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Sexual History and Epstein-Barr Virus Infection

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To determine the role of sexual contact in transmission of Epstein-Barr virus (EBV) and occurrence of infectious mononucleosis (IM), a cross-sectional study was undertaken of EBV serologic testing and histories of IM and sexual behavior among 1006 new students at Edinburgh University. Prevalence of EBV seropositivity was significantly greater among women (79.2%) than among men (67.4%; P < .001) and among those who had ever been sexually active (82.7%) than among those who had not (63.7%; P < .001). Having a greater number of sex partners was a highly significant risk factor for EBV seropositivity. Two thirds of IM cases, but only a tenth of asymptomatic primary EBV infections, were statistically attributable to sexual intercourse. The findings suggest that EBV transmission occurs during sexual intercourse or closely associated behaviors. Transmission in this way appears to account for most cases of IM but for only a minority of cases of asymptomatic EBV infection, which mainly occur at younger ages.

Epstein-Barr virus (EBV) is a tumorigenic herpes virus that is carried as a persistent infection by >90% of adults. Most persistently infected people produce EBV in their saliva, and transmission from infected to uninfected individuals is through close contact. EBV infection generally occurs early in life, often spread among family members, and primary infection with seroconversion usually occurs without clinical illness [1]. However, in Western countries where the standard of hygiene is high, primary EBV infection may be delayed until early adulthood, and this causes infectious mononucleosis (IM) in 45%–65% of cases [2, 3]. IM is a benign lymphoproliferative disease that is common among university students and can cause significant loss of working time because of prolonged illness and subsequent fatigue [4]. IM is also associated with an increased risk of subsequently developing Hodgkin disease [5].

Apart from one landmark study of US army recruits in the 1960s, which firmly established EBV as the cause of IM [6], most reports have been descriptive, and little is known about the detailed epidemiology of the virus. Because it is present in saliva, EBV is assumed (without direct evidence) to be spread among young adults through kissing. Two studies have reported EBV in male and female genital secretions [7, 8], but evidence on whether the virus is spread by this route is very limited. One study has suggested that the spread of a rare strain of EBV (EBV type 2) among homosexual men is through sexual contact [9], and a study of 98 Swedish teenaged girls gave evidence that EBV seropositivity was associated with previous sexual contact [10]. It is unclear, however, whether the risk among heterosexual individuals is related to age at first sexual intercourse or number of sex partners, and there is no information on whether this route of transmission can cause IM. This is a particularly important issue now that vaccines with the potential to prevent IM are being tested. The present study surveyed >1000 new university students to determine the relationship between sexual behavior and EBV infection in this population.

Subjects and Methods

Students who enrolled at the Edinburgh University Health Centre in October 1999 and who were enrolled in courses for which they would remain in Edinburgh for ≥4 years were invited to join the study. Most Edinburgh University courses last for ≥4 years, and the study was restricted to these students, because a major purpose is future follow-up of students who were initially EBV seronegative, to investigate risk factors for seroconversion. After reading an information sheet and discussing the study with one of the study coordinators, interested students were asked to sign a consent form.

All study participants were asked to complete an anonymous,
confidential questionnaire about their lifestyles before attending the university. This included information about demographic factors and history of sexual behavior (numbers and sexes of partners, age at first sexual intercourse, and use of contraception), sexually transmitted diseases, and IM. A blood sample taken from each participant was used to test for serum IgG antibodies to EBV capsid antigen by a routine indirect immunofluorescence assay [11].

Statistical analyses were conducted to investigate the relationship of each of the demographic and behavioral factors with the risk of EBV positivity and history of IM. Because these outcomes were not rare, calculation of odds ratios would give a biased estimate of relative risks, and, therefore, prevalence ratios were calculated instead, as the best estimate of relative risks [12]. The proportions of cases of IM and of asymptomatic seroconversion among sexually active students that were statistically attributable to sexual intercourse were determined by calculating the excess fraction (attributable risk percentage) [13].

The prevalence ratios shown in the tables are based on prevalences within the entire study group, for simplicity and consistency. This may not always be the best measure of the effects of sexual factors on seroconversion, however, because many students will have seroconverted asymptomatically in childhood, before their first sexual intercourse, and therefore will not actually be at risk of seroconversion from sexual intercourse. To allow for examination of the effects on the study results of different assumptions about childhood asymptomatic seroconversion, we reanalyzed the risks of IM, excluding the silent seroconverters from the denominator (i.e., taking the extreme assumption that all silent seroconversion had occurred before the first sexual intercourse). These results are outlined in the text.

