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Research Article

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Serological Prevalence of Latent Epstein-Barr Virus Infection in Children and Adolescents

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Abstract

Background: *Epstein-Barr Virus (EBV), first discovered in 1946, is one of the most common viruses, which found over the world causing latent infection or different undistinguishable symptoms ranged from mild to severe. This prevalence and age distribution of this latent infection varies significantly in different populations.*

Aim: *Serological detection of anti-EBV IgG antibodies in children and adolescents using of the sandwich enzyme-linked immunosorbent assay (ELISA), and estimating the association of seropositivity to sex of study population. Materials and Methods: A total of 182 serum samples were obtained randomly from the study population that involved children (5-12 years old) and adolescents (13-17 years old) of both sexes in Sulaymaniyah province (Iraq) during January-February (2023).*

Results: *Sera of an overall 29.12% study individuals were showed a serological positive reactivity to EBV-IgG antibodies. Significantly ($P<0.0289$), latent EBV was higher in adolescents (42.86%) than those observed in children (15.39%). Concerning the association of seropositivity to sex of study population, there was a significant increase ($P<0.0224$) in prevalence of anti-EBV-IgG antibodies among females (42.47%) than males (20.18%). Also, females were appeared significantly ($P<0.0001$) at higher risk of infection (2.104) than males (0.475).*

Conclusion: *The seroprevalence of latent EBV infection in female adolescent was higher than male children; however, additional investigations using of serological as well as molecular assays are of great importance to demonstrate the extent of infection and to define the full spectrum of EBV-associated diseases.*

Keywords: EPV, Enzyme-linked Immunosorbent Assay, Viral Diseases, IgG Antibodies, Iraq.

Introduction

EBV, also called Human gammaherpesvirus 4, is a double-stranded DNA virus in the *Lymphocryptovirus* Genus of *Orthoherpesviridae* Family that belongs to Heunggongvirae Kingdom [1,2]. The virus is known as one of the most common viruses in human which associated with various non-malignant, premalignant and malignant EBV-associated lymphoproliferative diseases as well as with the childhood disorders and developing of autoimmune diseases [3]. It spreads most commonly through bodily fluids, especially saliva, blood and semen during sexual contact, blood transfusions and organ transplantations [4]. Also, the virus can be transmitted through the contaminated fomites such as toothbrush and drinking glass as the virus survive for long time as the fomites moist [5]. After get primary infection, EBV remains in a latent state in B cells; but reactivation to lytic replication could occur which results in production large numbers of virions that infect other B-lymphocytes within the host and development of clinical symptoms of infection [6,7].

In children, few or no clinical symptoms could appear, but the development of disease in adults can cause fatigue, fever, inflamed throat, swollen lymph nodes in the neck, enlarged spleen and liver or rash [8,9]. Many different diseases (allergy, erythema, carcinoma, lymphoma and ulcers) and disorders (atrophy, dementia and Parkinson) were also thought to be linked to the EBV [10,11]. Hence, diagnosing of EBV based on clinical symptoms is challenging, and laboratory testing of blood to detect specific antibodies or antigens is necessary. Several serological tests such as indirect fluorescent antibody, rapid monospot tests and enzyme immune assays such as ELISA have been modified to diagnosis of EBV infection, and detection the type of antibodies to distinguish the acute and past infections [12].

Worldwide, several viruses were implicated primarily or secondarily in initiation of diseases in human population [13-16]. EBV is one of the viral pathogens that can result in a spectrum of diseases, with the host immune response playing a key role in shaping the clinical manifestations [17]. In Iraq, although different studies have been done to correlate the EBV with other diseases and disorders [1-20]; no available studies target detection of latent infection in apparently healthy population. Therefore, the current study was conducted mainly to detect the seroprevalence of latent EBV infection in children and adolescents in Sulaymaniyah province (Iraq) with estimating the association of seropositivity to sex of study population.

