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Research Article

Prevalence of Epstein-Barr Virus Infection in Paediatric Patients with Adenotonsilar Enlargement

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Abstract

Background: Epstein-Barr Virus (EBV) is a lymphotropic virus that is often responsible for various head and neck diseases. The virus typically remains dormant in lymphoid cells, including adenoid and tonsils, and can be reactivated later in life. Adenotonsilar enlargement is a common outpatient condition seen at the Ear Nose and Throat clinics. The common causes of adenotonsilar enlargement in children include allergy, exposure to chemical irritants and viral infections such as Epstein-Barr Virus. This virus has a trophic effect on nasopharyngeal tissues and has been implicated in the pathogenesis of diseases such as infectious mononucleosis, Burkitt's lymphoma and nasopharyngeal neoplasms.

Objective: This study was designed to determine the prevalence of EBV among paediatric patient with adenotonsilar enlargement who had adenotonsillectomy and compare with controls

Methodology: This was a comparative cross- sectional study that was carried out at the ENT clinic of University of Abuja Teaching Hospital, Gwagwalada. Abuja, Nigeria. All eligible paediatric patients diagnosed with adenotonsilar enlargement were recruited by convenience sampling technique, following ethical approval and consent/assent. A structured questionnaire was used to obtain participant's bio-data and clinical information from the parents or guardians of the participants. EBV status of the participants was evaluated serologically by Chemiluminescence Immuno Assay (CLIA) method. Data was analyzed using the Statistical Package for Social Sciences Version 24 and this was presented in prose, figures, tables and charts

Results: A total of 90 patients were recruited and grouped into the test (45) and control (45). They were age and sex matched $p = 0.480$ $\chi^2 = 0.498$. M: F was 3.3:1, and 2.2:1 in control group The mean age group was 51.7 ± 5.4 months for the test group and 66.5 ± 5.6 months in the control group.

EBV IgG prevalence for the test group was 91.1% and 8.9% were EBV negative.

In the control group EBV IgG prevalence was 82% with 17% being EBV sero-negative. There was also a significant difference between the mean values of the EBV IgG in the test (29.6 ± 2.8) and control (12.4 ± 1.4) groups using the T-test analysis. $T = 12.956$, $p = <0.001$. Only two (2) patients in the test group tested positive to EBV IgM and IgG.

Conclusion: The results suggests that EBV is just as prevalent in our environment as it is in western climes, even though the titers were significantly higher among the test group.

Keywords: Prevalence of EBV; Adenotonsilar Enlargement; Chemiluminescence Immuno Assay Method; Epstein-Barr Virus; Volumetric Analysis

Introduction

EBV is an ever-present virus that can be found in up to 85-95% of adults [1]. It is commoner in females between the ages of 0-25 years, and has a predilection for white ethnicity, non-smokers and underweight people [1,2]. A study done with saliva samples using the GACRIA reagent showed that 91% of school children aged 7 years already had the antibody the EBV which remained positive up to 11 years [2].

Epstein-Barr virus is named after Anthony M Epstein and Yvonne Barr who discovered the virus. The virus also known as Human Herpes Virus 4(HHV4), is a double stranded DNA virus. It consists of a linear double stranded core surrounded by a nucleocapsid which contains glycoproteins. EBV is now considered a category 1 human tumour virus by the International Agency for Research on Cancer (IARC) in 1997 [3].

The virus gains entry into the upper respiratory tract, the invasion may be symptomatic or asymptomatic. Most people infected with EBV gain adaptive immunity –about 95% and the virus persist in the B memory cells which are abundant in the adenoids [3] once the virus infects the host tissue, it proliferates, then enters into a latent phase. The latent virus may then be reactivated to re infect the same cells, other cells in the body or they can be transmitted to another individual.

Studies have been done to show that EBV can be found in the tonsil tissue and adenoid of patients who had adenotonsillectomy without any clinical feature of acute illness [4,5].

To specifically diagnose acute or chronic EBV infection, serology for EBV IgM (acute phase) EBV IgG (chronic phase) or EBV DNA antibodies will usually suffice. Other serological methods will include assay for Viral Capsid antigen IgA, IgM, IgG. Early Antigen IgA, IgM, and EBV DNA antibodies [6]. The GACRIA method can also be used for screening IgA in saliva in large populations [2].

