

Seroprevalence of Epstein–Barr virus among children and adults in Tehran, Iran

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Abstract

Epstein–Barr virus (EBV) as a herpes virus can be associated with numerous infections and cancers. The virus is known to cause infectious mononucleosis. There is no accurate estimation of the seroprevalence of EBV in Tehran, so this study aimed to estimate the seroprevalence of EBV among children and adults in Tehran, Iran. This descriptive–analytical study was conducted from 2015 to 2019 in Tehran province. In this study, 1220 people were selected by cross-sectional sampling, and blood samples and demographic data were collected by questionnaire. An anti-EBV-VCA ELISA kit was used to determine the seroprevalence of IgG against viral capsid antigen (VCA) among children and adults. In this study, most of the participants were in the age range of 20–29 years (349 individuals). The results of the ELISA test showed that the highest number of positive cases were in people over 40 years (94.8%), 30–39 years (92.5%) and 20–29 years were (92.1%), respectively. On the other hand, the seroprevalence of EBV infection in boys and girls up to 3 years was about 50%, and in adults, up to 40 years, it was about 95% ($p < 0.001$). The results of this study showed that in Tehran, the seroprevalence of VCA-IgG varied from 70% in primary school children to more than 90% in adults up to 40 years, indicating a broad spread of the virus. The results also indicate that the seroprevalence of EBV is high among men and women in Tehran.

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Introduction

Worldwide seroepidemiology studies have shown extensive Epstein–Barr virus (EBV) spread in different regions and communities around the world and the results show that in developing countries, most children up to the age of 6 years are infected with EBV and have the antibody against this virus [1,2]. However, studies show that in industrialized societies, >50% of people remain susceptible to EBV until they reach puberty [3,4]. Early transmission of EBV in humans occurs through the mouth, but research in developing and developed countries has

shown that in young adults and children, the virus can be transmitted through other ways such as infected fingers and saliva-contaminated fomites [5,6]. In children, the initial infection caused by the virus is often asymptomatic, but it can cause infectious mononucleosis in 35%–75% of adolescents and young adults [1,7]. By infecting B lymphocytes and epithelial cells, the EBV causes a chronic infection that persists throughout the lifespan of the individual, and on average, 20% of healthy adults spread this virus through oral saliva [8,9]. Nowadays, EBV has been extensively discussed as an aetiological agent in many human cancers, including Burkitt's lymphoma, nasopharyngeal carcinoma, Hodgkin's disease, and T-cell and B-cell lymphomas [10]. Burkitt's lymphoma is the most common cancer in children and adolescents with an average age of 7.7–10.5 years in different regions of the world [11,12], especially in the high incidence areas of tropical Africa [13]. Studies in African countries show that in >90% of cases, and in other parts of the world, about 20% of Burkitt's lymphoma cases, the EBV genome, nuclear antigen and high levels of

antibody IgG against EBV (anti-EBV IgG) have been identified [8,14]. Nasopharyngeal carcinoma is one of the rare cancers between the ages of 20 and 50 years, most commonly in males in the south of China, and the disease incidence has been reported in about ten cases in the 100 000 population [15,16].

On the other hand, in all cases of nasopharyngeal carcinoma, the EBV genome, anti-membrane, and nuclear genes have been observed [17,18]. In Iran, a study examined the incidence of nasopharyngeal carcinoma cases from 2004 to 2009, and results showed that the incidence was 0.33 per 100 000 persons; the overall incidence rate has increased annually [19]. Moreover, studies have also shown that in 80%–90% of individuals with Hodgkin's disease <30 years after the initial EBV infection, the EBV genome and its nuclear and membrane antigens have been identified [20,21]. Tehran, as the capital of Iran, is a large city in the northern part of the country that features a continental-influenced Hot-summer Mediterranean climate [22,23]. Tehran is the most populous city in Iran and Western Asia and has the second largest metropolitan area in the Middle East with a population of about 10 million in the city and 15 million in the larger metropolitan area of Greater Tehran. Tehran comprises several ethnic groups, including Iranian Azeris, Baloch, Assyrians, Arabs, Armenians, Georgians, Bakhtyaris, Talysh, Jews, Kurds and Circassians. However, the majority of people in Tehran identify themselves as Persians [24–27]. As the age and seroprevalence of EBV in different societies with different economic conditions are different, and the potential risk of this virus infection is related to lymphoproliferative malignancies in children and young people, we aimed to perform this sero-epidemiological study to determine the prevalence of IgG antibody against EBV in children, men and women. The results of this study can be used to identify the seroprevalence of EBV in children and adults of different ages in Tehran, Iran.