Adjustment for potential confounding was undertaken by Mantel-Haenszel stratification [14]. Ninety-five percent confidence intervals were Wald-based, and likelihood ratio tests for trend and heterogeneity were undertaken [14]. The calculations were done using the Stata statistical package [15].

Results

In total, 1950 new students (800 men and 1150 women) who registered with the Edinburgh University Health Centre for the autumn term of 1999 were eligible for the study. Of these, 1006 (52%) agreed to take part in the study, completed a questionnaire, and gave a blood sample. Of the remainder, about half declined, and half were not asked to take part because of the hectic nature of the general practitioner registration procedure during the first few days of term. This gives an overall response rate of 68%. Three hundred and seventy-one participants were men, and 635 were women; 945 (94%) were 19 years old, and 61 were ≥21 years old.

Overall, 753 students were EBV seropositive, and 253 were EBV seronegative. Of the EBV-seropositive students, 110 had a history of IM, and 643 had no such history. Ten of the EBV-seronegative students stated that they had a history of IM. The risk of being EBV seropositive was highly significantly greater among women, compared with men, with a greater likelihood of both IM and asymptomatic seroconversion among women (table 1). Adjustment for number of sex partners (data not shown) left these results virtually unchanged. For both men and women, there was a tendency, although not significant, for EBV positivity to be more prevalent at older ages.

Fifty-seven percent of men and 58% of women had been sexually active before coming to the university (data not shown). There was also similarity between the sexes in the age at first sexual intercourse and in the number of partners. Results for risk relative to sexual intercourse were similar for both men and women, and, therefore, both are combined in the analyses below.

Risks of EBV seropositivity overall, asymptomatic seroconversion, and IM were highly significantly greater among students who had ever been sexually active (table 2). Risks of EBV seropositivity were similar for heterosexual students and for those who were homosexual or bisexual, although there were wide confidence intervals for the latter groups. There was a complete absence of IM among homosexual students. On the basis of the likelihood ratio test, there was significant heterogeneity in risk of IM by sex of partner, although this must be interpreted cautiously because of the zero value in one of the cells in table 2. The proportions of cases of IM and of asym-

Table 1. Risk of Epstein-Barr virus (EBV) positivity and history of infectious mononucleosis, by sex and current age.

<table>
<thead>
<tr>
<th>Sex, age (years)</th>
<th>EBV negative</th>
<th>No history of infectious mononucleosis</th>
<th>History of infectious mononucleosis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of subjects</td>
<td>Prevalence, %</td>
<td>No. of subjects</td>
<td>Prevalence, %</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;19</td>
<td>77</td>
<td>36.0</td>
<td>120</td>
<td>56.1</td>
</tr>
<tr>
<td>≥19</td>
<td>44</td>
<td>28.0</td>
<td>98</td>
<td>62.4</td>
</tr>
<tr>
<td>Total</td>
<td>121</td>
<td>32.6</td>
<td>218</td>
<td>58.8</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;19</td>
<td>84</td>
<td>22.4</td>
<td>250</td>
<td>66.7</td>
</tr>
<tr>
<td>≥19</td>
<td>48</td>
<td>18.5</td>
<td>175</td>
<td>67.3</td>
</tr>
<tr>
<td>Total</td>
<td>132</td>
<td>20.8</td>
<td>425</td>
<td>66.9</td>
</tr>
</tbody>
</table>

NOTE. CI, confidence interval; RR, relative risk.

* P < .05.

b Female vs. male, adjusted for age.