Other methods for detecting EBV infection include In-situ Hybridization, Polymerase Chain reaction and the Chemiluminescent immunoassay (CLIA) and ELISA [7,8]. The CLIA method is a quantitative method which can give absolute value of viral copies present in the serum. It has a higher sensitivity compared with ELISA method. IgM 67,1% for ELISA, 92.2% FOR CLIA. Specificity of 93.8% for ELISA and 100% for CLIA [7].

The nasopharyngeal (adenoid) and palatine tonsils are part of a complex mucosal immune system which form the Waldeyers ring [9,10]. Other components include the lingual and tubal tonsils. Their main function is exogenous antigen sampling and stimulation of immune responses to these antigens to produce antibodies [9] the adenoid is located along the roof and posterior wall of the nasopharynx. The nasopharynx is a rigid conduit space that does not collapse, only a mass can obstruct it. Anteriorly is opens into the choana, the floor is formed by the palate, lateral to it are the fossae of Rossenmuller housing the opening of the Eustachian tubes. The palatine tonsils are located along each lateral oropharyngeal wall between the anterior and posterior faucial pillars. The tubal tonsils are located close to the Torus Tubarus on either side of the Eustachian tube in the lateral nasopharynx. The lingual tonsils are located at the base of the tongue and continuous with the palatine tonsils at the glosso-lingual sulcus [9,10]. Blood supply to the adenoid and tonsils are from the pharyngeal arteries with contribution from facial and internal maxillary arteries. Innervation is from branches of the Glossopharyngeal nerve and Vagus nerve [10,11].

Pathology of the adenotonsilar enlargement comes about in the following ways

- Infection from viral and bacterial agents results in inflammatory oedema, exudation and infiltration of the adenoid and tonsil gland by inflammatory cells. This is the situation in acute infection by bacterial or viral agents [10].
- Chronic reactive hyperplasia is seen in chronic presence of infective agent or chemical agents trapped by inflammatory cells within the adenoid tissue leading to proliferation of lymphoid cells of the gland. This results in increase in lymphoid cells without neoplastic changes. EBV virus commonly replicates and causes trophic effect in the fossa of Rossenmuller in the nasopharynx and the Tonsilar crypts in the palatine tonsils. The consequence of this is sustained increase in the glandular mass, causing obstruction of the nasopharyngeal airway. This is the predominant pathology in adenotonsilar hypertrophy in children [9,10].

The acute infective process alone does not give rise to sustained nasopharyngeal obstruction as the enlargement abates after treatment for infection and inflammation using antibiotics, mucosal

decongestants or anti allergic drugs. However, viruses like EBV tend to become dormant in the lymphoid tissues with recurrent periodic reactivation which can lead to a sustained enlargement that would cause symptoms of obstruction [5].

Functional adenoid tissue is present at birth. Clinically detectable enlargement of the gland is first noticed after the child has had first contact with environmental factors [11]. This is usually when oral feeding (spoon or bottle feeding) is introduced.

This is amplified when the child starts sitting and crawling picking dirt and putting in the mouth. This occurs between the ages three to six months. Within this period, the adenoid is immunologically challenged to play its defensive role.

At about nine to eighteen months, when the child starts walking and exploring wider environment, the child is in contact with new sets of chemicals, particles and biological agents of infection.

The adenoid and tonsils appropriately respond by reactively going hyperplastic in the process of meeting the new immunological challenge.

However, it is known that between the ages of six to eighteen months the adenoids spontaneously undergo hypoplastic changes (atrophy). At this time, the liver and spleen have fully matured to assume the role of immunological defense of the body. This is the situation in about 95% of population9. Only about 5% will have adenoids that are active and showing reactive hyperplasia presenting clinically with features of nasopharyngeal obstruction [11].

Adenotonsilar enlargement can occur due to infectious agents such as viruses or bacteria. It can also occur due to non-infectious agents such as exposure to tobacco smoke as second -hand smoke, gastroesophageal reflux disorders (GERD) and allergy [10]. Viral agents include EBV, CMV, Adenoviruses. Rhinoviruses, Herpes Viruses and Coronaviruses. Bacterial agents include; *Staphylococcus spp, Moraxella catarrhalis, Hemophilus Spp, Streptococcus Spp, Mycoplasma pneumonae, Neisseria Spp* and *anaerobes* such as *Fusobacterium, Pepto streptococcus* and *Prevotella Spp* [9].

The commonest clinical features include snoring, mouth breathing, refusal to eat due to nasal blockage and poor weight gain in younger children, failure to thrive in extreme cases, recurrent otitis media and its sequalae and chronic rhinosinusitis [12-14].

