Anti-HPV-E7 seropositivity and risk of esophageal squamous cell carcinoma in a high-risk population in China

Zhonghu He¹, Zhongyang Xu¹, Dong Hang³, Fangcen Guo, Amir Abizli, Noel S. Weiss¹, Longfu Xu¹,², Fangfang Liu, Tao Ning, Yaqi Pan, Chuanhai Guo, Yongmei Liang, Changdong Lu³, Lixin Zhang¹, Hong Cai⁶ and Yang Ke†³

Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Laboratory of Genetics, Peking University Cancer Hospital & Institute, No. 52 Fucheng Road, Beijing 100142, P.R. China; ²Department of Epidemiology, School of Public Health and ³Department of Pathology, School of Medicine, University of Washington, Seattle, WA, USA and ¹Anyang Cancer Hospital, No. 1 Yinhe Road, Anyang, Henan Province 455000, P.R. China

*To whom correspondence should be addressed. Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Laboratory of Genetics, Peking University Cancer Hospital & Institute, No. 52 Fucheng Road, Haidian District, Beijing 100042, P.R. China.

Tel: +86 10 88196762; Fax: +86 10 88196735; Email: dhcai@gmail.com

Correspondence may also be addressed to Yang Ke.
Tel: +86 10 88196762; Fax: +86 10 88196735; Email: keyang@bjmu.edu.cn

Results of previous serologic studies on the association of human papillomavirus (HPV) with esophageal squamous cell carcinoma (ESCC) have been inconsistent. From 2007 to 2010, the authors collected blood samples and relevant demographic data from 1435 patients with ESCC and 2071 age- and sex-matched normal controls from Anyang, China. HPV-16, 18 and 57 E7 antibodies were evaluated with the glutathione-S-transferase capture ELISA. The proportions of subjects who were positive for antibodies against these three HPV antigens in the case group were all significantly higher than those in the control group. In multivariate analysis, the presence of HPV-16 E7 antibody was associated with an increased risk of ESCC [odds ratio (OR) = 3.6, 95% confidence interval (CI): 2.5–5.0], whereas the presence of HPV-18 (OR = 1.1, 95% CI: 0.7–1.7) and HPV-57 (OR = 1.3, 95% CI: 0.9–1.9) antibodies were not significant after adjustment for HPV-16. In multiple cutoff value analysis, the lowest OR for HPV-16 was obtained with the standard cut point mean + 3 SD. This study provides serological evidence in support of HPV-16 infection playing a role in the occurrence of ESCC in a high-incidence area of China.

Introduction

In 2011, esophageal cancer was the fifth most common cause of cancer death in men worldwide and the eighth most common cause in women. The incidence of this tumor varies widely in different regions and populations (1). Extremely high rates (>50 per 100 000 per year) of esophageal cancer have been found in some parts of China. Over 90% of the esophageal cancer in these areas is squamous cell carcinoma. However, the principal risk factors are still poorly understood (2,3).

It is well established that oncogenic types of human papillomavirus (HPV) are necessary for carcinogenesis in cervical cancer (4), and HPV is also associated with some other anogenital and non-anogenital squamous-cell-derived carcinomas, such as penile cancer in males and oropharyngeal cancer (5–7). It was first suggested in 1982 that HPV is a potential etiologic factor in esophageal squamous cell carcinoma (ESCC) (8,9). Epidemiologic studies regarding this issue have focused on DNA-based and serologic studies. The location of HPV infection can generally be determined with DNA-based evidence, but DNA-based evidence may reflect only current infection status, whereas serologic status is a marker of past and cumulative exposure.

