Human Papillomavirus Infection and Esophageal Cancer: a Nationwide Seroepidemiologic Case–Control Study in Sweden

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Background: Infection with human papillomavirus (HPV) type 16 has been implicated as a risk factor for esophageal squamous cell carcinoma in three seroepidemiologic studies. We conducted a larger, population-based study to verify this association and to investigate possible confounding factors.

Methods: We performed a nationwide case–control study in Sweden of HPV16 or HPV18 infection and risk of esophageal squamous cell carcinoma or esophageal/gastroesophageal adenocarcinoma. Tumors were strictly classified by their location and histologic type. Case subjects with incident cancers and population-based control subjects donated blood samples and were interviewed in person about potential confounding factors. An enzyme-linked immunosorbent assay was used to detect HPV seropositivity. Multivariate analyses were conducted to study relationships between HPV seropositivity, level of education, smoking (all tobacco) status, alcohol consumption, and cancer risk.

Results: We compared 121 case subjects with esophageal squamous cell carcinoma and 173 case subjects with adenocarcinoma of the esophagus or gastroesophageal junction with 302 population-based control subjects. The age- and sex-adjusted odds ratios (ORs) for squamous cell carcinoma were 1.0 (95% confidence interval [CI] = 0.5–2.0) for persons seropositive for HPV16 and 0.5 (95% CI = 0.2–1.1) for persons seropositive for HPV18 in comparison with seronegative individuals. The corresponding ORs for adenocarcinoma were 1.2 (95% CI = 0.7–2.2) and 0.2 (95% CI = 0.1–0.7), respectively. Adjustments for smoking status, alcohol consumption, and level of education did not alter the results.

Conclusions: We found no evidence of a positive association between HPV16 or HPV18 infection and either form of esophageal cancer. Our results do not support conclusions from previous studies. [J Natl Cancer Inst 1999;91:156–62]
type 16, has recently been implicated as a possible risk factor for esophageal cancer in three seroepidemiologic studies (5–7). In two prospective studies (6,7), HPV16 seropositivity was associated with a more than sixfold excess risk. Although the number of observed cases did not permit separate analyses by histologic type, the association appeared to be the strongest in the case of squamous cell carcinoma (6,7). There was no excess risk associated with HPV18 seropositivity [(7); Dillner J: unpublished observation]. Also, a case–control study with hospital-based control subjects from a high-risk area (Shaanxi Province, China) found a statistically significant association (odds ratio [OR] = 4.5; 95% confidence interval [CI] = 1.8–11.9) between HPV16 seropositivity and esophageal cancer (mainly squamous cell carcinoma) (5). A dose–response gradient was observed between increased risk and increasing levels of HPV antibody (5).

An association between HPV and esophageal squamous cell carcinoma would seem biologically plausible by analogy with the association between bovine papillomavirus and esophageal cancer in cattle (8,9) and by analogy with the fact that infection with HPV, especially types 16 and 18, is well established as a risk factor for anogenital cancer in humans (10,11). Furthermore, although not universally confirmed (12,13), several groups (14–16) have reported detection of HPV DNA, predominantly type 16, in squamous cell carcinoma of the esophagus.

We performed a population-based, case–control study to investigate whether the association between HPV16 seropositivity and esophageal cancer remains consistent when the study size is expanded to include case subjects nationwide and if the association holds after adjustment is made for confounding risk factors. We analyzed the two histologic types of esophageal cancer separately, included analysis of HPV18 for comparison, and performed a detailed analysis of established risk factors that enabled us to control for possible confounding effects.

