Herbal Medicine Use, Epstein-Barr Virus, and Risk of Nasopharyngeal Carcinoma

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ABSTRACT

Herbal medicine use is thought to be linked to nasopharyngeal carcinoma (NPC) either through its ability to reactivate the Epstein-Barr virus (EBV) or through a direct promoting effect on EBV-transformed cells. To investigate this, 104 histologically confirmed NPC cases and 205 matched controls were studied in The Philippines. Blood was collected to assess antibody titers against EBV, and an interview was administered which elicited information concerning herbal medicine use and other risk factors for NPC. Subjects positively strong for anti-EBV antibodies (Epstein-Barr nuclear antigen [EBNA]) (titers ≥ 1:80) were at a 21-fold excess risk of disease (95% confidence interval, 8.4, 51.8). Herbal medicine use was also associated with NPC (relative risk, 2.5; 95% confidence interval, 1.4, 4.5). Associations persisted after adjustment for education, smoking, Chinese ancestry, and consumption of salted fish. Exposure to herbal medicines among subjects testing negative/weakly positive for anti-EBNA antibodies was not associated with an elevation in risk (relative risk, 0.6). Strong positivity to anti-EBNA antibodies in the absence of herbal medicine use was associated with a significant 16-fold excess risk of disease, and exposure to herbal medicines among subjects testing strongly positive for anti-EBNA antibodies was associated with a significant 49-fold excess risk of NPC when cases were compared to controls. Similar results were obtained when other serological measures of EBV exposure were used. Anti-EBV antibody titers were elevated in herbal medicine users compared to nonusers among cases but not among control subjects. This suggests that, if herbal medicines interact with EBV in the development of NPC, they do not do so by reactivating EBV infection but rather through a direct proliferative effect on EBV-transformed cells. Although the interaction between EBV and herbal medicines is biologically plausible, larger, more detailed studies need to be conducted to validate this preliminary finding.

INTRODUCTION

EBV has been implicated in the etiology of NPC. EBV DNA has been detected in nearly all NPC tumor specimens investigated to date (1), and recent evidence has demonstrated the transforming ability of the EBV latent membrane protein which is expressed in approximately 65% of NPC tumors (2). Given the ubiquity of this virus relative to the incidence of NPC, however, it is likely that other agents act as cofactors in the etiology of this disease. One such factor, phorbol esters present in extracts from herbal medicines and plants, has been hypothesized to be linked to NPC either through its ability to reactivate EBV infection (3) or through a direct promoting effect on cells transformed by the EBV (4, 5).

A case-control study of NPC was conducted in a Filipino population in The Philippines to examine risk factors for this disease in a country with incidence rates intermediate to that in Hong Kong (30.0/100,000 among males, 12.9/100,000 among females) and the United States (<1.0/100,000 among males and females). The age-standardized rate of NPC in Rizal Province in The Philippines in the period of 1978–1982 was 4.7/100,000 per year in males and 2.6/100,000 per year in females (6). As part of our case-control study, information was obtained concerning the use of herbal medicines, and EBV antibody status was assessed serologically. This has enabled us to examine the hypothesis that EBV and herbal medicines interact in the development of NPC.

PATIENTS AND METHODS

Incident cases of histologically confirmed NPC were recruited from the Philippine General Hospital. To be eligible for the study, cases were required not to have had previous treatment for NPC and to have resided on the island of Luzon for at least 6 months. All subjects presenting to the clinic with symptoms compatible with NPC and referred for a nasopharynx biopsy were invited to participate in the study. A total of 234 cases suspicious of NPC were identified during a 24-month period. Of these, 22 (9%) refused to undergo biopsy and were therefore considered ineligible for the study. Of the remaining 212, 104 individuals were pathologically confirmed to have NPC. All consented to participate in the study.

Two controls were selected for each confirmed NPC case. One control was selected from the hospital and a second from the community. Hospital controls were individually matched to cases on sex, age (± 5 years), and hospital ward type (private versus public). Hospital controls were chosen from the medical/surgical ward on the same day that a suspected case was confirmed by biopsy result. Medical rosters routinely kept by the hospital were used by the study coordinator to select hospital controls. This was done by reviewing the roster and selecting the first eligible subject as a control. Since this study was also designed to examine the effect of diet on risk of NPC, individuals with disorders thought to be associated with changes in dietary patterns were not eligible as controls. Two hundred cases with the following diagnoses were excluded from the study: gastrointestinal cancers, peptic ulcer, chronic cirrhosis, and gallbladder disease. A total of 104 hospital controls were selected, and all agreed to participate. Twenty-four hospital controls had diagnoses of urinary/renal disease, 15 with acute appendicitis, 11 with non-NPC/gastrointestinal cancers, 9 with respiratory impairment, and the remaining 45 with other conditions.