More than 100 DNA-based studies focusing on HPV and esophageal cancer have been published, but results have been markedly inconsistent, including results in studies conducted in the same high-risk area in China as the current investigation (10–12). In contrast, there have been only eight reported serologic studies on HPV and ESCC, but these results are also inconsistent (Supplementary Table, available at Carcinogenesis Online). Four studies were carried out in regions of low risk for ESCC. Among these, prospective studies in Finland [odds ratio (OR) = 13.1, 95% confidence interval (CI): 1.6–108] (13) and Norway (OR = 10, 95% CI: 1.0–510) (14) found a strong positive association between HPV-16 antibody and ESCC. However, retrospective studies in Sweden (15) and Holland (16) observed no increased risk with ORs of 1.0 (95% CI: 0.5–2.0) and 0.8 (95% CI: 0.3–2.0), respectively. Three other studies were conducted in high-risk regions. Among these, retrospective studies carried out in Shaanxi province in China (17) and in South Africa (18) found a positive association of HPV-16 seropositivity and ESCC, with ORs of 4.5 (95% CI: 1.8–119) and 1.6 (95% CI: 1.2–2.1), respectively. A prospective study conducted in Lixinian, China also showed a mild positive relation (OR = 1.6, 95% CI: 0.8–3.3) (19). All these studies adopted L1/L2 antibodies as the principal marker for HPV exposure except for a very recently reported study carried out by Sitas et al. They investigated L1, E6 and E7 antibodies in serum samples from six case–control studies conducted in either high- or low-risk regions. The presence of antibodies to E6 for HPV-16 (OR = 1.9, 95% CI: 1.1–3.3) and HPV-6 (OR = 2.5, 95% CI: 1.5–4.3), and of capsid antibodies to HPV-33 (OR = 1.3, 95% CI: 1.0–1.7), HPV-6 (OR = 1.2, 95% CI: 1.1–1.4) and HPV-11 (OR = 1.3, 95% CI: 1.1–1.6), were associated with an increased risk of ESCC (20). Different study populations and sampling methods, together with the relatively small sample sizes in most of these studies, may in part account for the inconsistency of the results. In addition, with the exception of the studies conducted by Sitas et al. (20) and Van Doornum et al. (16), all of these studies (13,15,17–19,21) tested only for antibodies against a baculovirus-derived virus-like particle containing L1/ L2 capsid proteins as a serologic marker for HPV exposure, rather than E6/E7 early protein, which is directly involved in carcinogenesis (22). This difference in methodology may result in underestimation of the association of HPV infection and the occurrence of ESCC and may also be a cause of discrepancy among studies.

We have recently reported a DNA-based case–control study of subjects from rural Anyang, which is a high-risk area for ESCC in China. A strong association between presence of HPV DNA, especially HPV-16, in the esophagus and occurrence of ESCC was observed (12). HPV-16 and 18 are the two most important oncogenic types of HPV in cervical cancer and other HPV-related carcinomas (5,6,23,24). Although HPV-57 is not oncogenic for cervical cancer, it was one of the most common non-oncogenic types found in esophageal cancer tissue in our previous studies (12,25). In this study, we conducted a larger case–control serologic study with 1435 ESCC patients and 2071 age- and sex-matched controls collected from the same area. Antibodies to HPV-16, 18 and 57 E7 were evaluated to determine
whether the seroprevalence of HPV-E7 antibodies is associated with an increased risk of ESCC.

Materials and methods

Subjects and serum sample collection

From January 2007 to March 2010, we recruited 1435 consecutive, newly diagnosed patients who had undergone esophagectomy at Anyang Cancer Hospital for histologically confirmed ESCC. To ensure comparability with the control group, only cases from the rural area of Anyang were enrolled, and all patients who were selected participated in our investigation. Demographic data and personal information, including age, sex, place of residence, tobacco use and alcohol consumption history, together with family history of esophageal cancer including immediate family and relatives within three generations, were obtained with a patient questionnaire and review of medical records.

A total of 2071 normal controls from rural Anyang were frequency matched by age (in 5 year intervals) and sex to study cases. Among these, 1470 controls of ages 31–65 were randomly selected from over 7000 participants in an ongoing population-based esophageal cancer cohort study, which is being carried out among a representative sample of rural Anyang (overall response rate > 85%) over the same time period (26,27). The protocol for subject recruitment for this cohort study has been described previously (27). Briefly, subjects were eligible if they were permanent residents in one of the targeted villages of rural Anyang, between ages 25 and 65, generally healthy and willing to participate in the survey. To identify controls for study cases with older patients, another 601 residents of ages 66–80 were recruited from the same villages using inclusion criteria identical to the cohort except for the age range. To increase statistical power, we adopted varied case–control matching ratios in different age groups of 1:1 in the 56–60, 61–65 and 66–70 age groups; 1:2 in the 51–55 and 71–75 age groups and 1:3 in the 31–35, 36–40, 41–45, 46–50 and 76–80 age groups. A questionnaire regarding information similar to that obtained for ESCC cases was completed for each control subject in a one-on-one interview. A blood sample from each case patient was collected within 1 week prior to esophagectomy, and blood from control subjects was collected during the course of field work for the esophageal cancer cohort study. All blood samples were centrifuged at 3000 r.p.m. for 15 min immediately after collection and was then separated and stored at −20°C. Within 2 weeks following collection, serum specimens were transported to our laboratory in Beijing and stored in ultralow temperature freezers (−70°C) pending testing for HPV antibodies. All serum specimens were processed in an identical manner and rigorous quality control procedures were employed to prevent contamination (26). An individual informed consent form was signed by every participant. This study was approved by the Institutional Review Board of Peking University School of Oncology, China.