Subjects and Methods

Study Design

This population-based, case–control study included all individuals in Sweden of ages below 80 years, who were born in Sweden and were still living there during the period December 1, 1994, through January 31, 1997. All individuals with incident adenocarcinoma of the esophagus or gastroesophageal junction and about half the case subjects with incident squamous cell carcinoma of the esophagus (born on even dates) were to be recruited as case subjects. The main purpose of our nationwide case–control study was to identify risk factors for adenocarcinoma of the esophagus (5,17) and to detect Barrett’s esophagus. Barrett’s esophagus is a columnar cell metaplasia of the esophagus, strongly associated with development of adenocarcinoma (17). In patients who underwent tumor resection, we obtained on formalin-fixed paraffin-embedded tumor tissue, detailed descriptions of the location of the tumor, and to detect Barrett’s esophagus. Barrett’s esophagus is a columnar cell metaplasia of the esophagus, strongly associated with development of adenocarcinoma (17). In patients who underwent tumor resection, we obtained on special forms from the surgeons and the pathologists detailed descriptions of the location of the cancer. In ambiguous cases, a panel of surgeons and pathologists using all available information classified the cases. To be classified as cancer of the gastroesophageal junction, the tumor had to have its center within 2 cm proximal to or 3 cm distal to the gastroesophageal junction. If Barrett’s esophagus was detected adjacent to the tumor, it was classified as esophageal, irrespective of location in the gastroesophageal junction. Squamous cell carcinomas were classified as esophageal even if they were located in the gastroesophageal junction.

Exposure Information

Case subjects and control subjects were interviewed in-person about possible risk factors. The interviews were conducted by specially trained, professional interviewers from Statistics Sweden (Orebro, Sweden). We were unable to blind the interviewers to the case or control status of the interviewees, but the interviewers were unaware of the study hypotheses and were trained to deal with the case subjects and control subjects in a similar manner.

Serum samples were collected from case subjects and control subjects in order to search for serologic evidence of current or previous HPV16 or HPV18 infection. To increase the number of case subjects with squamous cell carcinoma of the esophagus for this substudy, at 1 year after the start of the study, we collected sera not only from the patients with squamous cell carcinoma born on even dates but also from those born on odd dates. We did not interview the latter patients.

The blood samples were drawn for serum analysis during the patients’ initial hospital stay or during a follow-up visit at the outpatient department. The control subjects were asked to provide a blood sample at a local health care unit. The laboratory performing the analyses had no information about the identity of the samples.

To detect immunoglobulin G antibodies against HPV16 and HPV18 capsids (obtained from Dr. John T. Schiller, National Cancer Institute, Bethesda, MD), we used the validated enzyme-linked immunosorbent assay, which was identical to the one used in previous studies of HPV seropositivity and esophageal cancer (6,7). We included analysis of the same internal standards on each plate. Two cutoff levels were used for assigning seropositivity in conversion from continuous to categorical data (0.100 and 0.261 absorbance units). Any value above the cutoff level meant that the sample was positive for HPV; a value below this point was considered negative for the specific antibody. Relative to internal standards, these cutoff points were the same as those that had been used in previous studies of esophageal cancer (6,7), which meant that our results could be compared directly with findings from these previous studies. Both in this study and in our previous studies (6,7) the cutoff levels were preassigned, i.e., assigned before the start of the study. This is essential for statistical validity of categorical analysis of continuous data (18). The cutoff level of 0.100 absorbance units distinguishes HPV16-infected women from women who are not infected (19). The sensitivity of the method for detection of HPV16-infected women, with the use of detection of cervical HPV DNA by polymerase chain reaction as reference, is consistently at least higher than 50% (range, 95–98%) (19–21). In previous studies of HPV16 (6,7,22), however, the highest relative risk for esophageal or cervical cancer was found at a somewhat higher antibody level, corresponding to 0.261 absorbance units.

Statistical Analyses

A first simple comparison of HPV values (absorbance units) in different groups was performed by the Mann–Whitney test. Regression models adjusting for age and sex produced very similar results. We used logistic regression in both univariate and multivariate analyses of the relationship between HPV antibody levels, potential confounding factors, and cancer risk. The confounding factors were tobacco smoking status (categorized into three classes at a point 2 years before the interview: never smokers, previous smokers, and current smokers), alcohol intake (total alcohol in grams in three classes), and socioeconomic status (educational level in three classes). The case subjects in each cancer category were compared with all 302 control subjects. Model parameters were estimated by the maximum likelihood method (23). From these estimates, ORs with 95% CIs were computed. In the baseline model, adjustments were made for age and sex. We modeled the effect of HPV antibody levels in three ways. First, we used a dichotomous approach, with case subjects and control subjects classified as...
exposed (serum levels >0.100 absorbance units) or unexposed (serum levels ≤0.100 absorbance units), in age- and sex-adjusted models. Second, we divided the absorbance values into three categories (<0.100, 0.101–0.261, and >0.261 absorbance units). In these age- and sex-adjusted analyses, the exposed groups were compared with the group with antibody levels below 0.100 absorbance units. Finally, we estimated models with the HPV antibody levels in continuous form. In the multivariate modeling, with adjustment for the potential confounders smoking status (never smoker, previous smoker, and current smoker), alcohol intake (in grams per week), and socioeconomic status (measured as years of education), we could use only half of the squamous cell carcinoma case subjects, since the other half had not been interviewed. To retain statistical power in the multivariate analyses, we divided the subjects according to only two antibody levels (<0.100 versus >0.100 absorbance units).

