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Epstein-Barr Virus Infection Status Among First Year Undergraduate University Students

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Abstract

Epstein-Barr virus (EBV) is the cause of infectious mononucleosis, which disproportionately affects university students. This population has the potential to benefit from a prophylactic EBV vaccine trial. Our objectives were to determine EBV infection status and associated demographic/lifestyle factors among first year undergraduate university students at the beginning and end of first year. EBV infection status was assessed by testing for circulating IgG class antibodies against EBV viral capsid antigen. Of 198 starting students; 56.1% were positive for EBV antibodies with a higher rate in women (64.8%) than men (41.1%); p=0.002. A history of deep kissing was associated with a higher rate of EBV antibody positivity. On follow-up 8 months later at the end of freshman year, 22.4% had acquired EBV antibodies for a primary infection incidence of 33.6/100 person years. These findings indicate that our first year undergraduate population contains sufficient EBV-naïve subjects for a prophylactic vaccine trial.

Keywords

Epstein-Barr virus; university first year students; EBV seroprevalence; EBV antibody; immunity

Epstein-Barr virus (EBV) was discovered in 1964 by Epstein, Achong, and Barr studying cultured African Burkitt lymphoma cells, and shown to be the cause of infectious mononucleosis (mono) in 1968.[1,2] Mono is particularly prevalent in undergraduate university students and studies have confirmed that a major risk factor for acquisition of infection is deep kissing, as EBV is transmitted primarily through saliva.[3] The incubation

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period for mono is typically 5–7 weeks.[4] EBV infection resulting in mono can have a significant impact on undergraduate university students, and has been found to markedly reduce study time, physical exercise, and social activities.[5] Furthermore, mono is potentially life threatening in student-athletes if there is liver or splenic involvement.[6] Avoiding exposure to EBV is very difficult, as EBV is one of the most ubiquitous human viruses in the world, with at least 90% of adults infected.[7] Thus, prevention should be focused on prophylactic interventions, especially vaccines.

A candidate prophylactic gp350-based vaccine for EBV is under development at the University of Minnesota and is the only gp350 EBV vaccine to have undergone a successful phase two clinical trial.[8] A major impetus for this trial was to learn if the University of Iowa would be a suitable collaborator with Minnesota in a clinical trial. A prophylactic vaccine is designed to be given to EBV-naïve subjects. The prevalence of EBV antibodies varies widely by age and geographic location.[8–10] Therefore, prevalence data for EBV infection among entering first year undergraduate students is necessary to determine if this University has sufficient EBV-naïve subjects for a prophylactic vaccine trial. In addition to collecting prevalence data, we administered a questionnaire about demographic and lifestyle factors, which may be associated with acquisition of EBV infection and retested students 8 months later at the end of first year.

METHODS

Clinical Study Design

This was a cross-sectional study, approved by the Institutional Review Board of the University of Iowa, to determine EBV infection status and associated demographic and lifestyle factors among entering first year students at a large public Midwestern university.

Screening was conducted in a student co-educational residence hall during two consecutive days during the first week of September 2018. Recruitment efforts consisted of study posters posted in common areas of residence halls, campus broadcast email to first year undergraduate students, and a table set-up in the residence hall with study brochures and a study member to answer questions about the study from 10:00AM to 2:00PM. Students were asked if they were a first year student, between 18–19 years old, feeling well with no history of bleeding disorders, and planning to remain on campus next semester. Students who met all inclusion criteria and gave informed consent, completed a questionnaire and had 12mL of blood drawn via antecubital venipuncture for which the student received a $20 gift card. The questionnaire specifically asked their gender, race/ethnicity, and whether the students had ever been diagnosed with infectious mononucleosis and whether they had ever engaged in deep kissing.

Follow-up was conducted 8 months later the last week of April and first week of May 2019 at the same student co-educational residence hall as initial recruitment. Two weeks and the day before the follow-up visit period students who had tested negative for EBV IgG VCA antibody were emailed and texted to return for the follow-up visit between 10:00AM and 2:00 PM at the same place in the residence hall as enrollment on one of 5 days.

Students were asked to confirm previous participation by student ID and birth date. They completed a questionnaire, and if they were EBV VCA IgG antibody negative in September 2018, they had 12mL of blood drawn via antecubital venipuncture. All returning students received a $20 gift card. Students were notified of their EBV antibody status, and an explanation provided if they requested to be notified at enrollment or at follow-up.