Community controls were individually matched to cases on sex, age (± 5 years), and neighborhood. Selection of neighborhood controls was achieved by systematically selecting households either to the right or left of the subject's residence until an appropriate match was found. Replacement controls were obtained if the original control selected refused to participate. Thus, a total of 131 community controls were ascertained, of which 101 (77%) agreed to participate. Among those who refused, 76% did so because they refused blood extraction, and the remaining 24% declined all involvement.

A personal interview was administered to consenting subjects in their native language, Tagalog, by a trained nurse-interviewer. The interview obtained information concerning sociodemographic characteristics, Chinese ancestry, adult diet, occupation, smoking, and herbal medicine use. "Ever use" of herbal medicines was assessed, and for those who reported use, the names of the medicines used were assessed. Currency of use and total duration of use were not assessed. In addition, 5 ml of venous blood was collected from each consenting subject for anti-EBV antibody determination by immunofluorescence (7). Presence of the
following antibodies was assessed: VCA IgG; VCA IgA; EA IgG; EBNA IgG; EA, diffuse-type IgG; and EA IgA. Titers of antibodies were expressed as reciprocals of the maximum dilution of serum (2-fold from the initial 1:10 dilution) that gave fluorescence. Samples were tested without knowledge of the case-control status. Strong positivity was defined as follows: VCA IgG titer ≥ 1:160, VCA IgA titer ≥ 1:10, EA IgG titer ≥ 1:10, EBNA IgG titer ≥ 1:80, EA diffuse-type IgG titer ≥ 1:10, and EA IgA titer ≥ 1:10. These cutoff points were selected based on the titer distribution of controls so that as close to half of the control subjects as possible were categorized as strongly positive. Use of other cutoff points did not alter the conclusions.

Anti-EBV antibody geometric mean titers were calculated for cases and controls. The RR, as estimated by the odds ratio, was the measure used to assess associations between risk factors and disease. Significance levels were determined by use of the 95% CI. Conditional logistic regression was utilized to examine associations between exposures of interest and disease, while simultaneously adjusting for potential confounding factors (8). To examine the statistical interaction between two factors, indicator variables were created for each category of joint exposure and included in the conditional regression model (9). Deviation from an additive interaction was tested for significance utilizing the test for synergy described by Schlesselman (10). Deviation from an additive interaction was tested for significance by including the cross-product term in the logistic model (11).

Hospital and community controls were compared and found to be similar with respect to both factors of interest: anti-EBV antibodies and herbal medicine use. Therefore, these two groups were combined into one for purposes of analysis and are henceforth referred to as the control group.

RESULTS

The geometric mean titer of anti-EBV antibodies is presented in Table 1 for cases and controls. Regardless of the specific antibody test, cases were found to have a higher mean antibody titer (geometric mean titer ranging from 1:25-1:487) than controls (1:5-1:114). The percentage of control samples testing negative (titer < 1:10) for anti-EBV antibodies ranged from 0–94%, depending on the antibody test used (Table 1). The equivalent range for cases was 0–20%.

We assessed risk associated with being strongly positive for anti-EBV antibodies by dichotomizing EBV exposure and computing risk estimates, comparing cases to controls. Results indicate that individuals strongly positive for anti-EBV antibodies had relative risks ranging from 6–74 when compared to subjects testing negative/weakly positive (Table 1).

Next, we assessed the association between herbal medicine use and NPC. Thirty-eight % of cases and 21% of controls reported having used herbal medicines in the past. When cases and controls were compared, individuals who reported herbal medicine use were found to be at a 2.5-fold excess risk of disease (95% CI 1.4, 4.5) compared to individuals who reported never having used herbal medicines. This effect persisted after adjustment for potential confounding by education, salted fish consumption, Chinese ancestry, and smoking (RR 2.6; 95% CI 1.4, 4.6), factors found in previous studies to be associated with disease (12–14). The effect was also present when cases were compared separately to hospital (RR 2.1; 95% CI 1.1, 4.0) and community (RR 2.9; 95% CI 1.5, 5.8) controls.

We attempted to evaluate the specific herbal medicines used by study subjects to determine whether any specific type was primarily involved. This was accomplished by classifying specific herbal medicines reported by subjects into their respective phylogenetic family. The analysis was complicated by the fact that >40 different types of herbal medicines were reported, most were only used by one or two individuals, and many subjects reported use of multiple herbal medicines. Of the 40 cases and 44 controls who reported ever using herbal medicines, 29 cases and 32 controls were able to specify which herbal medicine(s) they used. We were then able to classify into families all but 6 herbal medicines reported by subjects. A list of the most frequently reported plant families is presented in Table 2. Overall, no specific family of plants appeared to be primarily responsible for the association observed between herbal medicine use and NPC. Three cases and no controls reported the use of plants in the Euphorbiaceae family, and no subject reported the use of plants in the Thymelaeaceae family, two families of plants known to contain phorbol esters (15).