Serologic analysis

We adopted glutathione-S-transferase (GST) capture ELISA for use in this study, as it has been proven to be sensitive and specific and appropriate in large epidemiologic studies examining immune responses to several HPV types in parallel (28,29).

GST–E7 fusion protein preparation

HPV-16, 18 and 57 E7 coding sequences were amplified by PCR and cloned into a pGEX vector. Escherichia coli BL21 cells were transformed with recombinant plasmids, and positive clones were selected and sequenced to ensure primary sequences were identical. E. coli BL21 cells transformed with the pGEX plasmid were then cultured at 37°C in Luria broth medium containing ampicillin. At an optical density (OD600) of 0.5, recombinant protein expression was then induced by adding 0.5 mM isopropyl-β-D-thiogalactoside at 25°C for 4 h. The bacteria were harvested by centrifugation at 4000g for 5 min at 4°C. Pelleted bacteria were resuspended in 1× phosphate-buffered saline (PBS) containing 1 mM phenylmethylsulfonyl fluoride and 1 mM phenylalanine, and then were incubated on ice for 20 min, followed by ultrasonication. The lysates were incubated on ice for another 20 min with 1% Triton 100 and cleared by centrifugation at 10 000g for 25 min at 4°C. Supernatants (containing soluble GST–E7 fusion protein) were stored at −70°C.

GST capture ELISA

Ninety-six-well polystyrene plastic plates were coated with glutathione casein, using 200 ng per well in 50 mM carbonate buffer (pH 9.6) at 4°C overnight. Wells were then incubated for 2 h at 37°C with 180 µl of blocking buffer per well (0.2% casein in PBS–Tween 20), followed by incubation for 1 h at 4°C with cleared bacteria lysates diluted in blocking buffer to a concentration of 0.25 µg/ml total lysate protein. Coated plates were then incubated for 1 h at room temperature in the presence of anti-human IgG polyclonal antibody, diluted to 1:5000 in blocking buffer and incubated for 40 min at 37°C. Freshly prepared o-phenylenediamine (0.34 mg/ml in 1× PBS, 0.05% H2O2) was used as a substrate. After 8 min, 50 µl 12.5% H2SO4 was added to stop the reaction, and absorbance was measured at 492 nm. After each incubation, plates were washed three to five times with 1× PBS containing 0.05% Tween 20.

Quality control and seropositive definition

Serum samples from children under 10 years of age and from cervical cancer patients were used as negative and positive controls, respectively. No positive control was used in HPV-57 E7 antibody evaluation as we did not find any obviously positive sample for HPV-57 E7 antibody in the control serum sample from cervical cancer patients. In every 96-well plate, 20 negative control serum samples, 1 positive control and 1 blank control (blocking buffer without sera) were included. Less than 1% of all positive and negative controls failed in the ELISA. The entire plate was retested when a positive control failed, and the falsely positive controls were discarded as outliers when calculating the cutoffs.

All samples were tested three times, and specimens, which were positive at least twice, were counted as positive. The mean + 3 SD of OD492, lead from the negative controls on the same plate after exclusion of positive outliers was adopted as the standard cutoff value for defining positive ELISA results in this study. In addition, to avoid arbitrary selection of cutoff values and to observe the trend of association between HPV seropositivity and ESCC with increasing cut off values, multiple cut points ranging from mean + 3 SD to mean + 5 SD (0.2 SD per step) were also adopted for this study.

Statistical analysis

In this study, we defined regular cigarette smoking as a smoking history of at least 18 packs over 1 year, and regular alcohol consumption was defined as drinking Chinese liquor (containing ~40% alcohol) at least twice a week for ≥12 months (consumption of other kinds of alcoholic beverages such as beer and red wine is very rare in the study area).

In this study, we defined regular cigarette smoking as a smoking history of at least 18 packs over 1 year, and regular alcohol consumption was defined as drinking Chinese liquor (containing ~40% alcohol) at least twice a week for ≥12 months (consumption of other kinds of alcoholic beverages such as beer and red wine is very rare in the study area).

Interaction terms were included in the full models in the stratified analysis to test the differences of the ORs derived from varied strata. All multivariate analyses were conducted with subjects with complete data only, and subjects with missing values in any of the analyzed variables were excluded. Statistical analysis was carried out using Stata 11.2 software for Windows (College Station, TX, StataCorp LP). All P values were two sided, and P < 0.05 was considered as statistically significant.