Materials and methods

This descriptive–analytical study was conducted in the eastern cities of Tehran province, including Firoozkooh, Damavand, Pakdasht, Varamin and the northern and eastern districts of Tehran. The study population was selected randomly using the multistage cluster sampling method from families living in these areas. From each family, one individual was selected at random. At the same time, a questionnaire containing demographic information of age and gender was completed for them by health workers in front of their houses. These questionnaires were prepared in five educational centres affiliated to Tehran, Shahid Beheshti and Iran Universities of Medical Sciences and were studied in the virology department of the Pasteur Institute of

Iran. A total of 1220 blood samples from children and adults in the age range of 1 month to ≥ 40 years old were collected, to study the EBV infection in Tehran, during the years 2015 to 2019. Five millilitres of the blood sample were gathered from each participant; the samples from the minors were taken after parental consent. The serum was separated and kept at -20°C until analysis.

Overall, 1220 serum samples were collected and tested in duplicate for IgG antibody against EBV using the Anti-EBV-VCA ELISA kit (Behring's kit, Marburg, Germany). Positive and negative standard sera accompanying the Anti-EBV-VCA ELISA kit were included in each assay, and the amount of colour intensity in microplates determined the presence or absence of the EBV IgG in the serum samples. Results were analysed by spectrophotometer at a wavelength of 405 nm. All steps and determination of the cut-off amount were performed according to the manufacturer's instructions.

Descriptive and statistical analyses were performed using SPSS 23 Package program (SPSS Inc., Chicago, IL, USA). All data were presented as frequencies or percentages. The Professional Ethics Committee of the Liver and Gastroenterology Research Centre of Shahid Beheshti University of Medical Sciences approved this project.

Results

A total of 1220 individuals were included, and their ages ranged from <1 month to ≥ 40 years, with a mean age \pm SD of 22.9 ± 2.76 years. Among the study participants, 28.6% ($n = 349/1220$) were in the age range 20–29 years (Table 1). Overall, 480 serum samples were collected from boys and girls up to 14 years of age, and 740 serum samples were collected

TABLE 1. Prevalence of anti-Epstein–Barr virus (EBV) IgG antibody according to age groups in population of Tehran, Iran

Age groups	Number of all tested serum samples in each group	Number of anti-EBV IgG VCA-positive subjects (%) in each group
<1 month	61	50 (82%)
1–3 month	45	24 (53.3%)
4–6 month	18	4 (22.2%)
7–11 month	13	4 (30.8%)
1–3 years	99	4 (50.5%)
4–6 years	82	58 (70.7%)
7–9 years	76	56 (73.7%)
10–14 years	86	66 (76.7%)
15–19 years	96	84 (87.5%)
20–29 years	349	321 (92.1%)
30–39 years	161	149 (92.5%)
> 40 years	134	127 (94.8%)
Total	1220	993 (81.4%)

Barr virus; VCA, viral capsid antigen.

from healthy adults without underlying disorders that were in the age range of 15–40 years.

In general, the ELISA test showed that the overall seroprevalence of IgG antibodies was 81.4%. The seroprevalence of EBV in the male and female groups, as well as in the different age groups <14 years, are shown in Fig. 1.

The highest seroprevalence of EBV was seen in individuals >40 years old ($n = 127/134$; 94.8%) (Table 1).

Table 1 shows the distribution of individuals and the presence of IgG antibodies against EBV in different age groups. The prevalence of IgG antibodies against EBV among infants was 82%, amidst 1 to 3 months old children was 53.3%, in 4 to 6 months old children was 22.2%, and the rate was 30.8% in children of 7 to 11 months old. Moreover, the seroprevalence of EBV among 4 to 6 years, 7 to 9 years, 15 to 19 years, 20 to 29 years, and 30 to 39 years were ($n = 58/82$; 70.7%), ($n = 56/76$; 73.7%), ($n = 84/96$; 87.5%), ($n = 321/349$; 92.1%), and ($n = 149/161$; 92.5%), respectively. The results also showed that the prevalence of IgG antibodies against the virus in children and adolescents of 10 to 14 years was 76.7%. On the other hand, the seroprevalence of EBV in boys and girls up to 3 years of age was c.50%, and in adults up to 40 years, it was about 95% ($p < 0.001$) (Figs. 1 and 2).

Discussion

From the discovery of EBV in 1964 and to understanding the aetiological role of the virus in causing infectious mononucleosis in 1968, large-scale epidemiological studies have been conducted in various parts of the world to investigate the epidemiology of EBV [28]. During the onset of the EBV infection, which occurs most

often in one form of infectious mononucleosis or asymptomatic infection, viral capsid antigen (VCA) -EBV-specific IgG class antibodies are formed in humans and remain stable over a lifetime [29]. Therefore, identifying and measuring the level of antibody titres to determine the incidence of EBV infection in different populations is an appropriate and standard method. EBV is spreading worldwide, and the prevalence of specific antibodies to the virus varies across regions and communities around the world [30]. In developing countries, EBV infection occurs at an early age, and most children become infected with EBV by the age of 6 years and develop stable immunity [1,2]. In industrialized societies, the incidence of the EBV infection is delayed in >50% of cases until adolescence, with approximately half of the adult infections, occurring in the form of infectious mononucleosis [3,4,6]. The results of this study indicate a high prevalence of maternally transmitted EBV antibody in the first months after birth, a decrease in maternal to neonatal passive immunity at 4–6 months of age, and the occurrence of primary EBV infection in the first year of life.