c P < .001.
There was some indication of an increase in EBV seropositivity with younger age at first sexual intercourse, and there was a significant trend in this direction for IM (table 3). The risk of seropositivity was highly significantly related to number of sex partners (table 3), and, again, this was more clearly present for IM than for asymptomatic seroconversion. The analyses shown are for heterosexual students; there were too few homosexual and bisexual students to conduct separate analyses for these groups. Adjusting the results on age at first sexual intercourse for number of sex partners reduced the significance of the trend in risk of IM from $P = .04$ to $P = .18$ (data not shown). Similarly, adjustment of number of sex partners for age at first sexual intercourse reduced the trend in risk of IM from $P = .02$ to $P = .08$ and the trend in risk of EBV seropositivity from $P = .001$ to $P = .002$. Repetition of the analyses of risk of IM in relation to number of sex partners and age at first sexual intercourse, with exclusion of seronegative subjects from the denominator (see Subjects and Methods), produced stronger trends than those seen without this exclusion; relative risks were $5.31$ for first sexual intercourse at age $<16$ years and $5.89$ for $\geq 5$ sex partners ($P = .02$ and $P = .002$, for the respective trends in risk; data not shown). Examination of the percentage prevalence of EBV seronegativity in table 3 in relation to number of sex partners shows, although not consistently, a $\sim 30\%$ reduction in seronegativity for each additional sex partner.

The questionnaire asked about long-term sexual relationships (i.e., those $\geq 1$ year in duration), as well as about any other sexual relationships. Among students who had had any sexual intercourse, the risk of EBV positivity was unrelated to the number of long-term relationships, both in crude analyses and in those adjusted for number of short-term relationships. When risks of EBV positivity were examined in relationship to cross-tabulated categories of number of sex partners and age at first sexual intercourse (table 4), both prevalences and relative risks increased with number of sex partners within each age stratum, but prevalences and relative risks for age at first sexual intercourse showed less consistent trends.

**Discussion**

EBV is a ubiquitous herpesvirus that is of clinical importance because of its association with IM and with a variety of human tumors. The frequency of IM and the high morbidity and mortality on a worldwide scale of EBV-associated malignancies make EBV a prime target for a vaccine to prevent primary infection. However, recent detailed information on the epidemiology of the virus, which is needed to plan a logical vaccine strategy, is lacking.

A previous study undertaken in the United Kingdom in the
1970s showed 2 peaks of EBV seroconversion, one at ages 6–7 years and another at ages 14–15 years [16, 17]. Although it has been suggested that the first peak of infection is due to virus spread by family members and the second due to virus spread through sexual contact, there is very limited evidence to support this supposition. One recent study showed that EBV type 2 infection, which accounts for only 1%–3% of infections in the general population, is related to sexual intercourse among homosexual men [9]. A second study showed an association between EBV seroconversion and previous sexual activity in 98 teenage girls in Sweden [10]. Our study was undertaken to gain up-to-date epidemiology, including more-detailed information on the role of sexual contact in EBV transmission, and information on risk factors for IM in a group of 1006 university students.

Because a cohort study from early childhood is not practical, the retrospective cross-sectional design of the present study is the only feasible alternative. However, this leads to the possibility that information may be inaccurate through recall. Although this may have led to underestimation of risk associated with sexual intercourse, such misclassification is likely to have been nondifferential (i.e., not of a different nature in serologically EBV-seropositive, compared with EBV-seronegative, individuals), especially since the students did not know their EBV serostatus. Misclassification of this type leads to dilution of true risks, not inflation or creation of them [13]. The major outcome examined in the study, EBV serological status, was a laboratory measure, not a recall variable. The information on the other outcome variable, IM, did originate from recall, but the findings were supported by the serologic test results, so could not be simply a recall artifact; for this variable, too, recall errors would be expected to be nondifferential and so to have led to underestimation, not inflation, of associations. Our ~68% response rate among eligible students who were approached to take part in the study is reasonably high for a study involving venepuncture and recruiting from a noninstitutionalized, healthy population. Again, this is likely to have been nondifferential and hence not to have led to artifactual associations, especially since EBV serostatus was unknown to respondents and nonrespondents alike.

We found a highly significant association between sexual intercourse and EBV seropositivity, with a correlation with increasing numbers of sex partners. These data strongly suggest that sexual contact, or a factor closely associated with it, is an important factor in acquisition of EBV during the teenage years. Because EBV has been found in genital secretions from healthy seropositive men and women [7, 8], direct spread of virus during sexual intercourse is possible. It is very difficult, however, to distinguish between this and transmission of virus by kissing or orogenital contact during sexual intercourse, and, therefore, an indirect association remains a possibility. Our results show some evidence of a reduction in risk with condom use, which would accord with a direct route of transmission, but the results are not clear cut and need repetition. It seems implausible that the association with sexual intercourse could be explained by confounding factors; certainly, there is no known nonsexual etiological factor that could provide such an explanation.