Table 3 presents results of the analysis which investigated the joint effect of EBV and herbal medicines. Only results for anti-EBNA antibodies are presented. EBNA was selected since its expression is associated with cell transformation and proliferation (16). However, findings consistent with the ones presented here were also obtained when the other anti-EBV antibody titers were examined. Among subjects testing negative/weakly positive for anti-EBNA antibodies, those who reported herbal medicine use in the past were not at increased risk of disease (RR 0.6; 95% CI 0.1, 3.5), while the converse (strong positivity for anti-EBNA antibodies in the absence of herbal medicines) was associated with a 16-fold excess risk of disease (95% CI 5.9, 43.7) when compared to subjects classified as negative/weakly positive for anti-EBNA antibodies and reporting no herbal medicine use. Subjects who reported herbal medicine use who were classified as strongly positive for anti-EBNA antibodies were at a 49-fold excess risk of disease (95% CI 13.8, 174.6). This finding suggests a greater than multiplicative interaction between EBV and herbal medicines, although the deviation from the multiplicative model was only marginally significant (P = 0.11).

Finally, we examined anti-EBNA antibody titer distribution by herbal medicine use among cases and controls separately. This analysis was performed to determine whether EBV antibody titers were higher among herbal medicine users compared to nonusers for both study groups. As shown in Fig. 1, an

<table>
<thead>
<tr>
<th>Anti-EBV Antibody:</th>
<th>Geometric mean titer</th>
<th>% negative</th>
<th>% strong positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCA IgG</td>
<td>1:487</td>
<td>0</td>
<td>92</td>
</tr>
<tr>
<td>VCA IgA</td>
<td>1:69</td>
<td>7</td>
<td>93</td>
</tr>
<tr>
<td>EA IgG</td>
<td>1:95</td>
<td>6</td>
<td>94</td>
</tr>
<tr>
<td>EBNA IgG</td>
<td>1:199</td>
<td>2</td>
<td>88</td>
</tr>
<tr>
<td>EA diffuse-type IgG</td>
<td>1:63</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>EA IgA</td>
<td>1:25</td>
<td>15</td>
<td>85</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Anti-EBV Antibody:</th>
<th>Geometric mean titer</th>
<th>% negative</th>
<th>% strong positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCA IgG</td>
<td>1:114</td>
<td>0</td>
<td>51</td>
</tr>
<tr>
<td>VCA IgA</td>
<td>1:80</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>EA IgG</td>
<td>1:37</td>
<td>4</td>
<td>30</td>
</tr>
<tr>
<td>EA diffuse-type IgG</td>
<td>1:53</td>
<td>94</td>
<td>80</td>
</tr>
<tr>
<td>EA IgA</td>
<td>1:15</td>
<td>43</td>
<td>57</td>
</tr>
</tbody>
</table>

Table 1 Anti-EBV antibody titers by case status and estimates of risk
When specific herbal medicines were examined, no single plant family appeared responsible for the association observed between herbal medicine use and NPC. Although it was expected that many cases would report use of medicinal plants from the Euphorbiaceae and Thymelaeaceae families, both of which are known to contain phorbol esters, only 3 of 40 cases who were herbal medicine users reported use of medicinal plants in these families. These results are similar to those reported by Yu et al. (21), who reported a lack of association between herbal medicines from the Euphorbiaceae family and NPC. This raises the possibility that agents in herbal medicines other than phorbol esters may also be involved in the etiology of NPC. In fact, results from a previous investigation conducted by Zeng et al. (3) suggest that plants from families other than the Euphorbiaceae and Thymelaeaceae families are capable of inducing EBV in vitro.

Of particular note is our finding of an apparent interaction between EBV and herbal medicines. Individuals who reported herbal medicine use in the past but who tested negative/weakly positive for anti-EBV antibodies were not found to be at an increased risk of disease, those who reported no previous use of herbal medicines but were strongly positive for anti-EBV antibodies were at a 16-fold excess risk of disease, and those who reported herbal medicine use and who were strongly positive for anti-EBV antibodies were found to be at a 49-fold excess risk of disease when compared to those who reported being nonusers of herbal medicines and who tested negative/weakly positive for anti-EBV antibodies. Although the possibility of a chance finding cannot be ruled out given the relatively small size of the present study, the magnitude of the effect was strong (and marginally significant) and warrants further consideration. Furthermore, the interaction between herbal medicines and EBV is biologically plausible, as shown