Materials and Methods

Ethical Approval

This study was licensed by the Scientific Committee of the Department of Microbiology, College of Medicine, University of Kirkuk (Kirkuk, Iraq).

Samples

A total of 182 apparently healthy individuals of both sexes and 6-18 years old were selected randomly from different areas in Sulaymaniyah province-Iraq, during January-February (2023). Venous blood samples collected under aseptic conditions into free-anticoagulant glass gel tubes were centrifuged (4000 rpm/3 minutes), and the obtained sera were transferred into a labeled Eppendorf tubes, and kept frozen until be tested by ELISA.

Serology

According to manufacturer's instruction of the Quantitative EBv-IgG ELISA Kit (SunLong Biotech, China), the serum samples and kit contents were prepared at room temperature, and then, the dilution of Standards was done at 48,32,16,8 and 4 ng/L. after adding of Standards in the microplate stripplate, the sera and dilution buffer were loaded to the sample wells, shacked gently, and incubated (37ºC/30 minutes). After washing the microplate, HRP-Conjugate was added, incubated (37ºC/30 minutes), and washed. After adding of Chromogen A and B, and incubating of microplate (37ºC/15 minutes), the stop solution was added and the absorbance was read at an optical density (OD) of 450 nm using ELISA Microplate Reader (BioTek, USA). The concentrations of EBV-IgG antibodies were calculated in the Standard Curve based on the values of known concentrations and ODs of the Standards as well as the ODs of the samples at a sensitivity of 0.1 ng/L.

Statistical Analysis

All obtained results were analyzed using the t-test and relative risk in the GrpahPad Prism Software (version 6.01), to detect significant variation between the study individuals and estimate association of positivity to sex and age factors. Differences between the values were considered significant at $P<0.05$ (*) and at $P<0.0001$ (****) [21].

Results

Sera of an overall 29.12% (53/182) study individuals were showed a serological positive reactivity to EBV-IgG antibodies (Figure 1). Significantly ($P<0.0289$), latent EBV was higher in adolescents (42.86% (39/91)) than those observed in children (15.39% (14/91)), (Figure 2).

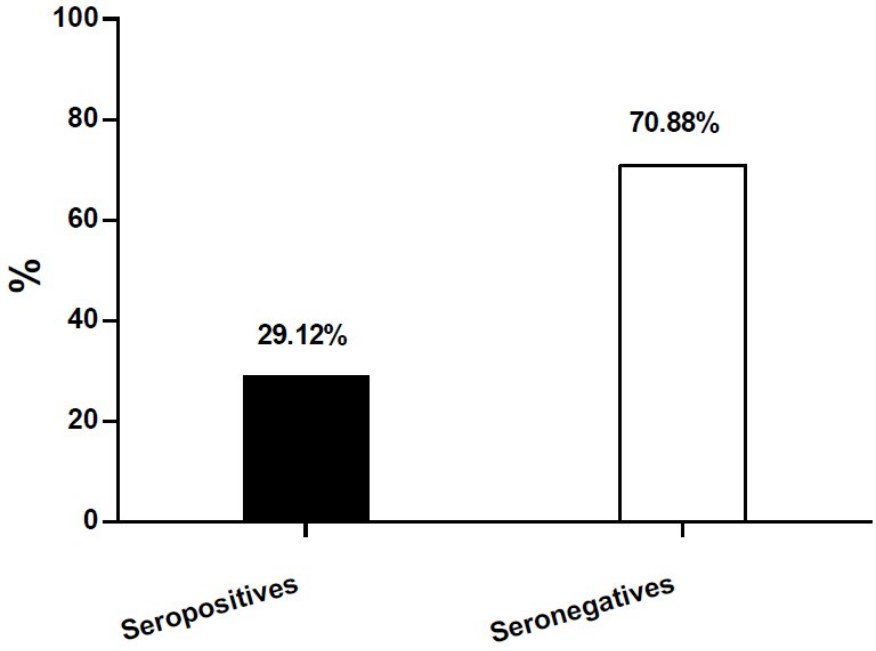

Figure 1: Total results for testing a totally 182 sera by sandwich ELISA.