The increasing risk of seropositivity with increasing number of sex partners would accord with a fixed risk per partner of

### Table 3. Risks of Epstein-Barr virus (EBV) positivity and infectious mononucleosis, by age at first intercourse and number of sex partners.

<table>
<thead>
<tr>
<th>Variable</th>
<th>EBV negative</th>
<th></th>
<th>EBV positive</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of subjects</td>
<td>Prevalence, %</td>
<td>No. of subjects</td>
<td>Prevalence, %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at first intercourse</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>153</td>
<td>36.3</td>
<td>248</td>
<td>58.8</td>
</tr>
<tr>
<td>≥18 years</td>
<td>31</td>
<td>20.1</td>
<td>106</td>
<td>68.9</td>
</tr>
<tr>
<td>16–17 years</td>
<td>51</td>
<td>17.1</td>
<td>197</td>
<td>66.1</td>
</tr>
<tr>
<td>≤15 years</td>
<td>11</td>
<td>11.6</td>
<td>64</td>
<td>67.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of sex partners</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>153</td>
<td>31.4</td>
<td>248</td>
<td>58.8</td>
</tr>
<tr>
<td>1</td>
<td>48</td>
<td>23.4</td>
<td>132</td>
<td>64.4</td>
</tr>
<tr>
<td>2–4</td>
<td>36</td>
<td>15.5</td>
<td>157</td>
<td>67.7</td>
</tr>
<tr>
<td>≥5</td>
<td>9</td>
<td>8.4</td>
<td>75</td>
<td>70.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOTE. Analysis was restricted to students with opposite sex partners only. CI, confidence interval; RR, relative risk.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a Adjusted for sex.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b P &lt; .05.</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>c P &lt; .01.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d P &lt; .001.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e Restricted to students who had ever had intercourse.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>f P for trend. The trend tests exclude the never/none category; if this were included, the trends would be stronger.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g Data not available for 3 students.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
ual intercourse may occur at older ages. It seems unlikely that
mon over time, or some seroconversion unconnected with sex-

17]; perhaps childhood seroconversion has become more com-
positivity at ages 10–12 years found in Britain in the 1970s [16,

presumably largely or entirely a consequence of seroconversion
positivity in students who had never had sexual intercourse is
intercourse are symptomatic. The 63.7% prevalence of sero-

gradient in prevalence of a history of asymptomatic seroconversion, im-

The increase, in percentage point terms, in prevalence of a

∼30% (see Results). This suggests that, on a statistical basis,
only in about half of instances does sexual intercourse with an
infected person lead to seroconversion. If EBV seropositive
partners infallibly transmitted the virus, then each new partner
should give a risk equivalent to the prevalence of seropositivity
among persons of that age (or perhaps greater, because partners
would, on average, have had more sexual experience than
would random persons of the same age), which would be ∼60%
[16, 17]. The analyses of risk in relationship to long-term sexual
relationships suggested that risk of infection by an EBV-positive
partner is not increased by greater numbers of episodes of sexual
intercourse.

The relationship of risk of IM to age at first sexual intercourse
is less clear. Without adjustment for number of sex partners,
the relationship was just significant; after adjustment, it
was not significant, so it may simply reflect confounding by
number of sex partners as the true risk factor. In cross-tabu-
lations by both factors, there was mixed evidence of an inde-
pendent effect of age at first sexual intercourse. If there is an
effect, it would be reminiscent of the relationship between the
sexually transmitted papilloma virus and cervical cancer and
might be due to greater susceptibility for exposures occurring at
younger ages. A larger study is needed to clarify whether age at first sexual intercourse is an independent risk factor.

The increase, in percentage point terms, in prevalence of a
history of IM with increasing numbers of sex partners (e.g.,
increasing by 7.2% from 0 partners [5.0%] to 1 partner [12.2%],
and by 4.6% from 1 partner [12.2%] to 2–4 partners [16.8%];
table 3) was somewhat greater than the corresponding increase
in prevalence of a history of asymptomatic seroconversion, im-
plying that a slight majority of seroconversions due to sexual
intercourse are symptomatic. The 63.7% prevalence of sero-
positivity in students who had never had sexual intercourse is
presumably largely or entirely a consequence of seroconversion
in childhood. It is somewhat higher than the 45%–50% sero-
positivity at ages 10–12 years found in Britain in the 1970s [16,
17]; perhaps childhood seroconversion has become more com-
mon over time, or some seroconversion unconnected with sex-
ual intercourse may occur at older ages. It seems unlikely that