Some patients also present with dental malocclusion with anterior overbite and hypoplastic mandible, due to the lack of the stimulus of the tongue in the oral cavity as these children are mouth breathers [9].

In severe cases there are features of obstructive sleep apnea, bed wetting, poor school work due to daytime somnolence and hyperactivity [11,13,15].

There seems also to be some higher incidence among patients with craniofacial abnormalities [13].

Aims and Objectives

Aim – To determine the prevalence of EBV infection in children with adenotonsilar enlargement and compare with controls.

Objective

To determine the prevalence of EBV infection among children with adenotonsilar enlargement and compare with prevalence among age and sex- matched controls.

Participants and Methods

The study was a comparative cross-sectional study on children 6months (6mo) -17years (17yrs) at the ENT clinic and theatre of University of Abuja Teaching Hospital Gwagwalada, Abuja between April 2022 and March 2023. It involves age and sex matched controls

The inclusion criteria were: Paradise., *et al*. criteria for tonsillectomy, Fujioka criteria >0.67 [16], patients with Brodsky grade 2-4 [17] and sequelae. Control group were age and sex matched children without adenotonsilar symptoms, Brodsky ≤1, who presented with conditions such as cerumen auris or foreign body in the ear or nose.

While the exclusion criteria included: Failure to give consent., acutely ill patients.

Assents were obtained from all participants by proxy via their parents or legal care givers since they were all minors (less than 18 years) before inclusion into the study.

Demographic and clinical information were obtained by proxy (via parents) for age below 12years, while the children 12 and above were guided by their parents to fill in the semi- structured pretested questionnaires.

Study population - Test group were 45 children between 6mo - 17yrs who had adenotonsilar enlargement. Control group were 45 age and sex matched children who did not have symptoms of adenotonsilar enlargement.

3mls of venous blood was withdrawn under aseptic condition into sterile plain sample bottles and immediately placed in a UN3373 cold chain box maintained at 2 to 8 degrees Celsius. This was then taken to the laboratory and then it was spun with a Biobase high speed centrifuge at 12000g for 5 minutes to extract the serum. The serum was placed in a Cryobase and stored at -80 degrees Celsius pending analysis.

Then 10µl of serum was placed into the Snibe commercial reagent kit which uses the CLIA method with the EBV reagent to run a quantitative analysis of EBV IgG and IgM- after thawing for 1 hour.

Daily lot to lot validation was done for each kit

Laboratory coats were washed and disinfected after each use and left in the laboratory. Proper hand washing was done and hands were sanitized with 75% alcohol spray after each procedure.

The results were then recorded in the excel sheet in the column for the corresponding patient.

Data Analysis was done using the Statistical Package for Social Sciences version 24. Quantitative variables – mean, median, standard deviation, range and CI. Qualitative variables are in frequencies.

Results were displayed in figures, texts and tables.

Results

In the study centre adenotonsillectomy accounts for about 48.7% of the surgeries performed in the ENT theatre. An average of 114 patients are operated annually. With M:F ratio of 2:1. The peak age at surgery was 3 years. The most common histology was reactive hyperplasia of lymphoid tissues, forming 90%.

In the test patient category, the mean age was 51.7 ± 5.4 months. The control group had a mean of 66.5 ± 5.6 months (P = 0.107)). See table 1.

	Group				
Variables	**Test n = 45 (%)**	**Control n = 45 (%)**	**Total N = 90 (%)**	**$\chi 2$**	**p-value**
Age (Months)					
0 – 36	22 (48.9)	12 (26.7)	34 (37.8)		
37 – 72	15 (33.3)	17 (37.8)	32 (35.6)		
73 – 120	5 (11.1)	12 (26.7)	17 (18.9)		
121 – 180 Mean ± SD Median	3 (6.7) 51.7 ± 5.4 42.0	4 (8.8) 66.5 ± 5.6 60.0	7 (7.7)	6.091	0.107
Sex					
Male	34 (75.6)	31 (68.9)	65 (72.2)		
Female	11(24.4)	14 (31.1)	25 (27.8)	0.498	0.480

Correlation with EBV IGG value	Correlation coefficient (r)	P-value
Gender	0.027	0.797
Age group	-0.135	0.204

Table 1: Age and Sex Distribution of Participants.

The peak age group among the test population was between 0-36months.

There was no statistically significant difference between the ages of the test and control groups, confirming the age matching of the participants in this study. $\chi 2 = 6.091$ p = 0.107.