**Ethical Considerations**

Sera were collected after all individuals provided a signed informed consent. The study was approved individually by all regional ethics committees in Sweden.

**RESULTS**

Of 507 case subjects with cancer of the esophagus or gastroesophageal junction observed in the study base, 294 patients (58%) were included in this study. Their characteristics are presented in Table 1. There were 121 patients with squamous cell carcinoma of the esophagus, of whom 62 underwent interview. Furthermore, we included 72 patients with adenocarcinoma of the esophagus and 101 patients with adenocarcinoma of the gastroesophageal junction, of whom only 155 were interviewed. The reason for nonparticipation was refusal in 10 patients (2%), a physical or mental condition prohibiting participation in the study (these patients often died shortly after diagnosis) in 86 patients (17%), and technical/logistic problems (e.g., failure of the hospital staff to remember to draw blood samples for the study) in 117 patients (23%). A total of 302 control subjects were included. The participation rate in the control group of 587 was 51% (158 [27%] refused to participate at all and 129 [22%] were interviewed but did not donate blood).

Table 2 gives the means, medians, and ranges for the HPV16 and HPV18 antibody levels in the three cancer groups and the control group. The only statistically significant finding was that the serum levels of HPV18 antibodies were lower in patients with adenocarcinoma of the gastroesophageal junction than in control subjects.

**Risk of Esophageal Squamous Cell Carcinoma**

When the serum antibody level was treated as a dichotomous variable, the age- and sex-adjusted OR for squamous cell carcinoma among 47 HPV16-seropositive subjects (serum antibody level >0.100 absorbance units), relative to 376 HPV16-seronegative subjects (combined case subjects and control subjects) (serum level =<0.100 absorbance units), was 1.0 (95% CI = 0.5–2.0). The corresponding OR for 35 HPV18-seropositive subjects, relative to 393 HPV18-seronegative subjects (combined case subjects and control subjects), was 0.4 (95% CI = 0.2–0.5). As expected, smoking emerged as a strong risk factor for squamous cell carcinoma, whereas alcohol intake and a low level of education were less strongly associated risk factors. The ORs among subjects seropositive for HPV16 were all close to unity, regardless of the cutoff point. Persons with serologic evidence of past or present HPV18 infection tended to have low ORs for squamous cell carcinoma, albeit statistically nonsignificant. Adjustment for smoking status, alcohol consumption, and educational level only marginally changed the HPV-associated risk estimates. Likewise, adjustment for HPV18 seropositivity did not have any important effect on risk estimates observed for HPV16-seropositive subjects and vice versa (data not shown). Models with the serum antibody levels as a continuous variable also did not reveal any statistically significant association between serum antibody level and risk of squamous cell carcinoma (data not shown).

**Table 2. Mean, median, and range values for HPV16 and HPV18 capsid serum antibodies (in absorbance units) in the three esophageal cancer groups and the control group**

<table>
<thead>
<tr>
<th>Group</th>
<th>HPV16 antibody absorbance values</th>
<th>HPV18 antibody absorbance values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Median</td>
</tr>
<tr>
<td>Case subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma of esophagus</td>
<td>0.054</td>
<td>0.021</td>
</tr>
<tr>
<td>Adenocarcinoma of esophagus</td>
<td>0.058</td>
<td>0.019</td>
</tr>
<tr>
<td>Adenocarcinoma of gastroesophageal junction</td>
<td>0.055</td>
<td>0.015</td>
</tr>
<tr>
<td>Control subjects</td>
<td>0.066</td>
<td>0.020</td>
</tr>
</tbody>
</table>

*HPV16 = human papillomavirus type 16; HPV18 = human papillomavirus type 18.
†Two-sided P values (Mann–Whitney test) pertain to comparisons with control subjects.
Risk of Adenocarcinoma of the Esophagus or Gastroesophageal Junction