Results

Table I shows the HPV seroprevalence data and demographic characteristics of the subjects included in this study. A total of 1435 ESCC patients and 2071 normal control subjects were enrolled, and the median ages of case and control subjects were 61 (range: 35–80) and 59 (range: 31–80) years, respectively. Regular cigarette smoking was common in this area, and no appreciable difference was found between the case and control groups (46.2 versus 45.1%, P = 0.538). However, there was slightly less regular alcohol consumption in the case group than in controls (18.5 versus 21.9%, P = 0.014), and a higher percentage of study cases had a family history positive for ESCC as compared with controls (28.8 versus 8.4%, P < 0.0001). The proportion of individuals in the case group in whom HPV-E7 antibodies were detected was significantly higher than in the control group (HPV-16: 12.4 versus 3.5%, P < 0.001; HPV-18: 5.2 versus 2.7%, P < 0.001; HPV-57: 7.9 versus 1.0%, P < 0.0001). The differences in demographic characteristics and proportions of subjects with detectable HPV antibodies in case and control groups were evaluated using the χ2 test. Conditional logistic regression analysis was carried out to estimate ORs and 95% CIs. Two kinds of multivariate logistic models, namely ‘reduced’ and ‘full’ models, were constructed in this analysis. In three reduced models, all potential confounding factors including age, cigarette smoking, alcohol consumption and family history of esophageal cancer were included, and the three type-specific HPV serologic variables were analyzed one at a time. In the full model, all confounders and serologic variables were included at once. Interaction terms were included in the full models in the stratified analysis to test the differences of the ORs derived from varied strata. All multivariate analyses were conducted with subjects with complete data only, and subjects with missing values in any of the analyzed variables were excluded.

Table I shows conditional logistic regression analysis of the association of HPV-16, 18 and 57 E7 antibodies and ESCC. In the reduced multivariate models in which these three antibodies were separately analyzed one at a time, HPV-16 (OR = 3.9, 95% CI: 2.8–5.3), HPV-18 (OR = 2.1, 95% CI: 1.4–3.0) and HPV-57 (OR = 2.0, 95% CI: 1.5–2.8) were also positively associated with increased risk of ESCC after controlling for potential confounders. In the subsequent full model analysis in which all the serologic variables were included at once, the presence of HPV-16 E7 antibody was positively associated with ESCC (OR = 3.6, 95% CI: 2.5–5.0), whereas HPV-18 (OR = 1.1,
in males than in females (OR: 4.8 versus 2.6; 95% CI: 1.5–10.3). No association was observed in the age group of ≥60 years, while a statistically significant difference was found in the age group of <60 years (OR: 3.5 versus 3.4; 95% CI: 1.9–6.2). In all previous studies except one [20], the ORs demonstrated the results of multiple cut point analysis. In Figure 1A, ORs for HPV-16 were found to be statistically significant for all cutoff values. The ORs first increased and then decreased with higher cutoff levels for seropositivity. The cut points corresponding to the lowest (OR = 3.6, 95% CI: 2.5–5.0) and highest (OR = 6.6, 95% CI: 3.8–11.5) ORs were mean + 3 SD and mean + 4 SD, respectively. In Figure 1B and C, the OR polylines of HPV-18 and HPV-57 were both relatively flat, and none of the ORs were statistically significant.

Table III shows the results of stratified analysis by age and sex based on the full models without interaction terms. In addition, full models including the interaction terms, namely HPV antibody variables and patient sex, and HPV antibody variables and age, were also fitted to test the differences between the ORs from each stratum. The association of HPV-16 seropositivity and ESCC was stronger in males than in females (OR: 4.8 versus 2.6; P (OR = 4.8, 95% CI: 1.9–10.3), ‘positive for HPV-16 and 57’ (OR = 3.7, 95% CI: 1.9–7.4) and ‘positive for HPV-16 and 18’ (OR = 2.2, 95% CI: 1.0–4.9). No statistically significant association was observed when HPV-18 was absent. To confirm these results, we further evaluated HPV-18 and HPV-57 among HPV-16 seronegative participants and obtained similar results (OR HPV-16: 1.5, 95% CI: 0.8–2.7; OR HPV-18: 1.4, 95% CI: 0.9–2.1).