There was a general male preponderance among all the patients who were studied with M:F ratio of 3.1:1 and 2.2:1 in the test and control groups respectively (See table 2).

Gender	Test n(%)	Control n(%)	χ2	P-value	Test n(%)	Control n(%)	χ2	P-value
			0.009	0.926			2.568	0.228f
Male	30(73.2)	26(72.2)			4(44.4)	0(0.0)		
Female	11(26.8)	10(27.8)			5(55.6)	4(100.0)		

Table 2: Comparing the IgG status of participants with the gender.

The lack of statistically significant difference between the sex for the test and control is a confirmation of the sex matching of patients in this study. P = 0.480.$\chi 2 = 0.498$.

Snoring 40(88.9%) followed by mouth breathing37 (82%), nasal blockage 37 (82%) and recurrent runny nose 30 (66.7%) were the commonest presenting complaints. This is similar to what is well established in the literature [12-14,20].

Whereas poor weight gain 9 (20%), speech impairment 4 (8.9%) and hearing loss 1 (2.2%) had the least responses. Other complaints noted were repeated sneezing 8(17.8%), ear tugging or pain 5 (11.1%) In terms of socioeconomic grouping, the most frequent was the patients of middle class parents 21(46.7%), followed by the upper class 14 (31.1%) then 10 (22.2%) patients from lower class families.

The prevalence of EBV in the test group was 91.1% (30 M:11F), while 4 F (8.9%) were EBV negative. The mean IgG- 29.6 ± 2.8 median of 30.0, Range = 0 - 50; CI = 23.9 -35.4 (see figure 1 and 2).

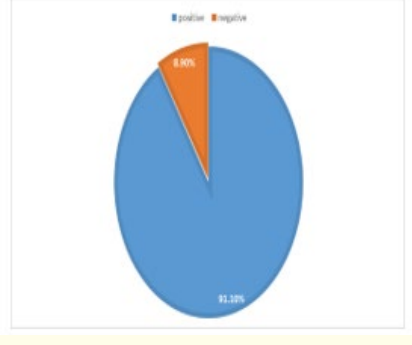

Figure 1: EBV IgG Status of test patients.

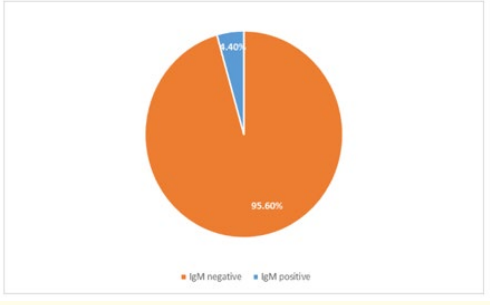

Figure 2: EBV IgM status of test patients.

In the control group prevalence was 82% (26 M:10 F) and 9 (17%) were IgG negative (5M:4F). The mean IgG -12.4 ± 1.4, median of 14.2 Range of 0 - 35.5; CI = 9.4 - 15.3.

There was a statistically significant difference between their mean titer values (t = 5.428; p = <0.001).

Similarly, when comparing the averages of the EBV IgG positives in the test vs control groups, there was a statistically significant difference using the T-test t = 12.956 and p value = <0.001.

Discussion

The peak age could be attributed to the fact that children under 5 years are more susceptible to environmental pathogens, this age also coincides well with the pathogenesis of adenotonsilar enlargement because around this age the adenoid enlargement begins to peak.

This finding was in keeping with Samdi [13] who found the mean age at surgery to be 3.5 ± 2.43 years and Adedeji., *et al*. [14],

but in contrast with Varkkhi [11], Tagliacani [20] and Endo., *et al*. [21] who found the peak age to be 5.6 years. This shows children are likely to have surgeries at least 2 years earlier in Nigeria than in western climes. This may be due to cultural and hygienic practices as well as climatic factors which cause children to be exposed earlier to viral pathogens and access to ENT surgeons early on in the disease progression. As most patients would have seen and ENT surgeon within 2 months of their complaint in our setting.

Similarly, the male preponderance was in keeping with findings by Adedeji., *et al*. [14], Chinawa., *et al*. [22], Samdi., *et al*. [13], Adegbiji., *et al*. [23] and Varkkhi., *et al*. [11]. The male preponderance could be as a result of male children in this age group being more active and adventurous and are likely more susceptible to infections than their female counterparts.

The most consistent clinical symptoms were snoring, mouth breathing and nasal blockage which is consistent with what is found in African and Western literature [12-14,23] However, Chinawa., *et al*. [22], found that most patients in his series had cough, catarrh and nasal allergies, poor weight gain and repeated sneezing as predominant symptoms. This could be as a result of the fact that the study was done in the paediatric clinic in acutely ill patients who had nasal allergies.