<table>
<thead>
<tr>
<th>No. of sex partners</th>
<th>Never sexually active</th>
<th>&gt;18</th>
<th>16–17</th>
<th>≤15</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prevalence, % RR (95% CI)</td>
<td>Prevalence, % RR (95% CI)</td>
<td>Prevalence, % RR (95% CI)</td>
<td>Prevalence, % RR (95% CI)</td>
</tr>
<tr>
<td>None</td>
<td>63.7 1.0</td>
<td>78.1 1.23 (1.08–1.39)</td>
<td>61.5 0.99 (0.64–1.51)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>— —</td>
<td>77.1 1.24 (1.10–1.40)</td>
<td>— —</td>
<td></td>
</tr>
<tr>
<td>2–4</td>
<td>— —</td>
<td>83.3 1.34 (1.16–1.54)</td>
<td>89.7 1.43 (1.26–1.59)</td>
<td></td>
</tr>
<tr>
<td>≥5</td>
<td>— —</td>
<td>86.7 1.36 (1.10–1.66)</td>
<td>95.3 1.48 (1.34–1.63)</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Analysis was restricted to students with opposite sex partners only. CI, confidence interval; RR, relative risk.

Table 4. Risks of Epstein-Barr virus positivity, by subcategories of age at first intercourse and number of sex partners.

There were appreciable numbers of students who had had sexual
intercourse but replied that they had not; the replies in the
questionnaires seemed to be very frank, and this is our expe-
rience of the students.

We calculated prevalences of EBV and IM history status in
relation to the whole student group as the denominator, but it
is arguable whether this provides the best assessment of the
effect of intercourse on these factors. Ideally, the analyses would
be restricted to individuals who had not seroconverted in child-
hood and, therefore, were at risk of seroconversion as a con-
sequence of sexual behavior. Previous studies [16, 17] indicate
that ∼50% of individuals in Britain seroconvert by age 12 years,
almost all without clinical illness. Thus, it is probable that many
seropositive individuals, if their serological status before their
first sexual intercourse had been known, should not have been
included in the denominator for the analyses.

Removal of these childhood seroconvertors from the analy-
sis, however, would be likely to increase the associations shown
in the tables, as illustrated by the reanalyses of IM risk shown
in Results. The prevalence of seropositivity at entry to univer-
sity was significantly greater among women than among men,
a difference not explained by age at first sexual intercourse or
number of sex partners. It might be related to the fact that
women tend to have sexual relationships with men older than
themselves; for those who have sexual intercourse before uni-
versity entrance, this might make them more likely to have
seropositive partners who would be able to transmit the virus.
The deficit of IM in homosexual students, with evidence of
heterogeneity in risk by sex of partner, is based on small num-
bbers, but raises the possibility that clinical severity of infection
may be affected by the sex of the partner, because there was
not a substantial deficit of overall seropositivity among ho-

The corollary of the finding that sexual contact is associated with
spread of EBV is that lack of previous sexual experience is a risk factor for EBV seronegativity. Indeed, among women,
three-quarters of seronegative students had never had inter-
course. To reach adult levels of seropositivity in the United
Kingdom, ∼90% of seronegative students will be infected with
EBV while at university, and \(~45\%–65\%\) of these will develop IM [2, 3]. Therefore, this group could benefit from a vaccine that prevents primary EBV infection.

Acknowledgments

We thank all the student volunteers; the staff of the Edinburgh University Health Centre for their help with this study; P. Hamill for her efficient organization of student recruitment; T. Alexander, B. Armstrong, A. Bommi-Reddy, T. Haque, C. Maguire, S. Morris, H. Nguyen, and J. A. Thomas, for their valuable help in recruiting study participants; Z. Qiao for computer programming; I. Anthony, A. Ashton, A. Bielski, E. Bonell, C. Boyd, D. Dombagoda, B. Forrest, R. Harper, D. MacDonald, I. McWilliam, and K. Matthews, for their valuable help in recruiting study participants; F. Burden, K. Chaggar, D. Keith, I. McAllister, I. Montgomery, and D. Porter, for phlebotomy; and J. Peto for valuable suggestions and advice regarding the analysis.

References