Table III shows the results of stratified analysis by age and sex based on the full models without interaction terms. In addition, full models including the interaction terms, namely HPV antibody variables and patient sex, and HPV antibody variables and age, were also fitted to test the differences between the ORs from each stratum. The association of HPV-16 seropositivity and ESCC was stronger in males than in females (OR: 4.8 versus 2.6; P (OR = 4.8, 95% CI: 1.9–10.3), ‘positive for HPV-16 and 57’ (OR = 3.7, 95% CI: 1.9–7.4) and ‘positive for HPV-16 and 18’ (OR = 2.2, 95% CI: 1.0–4.9). No statistically significant association was observed when HPV-18 was absent. To confirm these results, we further evaluated HPV-18 and HPV-57 among HPV-16 seronegative participants and obtained similar results (OR HPV-16: 1.5, 95% CI: 0.8–2.7; OR HPV-18: 1.4, 95% CI: 0.9–2.1).

Table III shows the results of stratified analysis by age and sex based on the full models without interaction terms. In addition, full models including the interaction terms, namely HPV antibody variables and patient sex, and HPV antibody variables and age, were also fitted to test the differences between the ORs from each stratum. The association of HPV-16 seropositivity and ESCC was stronger in males than in females (OR: 4.8 versus 2.6; P (OR = 4.8, 95% CI: 1.9–10.3), ‘positive for HPV-16 and 57’ (OR = 3.7, 95% CI: 1.9–7.4) and ‘positive for HPV-16 and 18’ (OR = 2.2, 95% CI: 1.0–4.9). No statistically significant association was observed when HPV-18 was absent. To confirm these results, we further evaluated HPV-18 and HPV-57 among HPV-16 seronegative participants and obtained similar results (OR HPV-16: 1.5, 95% CI: 0.8–2.7; OR HPV-18: 1.4, 95% CI: 0.9–2.1).

Table III shows the results of stratified analysis by age and sex based on the full models without interaction terms. In addition, full models including the interaction terms, namely HPV antibody variables and patient sex, and HPV antibody variables and age, were also fitted to test the differences between the ORs from each stratum. The association of HPV-16 seropositivity and ESCC was stronger in males than in females (OR: 4.8 versus 2.6; P (OR = 4.8, 95% CI: 1.9–10.3), ‘positive for HPV-16 and 57’ (OR = 3.7, 95% CI: 1.9–7.4) and ‘positive for HPV-16 and 18’ (OR = 2.2, 95% CI: 1.0–4.9). No statistically significant association was observed when HPV-18 was absent. To confirm these results, we further evaluated HPV-18 and HPV-57 among HPV-16 seronegative participants and obtained similar results (OR HPV-16: 1.5, 95% CI: 0.8–2.7; OR HPV-18: 1.4, 95% CI: 0.9–2.1).

In addition to the standard cut point, we further calculated ORs derived from the full models based on a series of increasing cutoff values from mean + 3 SD to mean + 5 SD (0.2 SD per step). Figure 1C demonstrates the results of multiple cut point analysis. In Figure 1A, the ORs for HPV-16 were found to be statistically significant for all cutoff values. The ORs first increased and then decreased with higher cutoff levels for seropositivity. The cut points corresponding to the lowest (OR = 3.6, 95% CI: 2.5–5.0) and highest (OR = 6.6, 95% CI: 3.8–11.5) ORs were mean + 3 SD and mean + 4 SD, respectively. In Figure 1B and C, the OR polylines of HPV-18 and HPV-57 were both relatively flat, and none of the ORs were statistically significant.

Table III shows the results of stratified analysis by age and sex based on the full models without interaction terms. In additional, full models including the interaction terms, namely HPV antibody variables and patient sex, and HPV antibody variables and age, were also fitted to test the differences between the ORs from each stratum. The association of HPV-16 seropositivity and ESCC was stronger in males than in females (OR: 4.8 versus 2.6; P (OR = 4.8, 95% CI: 1.9–10.3), ‘positive for HPV-16 and 57’ (OR = 3.7, 95% CI: 1.9–7.4) and ‘positive for HPV-16 and 18’ (OR = 2.2, 95% CI: 1.0–4.9). No statistically significant association was observed when HPV-18 was absent. To confirm these results, we further evaluated HPV-18 and HPV-57 among HPV-16 seronegative participants and obtained similar results (OR HPV-16: 1.5, 95% CI: 0.8–2.7; OR HPV-18: 1.4, 95% CI: 0.9–2.1).

Discussion

In this serological case–control study in Anyang, which is a high-risk region for ESCC in China, we observed a strong association between the presence of HPV-16 E7 antibody and increased risk of ESCC. However, there was no independent relationship between seropositivity for HPV-18 or HPV-57 and ESCC. This is the largest seroepidemiologic study conducted to date in a region with high risk for ESCC.