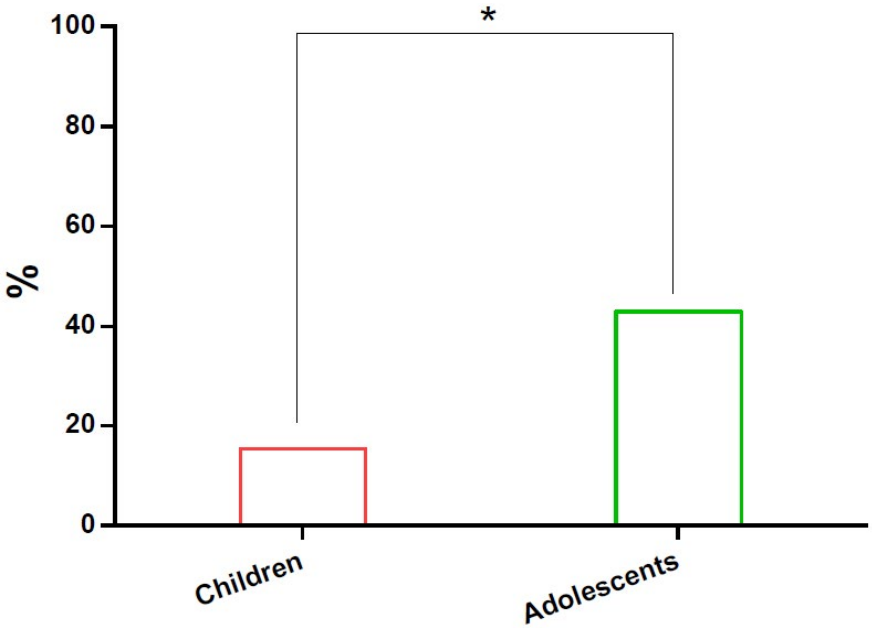

Figure 2: Distribution of seropositive results among children and adolescents.

Concerning the association of seropositivity to sex of study population, there was a significant increase (P<0.0224) in prevalence of anti-EBV-IgG antibodies among females (42.47% (31/73)) than males (20.18% (22/109)), (Figure 3). Also, females were appeared significantly (P<0.0001) at higher risk of infection (2.104) than males (0.475), (Figure 4).

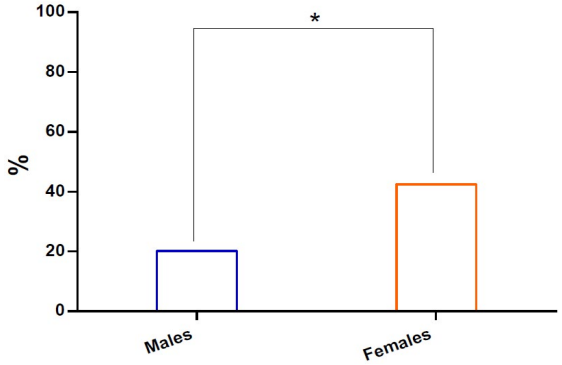

Figure 3: Distribution of seropositive results among females and males.

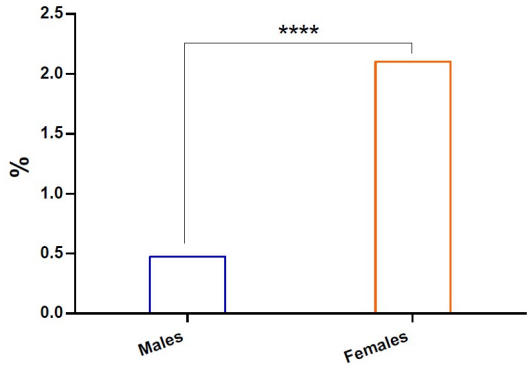

Figure 4: Results of relative risk among females and males.