Most primary EBV infection during the first year of life is asymptomatic or subclinical. In general, persisting maternal antibodies are present during early infancy, which leads to the asymptomatic EBV infection. Moreover, understanding the pattern of serological responses to EBV infection during infancy and early childhood is important for vaccine management [31]. The previously published study revealed that during the first year of life, male infants have a high susceptibility to EBV infection compared with female infants. This study states that the difference of immunity to EBV infection between female and male infants during the first year of age is the main factor in this high susceptibility [32]. As shown in Figs. 1 and 2, the seroprevalence of EBV was 64.8% among children and adolescents up to the age of 14 years. The results also showed

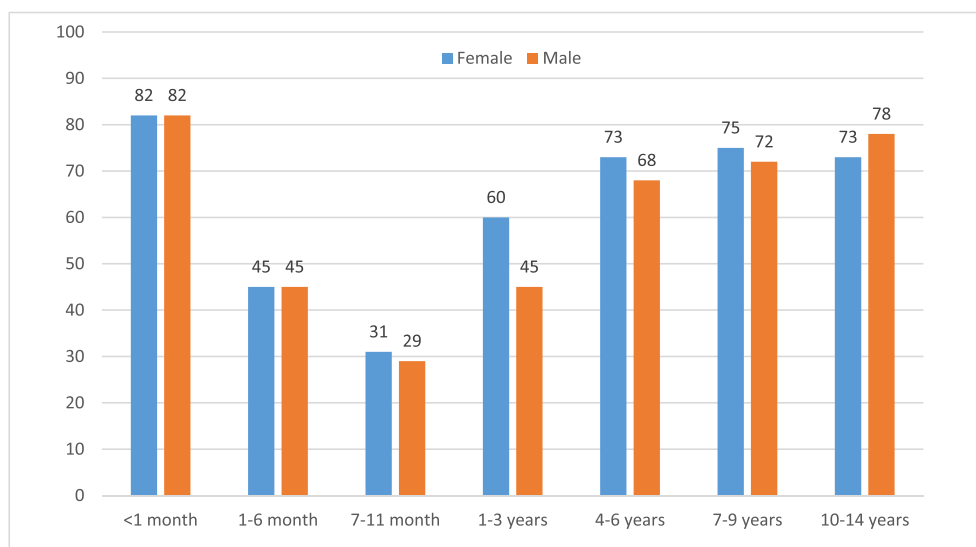


FIG. 1. Prevalence of anti-Epstein–Barr virus (EBV) IgG viral capsid antigen (VCA) antibody according to gender, and age groups in population under 10 years old in Tehran, Iran. The figure legends show the percentage of EBV seropositivity among different age groups.

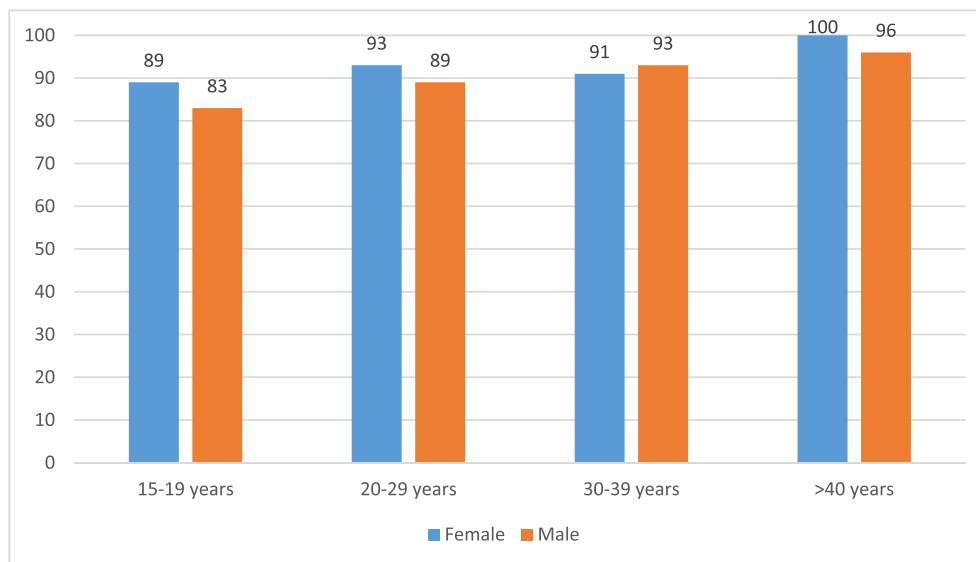


FIG. 2. Prevalence of anti-Epstein–Barr virus (EBV) IgG viral capsid antigen (VCA) antibody according to gender, and age groups among the population over 10 years old in Tehran, Iran. The figure legends show the percentage of EBV seropositivity among different age groups.