In contrast with Chinawa., *et al*. [22], where most of the patients were from the lower class, there was a preponderance of children from middle class homes in this study - maybe due to demographic of patients in the study centre, as most of them are children or wards of civil servants under the National Health Insurance Scheme thus underscoring the need for universal health coverage (See table 3,4).

Duration of complaint Correlation	Correlation coefficient (r)	P-value
Total Tonsil volume (ml)	0.409	0.009*
EBV IGG value (AU/ml)	0.315	0.048*

Table 3: Duration of complaint.

*Significant at 95%

Variables	Frequency (n = 45)	Percent
Lower Class	10	22.2
Middle Class	21	46.7
Upper Class	14	31.1

Table 4: Comparing socioeconomic class of parents in test group [27].

The high prevalence in this study- 91% in the test and 82% in the control may be attributed to the ubiquitous nature of the virus, its affinity for and ability to adapt to warm environment and immature immunity in this group of patients.

This was similar to findings by Crowcroft., *et al*. 2(91%) in a larger study group of school children between 7 to 11 years and Kuri., *et al*. 1(95%).

Sheishima., *et al*. found up to 60% of children less than 16 already had EBV infection, with a prevalence of 66% and 63% in their tonsils and adenoid respectively. Gunel., *et al*. [24] found that EBV was the commonest virus found in patients with recurrent tonsillitis and this was steady all year round in these patients with prevalence as high as 53.8% for recurrent tonsillitis and 32.0% in hypertrophied tonsils.

Xiaotong., *et al*. [25] found lower prevalence rates in his study group 51.9% among patients with adenotonsilar disease. The differences in this prevalence may be due to the larger sample size, differences in climates as well as the PCR methods used in viral studies. Studies that used serology found a higher prevalence than the PCR studies.

A study to compare the prevalence between tissue and serum in the same patients will be worthwhile.

Two (4.4%) of the participants from the test group were EBV IgM positive. This is indicative of an active infection; probably subclinical, as the patients were appropriately evaluated and found fit for surgery which they had with satisfactory outcome, this finding is supported by Jamiyan and Seishima., *et al*. [4,5]. They both had Brodsky grade 4 tonsils and adenoid nasopharyngeal ratio of 0.9. None of the patient in the control group had an active infection at the time of the study.

Since adenotonsilar enlargement is more of a chronic disease, it is understandable that the IgG antibody which connotes chronic

infection would be significant in these patients than IgM antibodies which depicts active or acute infection. with the virus. In the same line, the control participants were otherwise healthy children, hence the absence of acute infection in them.

The primary infection with EBV is the infectious mononucleosis which majorly focuses the tonsils. Also, the primary route of infection is oral route which may be why more patients with tonsil enlargement had a higher correlation than adenoid enlargement.

This study also provides backing data for screening and eventually vaccination of children against EBV infection. Also. Seeing as the prevalence is just as high as it is in western climes, there is need for emphasis on public health education on mode of transmission of the virus and treatment of the primary infections as well as re-educating health care workers on the potential risks of acquisition and spread of the virus through unsafe theatre and clinic practices. Lastly. There may be a need to review management protocols for Adenotonsillitis after screening particularly for those who fall into the lower socioeconomic class and cannot afford insurance or surgery.

Conclusion

In conclusion this study has shown that:

The prevalence of EBV infection among children with adenotonsilar enlargement is 91.1% whereas the prevalence in the control group was 82%.

Majority of the participants were male children under 5 years.

Conflict of Interest

The authors have no conflict of interest to declare.

Funding

The study was self-funded by the author

Research problem – Is EBV really ubiquitous in our setting? Is there a relationship between adenotonsilar enlargement and EBV sero-prevalence?

Research question – Is there a correlation between EBV status and enlarged adenoid and tonsil in children? What is the prevalence of EBV among children with adenotonsilar enlargement?

Justification – To bridge knowledge gap, establish prevalence in our setting and provide baseline data for further studies.

Limitation – The sample size may not be representative of a larger population of children with adenotonsilar enlargement as it was hospital based.

Ethical approval was obtained from the Ethics and Research Committee of the University of Abuja Teaching Hospital Gwagwalada, Abuja (UATH/HREC/PR/33) and West African College of Surgeons (EXM/PR/ORL/008/VOL 22).

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