Laboratory Procedures

Screening for EBV Antibodies—EBV infection status is usually assessed by testing for circulating IgG class antibodies against EBV viral capsid antigen (VCA IgG) or IgG class antibodies against EBV nuclear antigen-1 (EBNA-1 IgG). We chose to test for VCA IgG because a University of Minnesota study found that 5 of 62 prospectively followed subjects (8%) never developed EBNA IgG antibodies after primary EBV infection, whereas 66 of 66 developed antibodies against VCA IgG.[3]

EBV VCA IgG antibodies were assayed using commercially available EIA kits and a MAGO Plus Automated EIA Processor (Diamedix, ERBA Diagnostics, Inc, Miami Lakes FL).[11] An EIA index ≥1.10 was interpreted as positive, an EIA index ≤0.90 were interpreted as negative, and an EIA index 0.90–1.10 was interpreted as equivocal. For equivocal results, the test was repeated and confirmed with heterophile antibody against bovine erythrocytes via commercially available chromatographic immunoassay (Inverness Medical) and EBNA-1 IgG EIA.[3]

Statistics

Surveys were administered and data were entered using REDCap Cloud software (Encinitas, CA). Comparisons between categorical variables were assessed using Pearson's Chi-square test. Our type I error rate was set at $\alpha = 0.05$, and all hypothesis tests are reporting two-sided p-values Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA).

The enrollment goal of 200 was chosen to match the sample size of the Minnesota prospective studies, and also because that number was practical to obtain with the available resources. Our aim was to determine if we could enroll sufficient EBV-naïve participants who had a reasonably high risk of infection. Therefore, a typical power calculation that estimates the probability of rejecting the null under the alternative hypothesis is not needed. Instead, the goal was to estimate each prevalence with reasonable precision to inform future vaccine studies. A sample size of approximately 200 gives us adequate precision such that if the seroconversion rate during the first undergraduate year was 30%, 200 participants allow us to estimate a 95% confidence interval of approximately 0.24 to 0.36, in expectation.

RESULTS

Demographics

In this cross-sectional study, 200 students were enrolled, but peripheral blood was unable to be obtained from two students. Of the remaining 198 students (125 women, 73 men), 56.1% were positive for EBV VCA IgG antibodies at baseline (Table 1). The median age

was 18.6 years, ranging from 18.0 to 19.6 years. A statistically significant difference was observed between gender and EBV VCA IgG antibody prevalence with prevalence being higher among women than men (81/125; 64.8% versus 30/73; 41.1%; p=0.002).

Of the 198 students enrolled, 79.3% were white (157 subjects), 7.1% other (14 subjects), 6.1% Asian (12 subjects), 6.1% Hispanic (12 subjects), and 1.5% Black (3 subjects). Race/ethnicity had a significant relationship with the presence of EBV VCA IgG antibody and was significantly lower (p = 0.0014) among white students (79/157; 50.3%) than non-white students (32/41; 78.1%) (Table 1).

Risk Factors

Most students reported having engaged in deep kissing (149/197; 75.6%). Deep kissing had a marginally significant relationship with antibody prevalence (p=0.047). Of the students reporting engaging in deep kissing, 90 of 149 were antibody positive (60.4%) compared with 21 of 48 (43.8%) students who did not (p=0.047). Gender did not have a significant relationship with deep kissing: 75.0% of male (54/72) and 76.4% of female students (97/127) reported having engaged in deep kissing (Table 1). One student declined to respond.

EBV VCA IgG antibody prevalence was higher in students with a history of mono (11/14, 78.6%) compared with those who did not (99/183, 54.1%; p= 0.076) (Table 1).

Follow-up

Of the original 200 recruited students, 134 students (67%) returned for follow-up eight months later, and 58 of the returning students were initially negative for EBV VCA IgG antibody. The seroconversion rate of these students was 13/58, 22.4% for a primary EBV infection rate of 33.6/100 person years. Nine women and four men seroconverted, but there was no significant association between gender and seroconversion rate (p=0.115). Of the 13 students who seroconverted between baseline and follow-up, 11 claimed no history of an infectious mononucleosis diagnosis at baseline, and 2 students claimed a history of infectious mononucleosis at baseline. Of those 11 students with no history, 3 claimed to have had an IM diagnosis at follow-up in May.

DISCUSSION

We found that 43.9% of first year students entering the University of Iowa had not experienced an EBV infection as documented by the lack of circulating EBV VCA IgG antibodies. A 2017 study at the University of Minnesota reported that 43.7% of entering first year students (87/199) were EBV antibody negative.[12] Hence, the antibody prevalence of entering first year students at this university and the University of Minnesota was essentially identical. Follow-up data showed a seroconversion rate of 33.6/100 person years in these university first year students, and the University of Minnesota study found a similar rate of 24 cases/100 person years.[13] Demographic factors were also very similar. The study populations were both predominantly female (62% in Minnesota and 63.1% in this university) and predominantly white (88% in Minnesota and 79.3% in this university).[12]

