne and Junker (2005). Additionally, the decrease in prevalence(95.4%) among married women may be attributed to the presence of a loyal partner.

The prevalence of EBV infection in pregnant women correlated with the stage of pregnancy revealed that individuals in their first trimester exhibited the highest prevalence at 100%. This finding aligns with the assertion of Didier and Icart (2000), who indicated that most pregnant women experience an increase in EBV-specific antibodies during the first trimester. The second and third trimesters showed 96.7% and 92.3% prevalence rates, respectively.

Table 1: Distribution of EBV based on socio-demographic factors

Factor	Number of tested respondents	Number of positive respondents	%
Age			
19-20	2	2	100
21-25	18	17	94.4
26-30	14	14	100
31-35	9	8	88.8
36-40	2	2	100
Educational level			
Primary	9	9	100
Secondary	14	14	100
Tertiary	22	20	90.9
Occupation			
Student	1	1	100
Artisan	5	5	100
Business	22	21	95.4
Civil servants	11	10	90.9
Unemployed	6	6	100
Religion			
Islam	25	24	96
Christianity	20	19	95

Table 2: Distribution of EBV based on family and gynecological parameters

Factor	Number of tested respondents	Number of positive respondents	%
Marital status			
Married	44	42	95.4
Unmarried	1	1	
Nature of family			
Single	1	1	100
Monogamy	34	32	94.1
Polygamy	10	10	100
Parity			
≤2	21	19	90.5
>2	24	24	100
Age of pregnancy			
First trimester	1	1	100
Second trimester	31	30	96.7
Third trimester	13	12	92.3

Table 3: Distribution of EBV based on health-related criteria

Factor	Number of tested respondents	Number of positive respondents	%
Circumcision			
Yes	14	13	92.8
No	31	30	96.7
Blood transfusion			
Yes	2	2	100
No	43	41	95.3
Tattoo			
Yes	0	0	0
No	45	45	100
Incision			
Yes	45	43	95.5
No	0	0	0
Surgery			
Yes	2	2	100
No	43	41	95.3
Presence of rash			
Yes	0	0	0
No	45	43	95.5
Fever			
Yes	6	6	100
No	39	37	94.8

Table 4**:** Test of association between age group and EBV diagnosis

	Value	Df	Asymptotic Significance (2-sided)
Pearson Chi-Square	1.649[a]	5	0.895
Likelihood Ratio	2.228	5	0.817
N of Valid Cases	46		

Table 5**:** Test of association between occupation and EBV diagnosis

	Value	Df	Asymptotic Significance (2-sided)
Pearson Chi-Square	1.048[a]	4	0.902
Likelihood Ratio	1.434	4	0.838
N of Valid Cases	44		

Table 6: Test of association between religion and EBV diagnosis

	Value	Df	Asymptotic Significance (2-sided)
Pearson Chi-Square	43.985[a]	2	0.000
Likelihood Ratio	60.649	2	0.000
N of Valid Cases	46		

Table 7:Test of association between education level and EBV diagnosis

	Value	Df	Asymptotic Significance (2-sided)
Pearson Chi-Square	2.188[a]	2	0.335
Likelihood Ratio	2.960	2	0.228
N of Valid Cases	45		

Analysis of the prevalence of EBV infection among pregnant women in relation to their family structures revealed that the highest prevalence of 100% was observed in single and polygamous women, the latter referring to those simultaneously married to multiple husbands over their lifetime. This elevated prevalence may be associated with the sharing of personal items with individuals already infected with EBV. In contrast, women who maintained monogamous marital relationships throughout their lifetime exhibited the lowest prevalence of 94.1%.

Regarding educational background, the study found the highest prevalence rates(100%) at the primary and secondary education levels compared to the 90.9% rate recorded at the tertiary level. This can be attributed to lower educational attainment, possibly resulting from a lack of awareness about the virus's transmission mode. Additionally, the increased risk of EBV infection could be linked to contact with contagious oral secretions, particularly due to pre-chewing practices among children and between mothers and their wards. This observation aligns with the findings of Hjalgrim*et al.* (2007) and may be associated with a lack of sexual education among individuals with primary and secondary education.

Occupational distribution showed no significant correlation between maternal immunity and social class. Elevated prevalence rates were observed among the unemployed, students, and artisans, potentially linked to poor hygiene practices, interactions with diverse individuals in their work settings, and living conditions facilitating virus exposure. Factors like crowded living conditions and lower socio-economic status have been linked to an increased likelihood of being EBV seropositive in early life (Patel et al., 2021). Conversely, civil servants and business professionals displayed lower prevalence rates of 90.9% and 95.4%, respectively, likely influenced by the characteristics of their respective occupations. This observation aligns with prior research suggesting a delayed onset of EBV infection in higher socioeconomic groups. This finding correlates with the work of Pender (2010).

Furthermore, women who had undergone blood transfusions exhibited a prevalence rate of 100%, aligning with the findings of Junker (2005), which highlighted blood transfusion as a significant factor in EBV transmission.

CONCLUSION

In conclusion, the study's findings indicate the presence of EB-NA (IgG) antibodies among the pregnant women screened. IgG antibodies, particularly those targeting specific EBV antigens like VCA and EBNA-1, are essential for diagnosing and understanding the immune response to EBV infection. High seroprevalence of EBV can have far-reaching implications for immunity, maternal health, and fetal development, including severe fetal growth restriction and even stillbirth in some cases. The analysis of EBV infection prevalence concerning pregnancy stage, family structure, educational background, occupational distribution, and history of blood transfusion offers valuable insights into the transmission dynamics in this demographic. These insights enhance our understanding of EBV infection epidemiology in pregnant women, underscoring the importance of tailored interventions and educational initiatives to reduce transmission risks and improve maternal and fetal health outcomes.

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