The age- and sex-adjusted OR for adenocarcinoma of the esophagus or the gastroesophageal junction in 54 HPV16-seropositive persons (antibody levels >0.100 absorbance units), relative to 421 HPV16-seronegative subjects (combined case subjects and control subjects) (=0.100 absorbance units), was 1.2 (95% CI = 0.7–2.2); however, in the 33 persons seropositive for HPV18, relative to 447 HPV18-seronegative subjects (combined case subjects and control subjects), it was statistically significantly low (OR = 0.2; 95% CI = 0.1–0.7). Table 4 shows the age- and sex-adjusted ORs after the serum antibody levels were divided into three categories and after multiple adjustments were made for other suspected risk factors. Use of a higher cutoff level for HPV16 seropositivity (>0.261 absorbance units) also did not reveal any association. The multiple adjustments shifted the point estimate slightly (OR = 1.4; 95% CI = 0.7–2.6). HPV18 seropositivity was inversely associated with adenocarcinoma also in the multivariate model (OR = 0.2; 95% CI = 0.1–0.7). Tobacco smoking was a moderately strong risk factor, whereas a high socioeconomic status (high educational level) tended to be protective, without reaching statistical significance. Alcohol intake showed no consistent association with age, sex, distribution, tobacco smoking status, alcohol intake, educational level, or place of residence (countryside or city) (data not shown).

Characteristics of Nonparticipants

We compared the persons who did not give a blood sample in connection with the interview with those who did, with regard to general characteristics (median age and sex distribution), socioeconomic factors (level of education and place of residence), and exposure to known risk factors for squamous cell carcinoma of the esophagus (tobacco smoking status and alcohol consumption). However, with respect to these characteristics, the nonparticipants and the participants were similar, and no statistically significant differences were found (data not shown).

Discussion

In this large population-based, case–control study, we did not detect any association between HPV type 16 or 18 seropositivity and risk of squamous cell carcinoma of the esophagus, and we found no association between HPV16 and adenocarcinoma of the esophagus or gastroesophageal junction. An unexpected finding was that HPV18 was negatively associated with adenocarcinoma.

Our results differ from those of the three previously published seroepidemiologic studies on esophageal cancer and HPV16 (5–7). In these previous studies, the tumors were not distinguished by histologic type, and the number of case subjects [90 (5), 39 (6), and 57 (7)] and control subjects [121 (5), 78 (6), and 171 (7)] were smaller than in our study (121 patients with squamous cell carcinoma, 173 patients with adenocarcinoma, and 302 control subjects). The seropositivity rates among control subjects were lower [7% (5), 3% (6), and 5% (7)] than those in our study (11%). However, the validity of the seropositivity rates was hampered by small numbers in the previous studies.
(the calculations were based on two to nine seropositive control subjects compared with 33 in our study), which meant that the point estimates of seropositivity in these previous studies had a low precision. In all studies, the subjects were predominantly males, but the male predominance was stronger in the Norwegian prospective study (7). The median age was higher in our study and in the Chinese case–control study (5), compared with two prospective studies (6,7). In the case–control study from China (5), the data were not adjusted for smoking status, alcohol consumption, or other known or suspected risk factors for the disease, and in none of the two prospective seroepidemiologic studies (6,7) were the data adjusted for alcohol consumption. The possibility that the association reported in previous studies was attributable to confounding is suggested by findings of an association of smoking and alcohol consumption with HPV positivity in some populations (6,24). In our study, HPV seropositivity was not significantly associated with any of these risk factors.