Female students in this study were significantly more likely to be EBV antibody positive compared with male students. The antibody prevalence was also higher among women than men among the University of Minnesota subjects, but the difference was not statistically significant.[13]

Our study found that race/ethnicity was a significant factor for EBV antibody prevalence. Although our sample size was small, with only three Black students and twelve Hispanic students, it is consistent with the National Health and Nutrition Examination Surveys data on 9,292 American children ages 6–19 from 2003–2010, which showed that age-specific EBV VCA IgG antibody prevalence was significantly higher for Mexican-Americans and non-Hispanic Blacks as compared with non-Hispanic Whites.[14]

Deep kissing was associated with a higher rate of EBV VCA antibody positivity compared with no history of deep kissing. This finding is consistent with what other studies have found. Crawford *et al*[15] found that deep kissing was a risk factor that was enhanced by penetrative sexual intercourse, although Balfour *et al*[3] later found that deep kissing with or without coitus was the only significant risk factor for EBV infection. The p-value for the risk of deep kissing in our study was 0.047, compared with the Balfour study value of p= 0.02. In the Balfour study, other lifestyle factors, such as alcohol consumption, energy level, and stress were not risk factors for acquiring an EBV infection.

It is interesting that more than half of participating students with no history of mono were EBV VCA antibody positive. The likely explanation is the wide variability in presentation of primary EBV infection, which ranges from a mild viral prodrome to the classic presentation of mono with significant symptoms. Conversely, two students who were EBV VCA IgG antibody negative at baseline claimed they had had a diagnosis of infectious mononucleosis at baseline which may have been based only on clinical symptoms versus confirmation testing. Our results reinforce the need to serologically confirm EBV exposure status before enrolling subjects into a vaccine trial. Additionally, while the students were filling out their questionnaire, questions most often arose about “infectious mononucleosis,” indicating a poor understanding of the term. However, most students understood the term once they realized it was “mono.” High risk populations, such as adolescents in high school and young adults in college or the military, would benefit from better education on this disease.

There were several limitations in this study. While the diversity of the study population closely reflected the student population overall, the number of African-American and Hispanic students was relatively low compared to the rest of the country. Second, the prevalence of EBV infection in students in other regions of the United States may well be different than the Mid-west. Third, the endpoint of the study was EBV VCA IgG seroprevalence and seroincidence as opposed to a clinical diagnosis of symptomatic infectious mononucleosis which would have required much more intensive and frequent monitoring which would be done in the vaccine trial. Given the high percentage of symptoms associated with EBV infection in similar university students[3,11], our high seroincidence rate gives us confidence that we would have sufficient clinical endpoints in a vaccine trial.

CONCLUSIONS

Mono disproportionately affects university students. It can result in hospitalization or significant time off of school and may result in withdrawing from college completely.[5] As a result, developing a prophylactic vaccine is an important step in reducing the incidence of this disease. Scottish and University of Minnesota studies have shown the potential benefit of a gp350-based vaccine.[3,12,13,15] The University of Minnesota is developing a prophylactic EBV vaccine based on adjuvanted, soluble, subunit EBV gp350.[8] A major objective of this study was to learn if the University of Iowa would be a suitable collaborator with Minnesota in a clinical trial. The vaccine being developed in Minnesota is similar to the one that showed efficacy in preventing infectious mononucleosis among Belgian college students,[8] and is essentially identical to the vaccine described by Servat and colleagues.[16] The reason for selecting gp350 as the immunogen is that it is the major surface glycoprotein of EBV, which preferentially binds to CD21 on the surface of B cells, thus initiating infection. Antibody against gp350 effectively blocks infection of B cells by EBV. Several clinical sites are needed for the first human trial of the University of Minnesota vaccine. Finding that first year students at the university of Iowa are similar to those in Minnesota, in terms of proportion naïve to EBV at the beginning of fall semester and their incidence of primary EBV infection during the first year at University, makes this site an attractive clinical trial site along with Minnesota.

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Table 1:

EBV seropositivity in 198 freshman students in September 2018 in relation to demographic/risk factors

	# of EBV antibody positive	Total	% of EBV antibody positive	p-value
Gender				p=0.002
Male	30	73	41.1%	
Female	81	125	64.8%	
Race				p=0.0014
White	79	157	50.3%	
Non-White	32	41	78.1%	
Black	2	3	66.7%	
Asian	8	12	66.7%	
Hispanic	11	12	91.7%	
Other	11	14	78.6%	
Deep Kissing:				p=0.047
yes	90	149	60.4%	
no	21	48	43.8%	
History of Infectious Mononucleosis:				p=0.076
yes	11	14	78.6%	
no	99	183	54.1%	
Overall EBV antibody status				
positive		111	56.1%	
negative		87	43.9%	