In all previous studies except one [20], HPV antibody was evaluated with ELISA in order to detect antibodies against baculovirus-derived virus-like particle containing L1 or L1/L2 proteins [30,31]. In the present study, E7 was used as serologic marker for HPV infection [32]. We expect this approach will overcome underestimation of the real causal association, as HPV- E7 is directly involved in carcinogenesis. A previous study has demonstrated that anti-E6/E7 antibody
Table II. Conditional logistic regression analysis of ESCC and the presence of E7 antibodies against HPV-16, 18 and 57 and ESCC, Anyang, China, 2007–10

<table>
<thead>
<tr>
<th>Variables</th>
<th>Case/control (n)</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted OR(^a) (95% CI)</th>
<th>Adjusted OR(^b) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV-16 E7 antibody</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>1257/1998</td>
<td>1.0 (referent)</td>
<td>1.0 (referent)</td>
<td>1.0 (referent)</td>
</tr>
<tr>
<td>Positive</td>
<td>178/73</td>
<td>3.7 (2.7–4.9)</td>
<td>3.9 (2.8–5.3)</td>
<td>3.6 (2.5–5.0)</td>
</tr>
<tr>
<td>HPV-18 E7 antibody</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>1360/2016</td>
<td>1.0 (referent)</td>
<td>1.0 (referent)</td>
<td>1.0 (referent)</td>
</tr>
<tr>
<td>Positive</td>
<td>75/55</td>
<td>2.0 (1.4–2.9)</td>
<td>2.1 (1.4–3.0)</td>
<td>1.1 (0.7–1.7)</td>
</tr>
<tr>
<td>HPV-57 E7 antibody</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>1322/1986</td>
<td>1.0 (referent)</td>
<td>1.0 (referent)</td>
<td>1.0 (referent)</td>
</tr>
<tr>
<td>Positive</td>
<td>113/85</td>
<td>1.9 (1.4–2.6)</td>
<td>2.0 (1.5–2.8)</td>
<td>1.3 (0.9–1.9)</td>
</tr>
<tr>
<td>HPV-E7 antibody status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative for all three types</td>
<td>1183/1913</td>
<td>1.0 (referent)</td>
<td>—</td>
<td>1.0 (referent)(^f)</td>
</tr>
<tr>
<td>Only positive for HPV-16</td>
<td>97/38</td>
<td>4.0 (2.7–5.9)</td>
<td>4.7 (3.0–7.2)(^e)</td>
<td></td>
</tr>
<tr>
<td>Only positive for HPV-18</td>
<td>21/22</td>
<td>1.4 (0.8–2.7)</td>
<td>1.8 (0.9–3.5)(^e)</td>
<td></td>
</tr>
<tr>
<td>Only positive for HPV-57</td>
<td>45/51</td>
<td>1.3 (0.9–2.1)</td>
<td>1.6 (1.0–2.5)(^e)</td>
<td></td>
</tr>
<tr>
<td>Positive for HPV-16 and 18</td>
<td>21/13</td>
<td>2.3 (1.1–4.7)</td>
<td>2.2 (1.0–4.9)(^e)</td>
<td></td>
</tr>
<tr>
<td>Positive for HPV-16 and 57</td>
<td>35/14</td>
<td>3.8 (2.0–7.3)</td>
<td>3.7 (1.9–7.4)(^e)</td>
<td></td>
</tr>
<tr>
<td>Positive for HPV-18 and 57</td>
<td>8/12</td>
<td>1.4 (0.5–3.5)</td>
<td>1.3 (0.5–3.5)(^e)</td>
<td></td>
</tr>
<tr>
<td>Positive for all three types</td>
<td>25/8</td>
<td>4.7 (2.1–10.7)</td>
<td>4.4 (1.9–10.3)(^f)</td>
<td></td>
</tr>
<tr>
<td>Regular cigarette smoking(^d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>738/1135</td>
<td>1.0 (referent)</td>
<td>—</td>
<td>1.0 (referent)</td>
</tr>
<tr>
<td>Yes</td>
<td>634/934</td>
<td>1.1 (0.9–1.4)</td>
<td>1.3 (1.0–1.6)</td>
<td></td>
</tr>
<tr>
<td>Regular alcohol consumption(^d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1117/1615</td>
<td>1.0 (referent)</td>
<td>—</td>
<td>1.0 (referent)</td>
</tr>
<tr>
<td>Yes</td>
<td>253/454</td>
<td>0.8 (0.7–1.0)</td>
<td>0.8 (0.7–1.0)</td>
<td></td>
</tr>
<tr>
<td>Family history of ESCC(^d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>974/1895</td>
<td>1.0 (referent)</td>
<td>—</td>
<td>1.0 (referent)</td>
</tr>
<tr>
<td>Yes</td>
<td>393/173</td>
<td>4.6 (3.7–5.7)</td>
<td>—</td>
<td>4.5 (3.6–5.7)</td>
</tr>
</tbody>
</table>