Discussion

EBV is thought to play an important role in development of different diseases. From the discovery of EBV in 1964, numerous countries throughout the world have perormed extensive epidemiological researches to obtain more data about the EBV [22]. In this study, the findings revealed that 29.12% of study population was positive serologically for latent infection. In comparison to other national studies, the seroprevalence of EBV-IgG antibodies was 18.3% in Mosul [23], 92.73% in Anbar [24], 51.74% in Kirkuk [25], 70.6% in Diyala [26], 92.5-100% in Basrah [27], and 12.38% in Erbil [28]. Internationally, there were 12.5% in Sweden [29], 45% in Egypt [30], 47.1% in Ghana [31], 97.4% in Spain [32], 97.9% in Qatar [33], 70.3% in Bahrain [12], 82% in Iran [34], 41.7% in Syria [35], 71% in Saudi Arabia [36], and 21.4-9% in China [37]. Moreover, although this study targets random detection of EBV in study population, almost studies were carried out to identify the prevalence of EBV in patients with other diseases and disorders as reported as 83% in lymphoplastic leukemia compared to 95% in control [38], 32.3% in leukemic patients compared to 2.3% in control [39], 28-45% in breast cancer disease [30], 86-97% in lymphoma [40], 52.73% in periodontitis [41], 33% in renal transplant recipients [42], 12.33% in thalassemia [19], 85.7% in laryngeal carcinoma [25], 48.37% in myeloma patients compared to 5.16% of control [43], 11.66-100% in malignant lymphoid solid [27], 96.67% in inflammatory bowel [44], and 57.5% in Burkitt lymphoma patients (Al-Khreisat et al., 2023).

Our findings revealed the higher prevalence of anti-EBV IgG antibodies in adolescents than children. In developing societies, virus transmits to a high percentage of children that become infected at 6 years old and develop stable immunity [33,45]; whereas in industrialized countries, EBV has delayed until adolescence [45-47]. Căinap et al. [31] found that the seroprevalence of EBV was 100% in patient having ≤18 years old. In USA, Balfour et al. [48] reported that individuals of 6-8 years and 18-19 years having respectively low (50%) and high (89%) rate of infection; while, Condon et al. [49] revealed that 26-74% of people becomes infected at an age of 1.5-19.9 years old. Sharifipour and Rad [34] recorded that 50% of children become infected at 3 years, and the percentage reaches 95% at 40 years old. Also, they recorded that incidence of EBV increases post 15 years to reach >80% at 20 years and >90% at 40 years. Salih et al. [26] demonstrated that 39.1%, 79.5%, 74.1% and 64% of individuals having the infection at <1, 1-4, 5-9, and 10-14 years old, respectively. Ghazi et al. [44] reported that the IgM is more prevalent in younger (<16 years) than older (□40 years) but IgG was more prevalent in older (≤17-40 and >40 years) than younger (≤16 years). However, the results of current study disagree other authors who recorded no significant differences between age groups; ≤35 and ≤35 years old [24,32]. While, Zaki [36] found the seroprevalence of non-typical clinical symptoms of EBV was more prevalent in children than adolescents.

Our findings were similar with that detected by Farid and Al-Biltagi [12], Salih et al. [26] and Zaki [36]; but, in contrast to results of Hassan et al. [50] who seen that the seroprevalence of EBV in males was greater than females; and, Ghazi et al. [44] who show no significant differences were found between females and males. Sharifipour and Rad [34] demonstrated that male infants having a higher rate of susceptibility than females, which might occur due to difference in immunity between female and male. The increasing of EBV seropositivity among females may be due to existence of other herpes viruses and incidence of silent primary EBV infection.

Conclusion

The seroprevalence of latent EBV infection in female adolescent was higher than male children; however, additional investigations using of serological as well as molecular assays are of great importance to demonstrate the extent of infection and to define the full spectrum of EBV-associated diseases.

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Conflict of Interest

There is no conflict to be interested.

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