In our study, we found a statistically significant negative association between HPV18 infection and adenocarcinoma of the esophagus. It is hard to imagine any plausible mechanism for this negative association, and the possibility of bias in our study should be considered. Such bias might have arisen from the selection caused by nonparticipation. However, nonparticipation among the case subjects was explained most often by forgetfulness on the part of the staff at the participating hospitals and it is unlikely that such forgetfulness is in any way linked to HPV exposure in the patients. Some patients were unable to participate because their disease was advanced at the time of first diagnosis and they died early, but again it seems unlikely that the HPV exposure is linked to the tumor stage at presentation. In advanced stages of the cancer, disease-induced influences on the classification of the exposure status are likely to be increased, either as a result of a decreased ability of the immune system to respond with antibodies (anergy) (25) or as a result of reactivation of latent HPV infection; therefore, the loss of such patients is not likely to have severely affected the internal validity of the study. Nonparticipation among control subjects was mainly due to their unwillingness to be interviewed and/or to take the trouble to go to a health care unit to give a blood sample. Although HPV infection of the esophagus is not likely to cause symptoms, the possibility that control subjects having been infected with HPV might have had some symptoms in connection with this infection, making them more willing to follow through a blood donation, must be considered. But, again, we do not believe that this possible selection would explain the lack of association between HPV16 and cancer risk. Those who took the trouble to donate a blood sample are likely to be more health conscious than those who did not. Therefore, the nonparticipants may have been more, rather than less, likely to be infected with HPV than the participants. Furthermore, when we compared general characteristics, socioeconomic factors, and known risk factors of esophageal cancer among the persons who did not give a blood sample with those who did, we found no important differences.

Another possible reason for the discrepancy between the seroepidemiologic studies is the different study designs used. Prospective studies nested within closed cohorts with negligible loss to follow-up and ascertainment of outcome that is truly independent of exposure have the advantage of low probability of bias. On the other hand, a disadvantage of prospective studies done within historical cohorts, where accrual of subjects and collection of exposure information were organized for purposes other than to test the current hypothesis, is the possible inadequacy of information about exposure to suspected confounding factors. In a case like this, when a possible causative exposure is expected to have occurred long before the disease development, sampling at a time point closer to the exposure is likely to give a more accurate exposure assessment than what is attained when
the disease has already occurred. The previous studies (6,7) had a median follow-up of about 14 years. Although HPV antibodies have in general been stable over time in long-term follow-up (26), it is possible that some HPV capsid seropositivity may be lost if several decades pass between exposure and cancer diagnosis. Our case–control study design meant that blood testing was not performed until after the cancer diagnosis. The disease in the case subjects may have affected the antibody levels, but it is uncertain how this might have influenced the risk estimates. Differences in study design cannot explain the discrepancy with the Chinese case–control study (5), which was also based on cases sampled after diagnosis. It should be noted that the same control group was used in the analyses of both cancer types, which is a potential source of inflated type I error compared with the situation with just one case type. However, epidemiologic studies often include tests of many models as parameters, which means that, in a strict sense, the type I error is typically inflated. Furthermore, because our study gave negative results, problems caused by false statistically significant findings were not the most critical to exclude.

After having considered all the above noted possible reasons for the discrepancy between our study and the previous seroepidemiologic studies, we find it likely that the main reason was the lack of control for important confounding factors in previous studies.

Strengths of our study include the population-based design and the comparatively large number of case subjects and control subjects; to our knowledge, the number of exposed subjects in our study was larger than in any previous seroepidemiologic study on HPV and esophageal cancer. Patients with histologically verified squamous cell carcinoma were compared not only with population-based control subjects but also with patients with adenocarcinoma of the esophagus or gastroesophageal junction. Great efforts were made to standardize and optimize the diagnosis to reduce misclassification. The interview data enabled us to control for important confounders.

The HPV serologic method applied has been extensively validated as a marker of HPV16 and HPV18 infection (20,21). Since this method captures both past and present HPV infection, it could explain our negative results. Also, HPV seropositivity is much more common in patients with cervical and anogenital cancers than in patients with esophageal and gastroesophageal cancer. Great efforts were made to standardize and optimize the diagnosis to reduce misclassification. The interview data enabled us to control for important confounders.

The occurrence of HPV DNA as detected by modern polymerase chain reaction methods is considerably lower in esophageal tumors (generally 0%–10%; range, 0%–60%) (13,27,28) than in cervical tumors (consistently >90%) (28). Also, HPV seropositivity is much more common in patients with cervical and anogenital cancers (22,29) than in those with esophageal cancers. Therefore, it appears that any association between HPV and esophageal cancer, if it exists, would be limited to a minority of cases.

In conclusion, our large population-based study, with strict classification of incident cases and with control for confounding factors, found no evidence of a role of HPV16 infection as a risk factor for esophageal cancer—be it squamous cell carcinoma or adenocarcinoma.

REFERENCES


NOTES

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