\(^a\) Adjusted ORs of the ‘reduced models’ for subjects with complete data adjusted for age, regular cigarette smoking, alcohol consumption and family history of esophageal cancer.

\(^b\) Adjusted ORs of the ‘full model’ for subjects with complete data, in which all the listed variables in the table and age were included simultaneously.

\(^c\) ORs and 95% CIs adjusted for age, regular cigarette smoking, alcohol consumption and family history of esophageal cancer.

\(^d\) The category entries in sum are not equal to the sample size due to missing values.

Table III. Conditional logistic regression analysis of the association between the presence of E7 antibodies against HPV-16, 18 and 57 and ESCC, by gender and age, Anyang, China, 2007–10

<table>
<thead>
<tr>
<th>Variables</th>
<th>Male Case/control (n)</th>
<th>Adjusted OR(^a) (95% CI)</th>
<th>Female Case/control (n)</th>
<th>Adjusted OR(^a) (95% CI)</th>
<th>&lt;60 years Case/control (n)</th>
<th>Adjusted OR(^a) (95% CI)</th>
<th>≥60 years Case/control (n)</th>
<th>Adjusted OR(^a) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV-16 E7 antibody</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>782/1279</td>
<td>1.0 (referent)</td>
<td>475/719</td>
<td>1.0 (referent)</td>
<td>528/1009</td>
<td>1.0 (referent)</td>
<td>729/989</td>
<td>1.0 (referent)</td>
</tr>
<tr>
<td>Positive</td>
<td>100/31</td>
<td>4.8 (3.0–7.8)</td>
<td>78/42</td>
<td>2.6 (1.6–4.4)</td>
<td>78/33</td>
<td>3.5 (2.1–6.0)</td>
<td>100/40</td>
<td>3.4 (2.1–5.5)</td>
</tr>
<tr>
<td>HPV-18 E7 antibody</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>837/1282</td>
<td>1.0 (referent)</td>
<td>523/734</td>
<td>1.0 (referent)</td>
<td>575/1008</td>
<td>1.0 (referent)</td>
<td>785/1008</td>
<td>1.0 (referent)</td>
</tr>
<tr>
<td>Positive</td>
<td>45/28</td>
<td>1.4 (0.7–2.5)</td>
<td>30/27</td>
<td>0.8 (0.4–1.6)</td>
<td>31/34</td>
<td>0.8 (0.4–1.5)</td>
<td>44/21</td>
<td>1.6 (0.8–3.1)</td>
</tr>
<tr>
<td>HPV-57 E7 antibody</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>809/1262</td>
<td>1.0 (referent)</td>
<td>513/724</td>
<td>1.0 (referent)</td>
<td>552/999</td>
<td>1.0 (referent)</td>
<td>770/987</td>
<td>1.0 (referent)</td>
</tr>
<tr>
<td>Positive</td>
<td>73/48</td>
<td>1.5 (1.0–2.5)</td>
<td>40/37</td>
<td>1.1 (0.6–1.9)</td>
<td>54/43</td>
<td>1.7 (1.0–2.9)</td>
<td>59/42</td>
<td>1.1 (0.7–1.9)</td>
</tr>
</tbody>
</table>

\(^a\) Adjusted ORs of the ‘full model’ for subjects with complete data, in which all the three antibody variables, age, regular cigarette smoking, alcohol consumption and family history of esophageal cancer were included simultaneously.

positivity confers a 7– to 10-fold higher risk for an HPV-related cancer than antivirus-like particle antibody positivity (22).