**DISCUSSION**

Results from the present study conducted on a Filipino population confirm that EBV is strongly associated with NPC in The Philippines (RR 6–74), as has previously been observed in other areas (17, 18). Also, use of herbal medicines was found to be associated with disease in this population (RR 2.6), independently of educational achievement, smoking, Chinese ancestry, and consumption of salted fish. Previous studies which examined the association between herbal medicine use and NPC in Chinese populations had conflicting results. While one study conducted in Taiwan observed an effect of similar magnitude to that presented here (14), studies conducted by Yu et al. (19–21) in Mainland China failed to detect an independent effect of herbal medicine use on risk of NPC. Although it is possible that the association detected between herbal medicine use and NPC reflects increased use of herbal medicines by cases because of their symptoms, the fact that the association was still present and statistically significant when cases were compared to hospital controls (RR 2.1) suggests that this might not be the case. Nevertheless, an interpretation of causality should be made with caution. The possibility that herbal medicine use is a marker for a traditional lifestyle which predisposes to NPC cannot be ruled out, despite our attempts to control for possible confounding by education, smoking, Chinese ancestry, and consumption of salted fish. Also, herbal medicines are very heterogeneous, and it is unlikely that all herbal medicines increase risk of NPC.

**Table 2** Plant families most frequently reported by subjects reporting use of herbal medicines and respective frequencies, by case status

<table>
<thead>
<tr>
<th>Family name</th>
<th>No. of cases*</th>
<th>No. of controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myrtaceae</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td>Lamiaceae</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Arecaee</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Anonaceae</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Piperaceae</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Zingiberaceae</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Euphorbiaceae</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Lythraceae</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Leguminosae</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Musaceae</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Total no. of subjects: 104
Total no. reported use: 40
Total no. reporting specific herbs: 29

* Several individuals reported use of more than one herbal medicine and may therefore be listed more than once.

**Table 3** Joint effect of EBV and herbal medicines

<table>
<thead>
<tr>
<th>EBV:</th>
<th>Never</th>
<th>Ever</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>1.0*</td>
<td>0.6</td>
</tr>
<tr>
<td>Positive</td>
<td>16.1</td>
<td>49.2</td>
</tr>
</tbody>
</table>

Test for departure from multiplicative model: $P = 0.11$

Test for departure from additive model: $P = 0.09$

* RR.

95% CI.

* Cases/controls.

An upward shift in the antibody titer distribution was observed among herbal medicine users compared to “never users” for cases but not for control subjects. Similar findings were observed when antibody titers directed against the other EBV antigens were examined.
by investigations demonstrating that extracts from herbal medicines and plants are capable of promoting either EBV or EBV-transformed cells (3, 4). Finally, a previous epidemiological study conducted in Taiwan (22) revealed an interaction between EBV and herbal medicine use which was more than additive but less than multiplicative.

Our finding that anti-EBV antibody titers were higher among herbal medicine users compared to nonusers only among cases and not among controls suggests that, if the interaction observed is real, herbal medicines do not interact with EBV through an ability to reactivate EBV infection. Rather, it is more likely that herbal medicines interact with EBV in the development of NPC through a promotional effect on cells transformed by the virus. However, it is also possible that the interaction observed in this study is spurious. It is feasible that subjects who are more severely ill are more likely to use herbal medicines. Also, it has been demonstrated that EBV antibody titers increase with increasing severity of disease among NPC patients (16). Therefore, the finding of increased antibody titers among cases who are herbal medicine users compared to cases who are not may reflect the increased use of herbal medicines among more severely ill individuals rather than a true biological effect.

We were unable to directly test the hypothesis that more severely ill NPC patients are more likely to use herbal medicines since information concerning clinical stage of disease at the time of diagnosis was not obtained. Also, we are unable to precisely define the temporal association between EBV and herbal medicine use among control subjects, because no distinction was made between past and current herbal medicine use. If herbal medicines do indeed re activates EBV infections, one would expect individuals who are currently using these medicines to have higher levels of antibodies than individuals who were users of herbal medicines in the past but who are not current users. Our inability to distinguish these two groups of individuals might have masked an association between herbal medicine use and EBV antibody titers among control subjects.

Other limitations of the present study include the inability to examine the effect of duration and intensity of use of herbal medicines on risk of NPC and the relatively small sample size of our study. Although 104 cases and 205 controls participated in the study, the power to detect the observed 5-fold deviation from a multiplicative interaction was 60%.

In summary, this study confirms an association of anti-EBV antibodies and herbal medicines with NPC in The Philippines. In addition, an interaction between anti-EBV antibodies and herbal medicines was observed. Although the interaction is biologically plausible as demonstrated by laboratory studies, we are not able to determine the validity of this finding because of the small sample size and the unavailability of detailed information concerning timing and intensity of herbal medicine use.

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