that the seroprevalence of EBV in children up to 3 years was about 50%, and in adults up to 40 years was about 95% ($p < 0.001$). Seroepidemiological studies conducted around the world have shown that during the first months of birth, the prevalence of EBV antibodies decreases, and antibody titres against the virus in children with 1–6 months of age are often low or not observed at all. However, studies have shown that the level of EBV antibodies increases after 7 months [33,34]. In this study, the prevalence of IgG antibody against EBV in infants was 5%, and in infants of 1–3 months, 1–6 months and 6–11 months, it was 53.3%, 22.2% and 30.8%, respectively. It can be inferred that the high prevalence of antibodies against EBV in the first months of birth is due to passive immunity from the mother [35]. Also, low levels of the virus IgG antibody at 2–6 months of age are associated with the disappearance of maternal to neonatal IgG antibodies [35,36]. On the other hand, the increase in EBV antibody levels after 6 months is due to the occurrence of EBV infection in the first year of life, which indicates a low age of EBV infection in Tehran. The study found that about 70% of children up to the age of 6 years present anti-EBV, and the seroprevalence did not change significantly between the ages of 7 and 14 years and to the age of 14 years. The results of this study are similar to the seroepidemiological studies in developing countries; and in the communities in the tropical regions, where the prevalence of EBV antibodies in children aged 4–6 years is as high as 80% [3,4,8,37]. Also, other studies in the USA have reported that the prevalence of IgG antibodies against EBV among children aged 18 months to 19.9 years is 26%–74% [38]. The result of a study in the USA revealed that the prevalence of IgG antibodies against EBV is varied among different age groups. This study revealed that people aged 18–19 years (89%) and 6–8 years (50%)

had the highest and lowest frequencies of EBV IgG antibodies, respectively [39]. Dowd et al. reported that Overall EBV seroprevalence was 66.5%, and the seroprevalence of EBV increased with age, ranging from 54.1% for 6- to 8-year-olds to 82.9% for 18- to 19-year-olds [3]. On the other hand, Levine et al. revealed that overall seroprevalence rates were 87% for EBV among individuals with an age range from 17.6 to 26.1 years [40]. Nowadays, the prevalence of EBV infection varies depending on the socio-economic conditions of different communities. Studies in the USA have reported the seroprevalence of antibodies against EBV in children aged 5–10 years of low socio-economic status of 60%, and in children with high socio-economic level, the prevalence of these antibodies is about 20% [8,41,42]. In general, EBV infection in developed countries such as the USA is often delayed until adolescence, whereas, in developing countries, the EBV infection acquired in the first few years of life and general seroconversion is frequently seen by the age of 3–4 years [43]. Moreover, in developing countries such as Thailand and Turkey, several factors, including crowded family conditions and low income, have also been found to increase the possibility of being EBV seropositive in children [44,45]. In China, about 100% of children between the ages of 10 and 15 years have EBV antibodies, and it has been observed that in large areas of China, nasopharyngeal carcinoma is common in the age group of 20–40 years, possibly due to activation of primary EBV infection [46]. Overall, the results of our study showed that the seroprevalence of EBV among men and women in Tehran increased after the age of 15 years, and >80% of adults up to 20 years and >90% of men and women up to 40 years have the IgG antibodies against EBV. The prevalence of the antibody against the virus was similar between adults. However, the result of a study in

France showed the seroprevalence of EBV decreased for different age classes (<10, 15–19, 20–30, and 30–40 years old), over the studied period (2001–2015). This study revealed that the age at the EBV primary infection has recently increased in some developed countries [47]. In this study, unfortunately, we could not obtain other demographic information such as occupation, race/ethnicity for each individual, and this was one of the main limitations in our study.

Conclusion

The results of this study show that the seroprevalence of EBV in Tehran varies from 70% in primary school children to >90% in adults up to 40 years, indicating a broad spread of the virus. Also, the high seroprevalence of EBV in the first years of life indicates that the age of occurrence of EBV infection in Tehran's children is precocious. Overall, the results of this study showed that the seroprevalence of EBV was high among men and women in Tehran.

Conflict of interest

All of the authors declare that there are no commercial, personal, political or any other potentially conflicting interests related to the submitted manuscript.

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