Two previous studies have evaluated the association of HPV-E7 antibody and ESCC (16,20). In Sitans et al.’s study (20), serum samples collected from six separate case–control studies were analyzed, one of which was conducted in Shanxi Province in China, which is a high-risk area neighboring our study area. A positive association (OR = 16.73, 95% CI: 0.55–512.24) was observed in this population, despite the wide CI that may have been due to limited sample size. However, on the other hand, the investigators reported that the pooled OR for HPV-16-E7 was not significant (OR = 0.82, 95% CI: 0.46–1.46). A possible explanation for this discrepancy is that only a proportion of ESCC may be attributable to HPV infection, as only 12.4% of ESCC cases were seropositive for HPV in this study and only 31.0% of cases were HPV DNA positive found in our previous study (12). Other population-specific environmental risk factors as well as genetic susceptibility may also play an important role in the development of ESCC, which is thought to possibly be a principle reason for the geographical heterogeneity of ESCC worldwide. That is, although HPV infection is common in different populations, the risk attributable to HPV in the occurrence of ESCC can vary dramatically. As a result, the effect of HPV-E7 was neutralized in pooled analysis.
In the other study conducted in Holland, Van Doornum et al. (16) found no E7 positivity in ESCC cases. In this study, only L1-positive sera were further tested for HPV-16 E7 antibody. In contrast, we analyzed HPV-16 E7 antibody in all cases and controls regardless of L1 status. The results thus cannot be compared directly.

The proportion of subjects with detectable antibody to E7 in HPV-16, HPV-18 and HPV-57 in the case study group was all higher than the control group. But in the ‘full’ model in which all three serological markers were included together for analysis, only HPV-16 was associated with ESCCC. Taken together with the fact that about half of the cases positive for HPV-18 or 57 were also positive for HPV-16 (data not shown), we believe that HPV-18 and HPV-57 are associated with ESCC only as a result of their coexistence with HPV-16. Bjorgo et al. (14) observed a similar result in their serologic study, in which both type 16 and type 33 were initially associated with an increased risk of non-cervical anogenital cancers, but the association with type 33 was not found after adjustment for HPV-16. Four previous serologic studies have examined the association between HPV-18 and esophageal cancer, all of which also obtained a negative result (14,15,19,20).

Most previous ELISA-based studies have adopted a preassigned or fixed cutoff value obtained from the optical density of all negative control sera, for example mean + 3 or 4 SD of the absorbance value of the control serum (18,21). Kamangar et al. (19) adopted continuous cut points in their study, but analyzed only 99 esophageal cancer cases, which limited the validity of this statistical procedure. To eliminate variability among testing plates and bias induced by an arbitrary selection of a single cut point, we adopted plate-specific and multiple seropositivity classification cut points in addition to the single standard cutoff value mean + 3 SD. As shown in Figure 1, the associations between HPV-16, 18 and 57 seropositivity and ESCC were all relatively stable with increasing cut points. The lowest OR of HPV-16 E7 antibody to ESCC derived from the standard cutoff value (mean + 3 SD), which was more likely to lead toward acceptance of the null hypothesis, was used to ensure a valid estimate.

In this case–control study, we evaluated anti-HPV-E7 seropositivity and risk of esophageal cancer in a high-risk population in China. Higher proportions of seropositivity for HPV-16, 18 and 57 were found in ESCC cases as compared with the control group, but only the presence of serum antibody to HPV-16 E7 protein was independently associated with an increased risk of developing ESCC in multivariate analysis.

Supplementary material

Supplementary Table can be found at http://carcin.oxfordjournals.org/

Funding

Natural Science Foundation of China (30872937, 30930102); ‘973’ Project of National Ministry of Science and Technology Grant (2011CB504300, 2012CB910800); ‘863’ Key Projects of National Ministry of Science and Technology Grant (2006AA2Z467); Charity Project of National Ministry of Health (201202014, 200902002); Natural Science Foundation of Beijing (7100001) to Y.K.

Acknowledgements

We would like to thank Yuqin Song, Xiuyan Tian, Huirong Ding, Chunfeng Zhang, Yue Zhou, Wenjun Yang, Li Zheng, Fang Lu, Xueqian Wang, Yanlan Zhang, Yiqiang Zhao, Ke Chen, Lei Gao, Min Sun, Ying Liu, Luyan Shen, Qiyan Wang, Na Shen, Jingjing Li, Yong Li and Haoran Wei who contributed to the field work. We also thank Dr Michael A. McNutt for editing and correction of this manuscript.

Conflict of Interest Statement: None declared.

References


Figure 1. Adjusted ORs and CIs derived by ‘full models’ for seropositivity to (A) HPV-16, (B) HPV-18 and (C) HPV-57 by different cutoff values, including 1435 ESCC cases and 2071 age- and sex-matched controls from Anyang, China, 2007–10.

Received June 19, 2013; revised November 21, 2013; accepted December 14, 2013.