Dietary Intake of Benzo(a)pyrene and Risk of Esophageal Cancer in North of Iran

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One etiologic factor for high incidence of esophageal squamous cell carcinoma (ESCC) in Golestan (Northeastern Iran) might be exposure to polycyclic aromatic hydrocarbons. We examined whether food and water are major sources of benzo(a)pyrene (BaP) exposure in this population. We used a dietary questionnaire to assess the daily intake of staple food (rice and bread) and water in

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3 groups: 40 ESCC Golestan cases, 40 healthy subjects from the same area, and 40 healthy subjects from a low-risk area in Southern Iran. We measured, by high-performance liquid chromatography combined with fluorescence detection, the BaP concentration of bread, rice, and water in samples obtained from these 3 groups and calculated the daily intake of BaP. Mean BaP concentration of staple foods and water was similar and within standard levels in both areas, but the daily intake of BaP was higher in controls from the high-risk area than in controls from the low-risk area (91.4 vs. 70.6 ng/day, P < 0.01). In the multivariate regression analysis, having ESCC had no independent effect on BaP, whereas residence in the low-risk area was associated with a significant decrease in total BaP intake. Polycyclic aromatic hydrocarbons might, along with other risk factors, contribute to the high risk of ESCC in Golestan.
INTRODUCTION

Esophageal cancer is the eighth most common cancer worldwide and the sixth most common cause of death from cancers. Geographic variation in its incidence is very striking with more than a 20-fold variation between high-risk and low-risk areas. These differences are observed both among countries and within countries (1). In Golestan, a province in Northeastern Iran, although the age-standardized incidence rate of esophageal squamous cell carcinoma (ESCC) has declined in the past 30 years, it is still the most common cause of cancer in both men and women in this region, with rates of approximately 40/100,000 (2). In contrast, rates in the southern part of Iran are only about 3/100,000 (3).

Polycyclic aromatic hydrocarbons (PAHs) are a class of known carcinogenic compounds that originate mainly from incomplete combustion of organic matter (4), such as tobacco, and have been implicated as possible etiologic reasons in some high-risk areas of the world for ESCC. In Linxian, China, a high-risk area for ESCC, raw and cooked cereals were shown to have high level of carcinogenic PAH (5). Further studies in this area detected PAH-DNA adducts in archived esophageal endoscopic biopsy samples (6).

In a recent study from high-risk areas of northeastern Iran (7), urinary markers of benzo(a)pyrene (BaP), a major subgroup of PAHs, were elevated in 80% of the study population. Both smokers and nonsmokers had elevated levels, and the source of exposure was unclear. Dietary intake of PAHs via food and water may be an important source of exposure to PAHs. Diet the primary source of PAH exposure in non-smokers (8). For smokers, the contribution from smoking and food may be of a similar magnitude. The contribution of passive smoke and inhalation of indoor and outdoor air to total PAH intake is very small in comparison with dietary intake and active smoking (9). Therefore, we speculated that food and water might be the source of high levels of PAH exposure in Golestan.

As a first step to confirm or refute this speculation, we measured the food (rice and bread) and water concentration of BaP in samples collected from ESCC cases and control subjects from Golestan as well as in samples collected from healthy subjects from a low-risk area in southern Iran. We also estimated the daily intake of water, bread, and rice and multiplied these intakes by the BaP concentration to estimate the daily BaP intake from these sources.

MATERIALS AND METHODS

This study was conducted between March 1 and August 31, 2005. Forty ESCC cases and 40 control subjects were selected in Atrak clinic in Gonbad, a referral clinic for upper gastrointestinal tract diseases in Golestan, and 40 control subjects were selected in a gastrointestinal research center located in Shiraz in low-risk Fars province.

Cases and Control Eligibility Criteria

Case eligibility criteria included histologically confirmed ESCC, diagnosis made within the previous 6 mo, residence in the area for at least 5 years, and sufficiently good health status to give answers to the questionnaires. Eligibility criteria for controls included matching to cases for gender and age (± 5 yr), admission to the same clinic (in the high-risk area) during the same period as the corresponding cases and histologically confirmed no upper gastrointestinal diseases (for the high-risk area) and no clinical evidence of upper gastrointestinal disease (for the low-risk area), no family history of esophageal cancer in the first-degree relatives, residence in the area for at least 5 yr, and sufficiently good health status to give answers to the questionnaires.

Questionnaires

All study participants (n = 120) completed a questionnaire on demographic characteristics, tobacco smoking, opium use, type of fuel used for heating and cooking at home, use of wood or charcoal as fuel for bread cooking during the last 5 years, and exposure to stubble burning within 500 m of their house. Their diet was assessed using a validated food frequency questionnaire (10). Questions on bread and rice intake were expanded and were asked separately for breakfast, lunch, dinner, and snacks. Subjects were also asked about their water and tea consumption. Total bread and rice intakes were estimated as g/day. All participants were interviewed face-to-face by a nutritionist. Daily BaP intake from bread and rice and water was estimated by multiplying the average consumption of each food by the mean concentration of BaP in the corresponding area.

The study was approved by the Ethics Review Board of Digestive Disease Research Center and Tabriz University of Medical Science.

Food Sampling

Ten subjects from each of the three groups were randomly selected to provide samples of their bread, rice, and water. The subjects were called 1 day before being visited and were asked to set aside a piece of bread bought and 2 spatulas of rice (1 from the top of pot and 1 from the bottom of the pot) cooked in the day of visit and provide these samples to the research team. Food samples were collected in plastic bags and were transferred in an ice pack container till transferred to a −20°C freezer. In addition, water samples from drinking water were collected in plastic bottles. Because each study subject provided 3 samples (rice, bread, and water), a total of 90 samples were analyzed in this phase.

Measurement of Benzo (a) Pyrene in Food and Water Samples

All organic solvents were purchased from Merck®, Germany. BaP standards were purchased from Acros Organics, Belgium.
BaP detection was performed by the high-performance liquid chromatography (HPLC) combined with fluorescence detection (FL; HPLC-FL) technique.

A total of 50 g of each food sample was used for BaP analysis by HPLC. Sample extraction and cleanup was conducted according to the method described by Ahmed et al. (11). In brief, each sample was reflux extracted for 10 h with n-hexane, concentrated to 2 ml by a rotary evaporator, saponified in methanolic KOH, and refluxed again for 2 h. Then, the saponified material was separated to aqueous and organic layers. The aqueous layer was extracted again with n-hexane. The organic layer was evaporated by a rotary evaporator and concentrated to 2 ml, which was then passed onto a Sep Pak Silica classic cartridge and eluted with 10 ml of hexane at the flow rate of 1 ml/min. The eluted hexane was evaporated to dryness, and the residue was redissolved in 1 ml of acetonitrile, which was then filtered. The concentrated sample was wrapped in aluminum foil until analysis.

For water samples, 100 ml of sample was extracted with 100 ml of n-hexane 3 times, and the remaining steps were as described for bread and rice samples. An aliquot (20 ml) of the acetonitrile solution was injected into the HPLC system and eluted with acetonitrile:water (85:15, vol:vol) at a constant flow rate of 1 ml/min, and the detector was set at the excitation wavelength 294 nm and the emission wavelength 404 nm.

For HPLC validation study, 50 g of a single laboratory control bread sample was fortified with 5, 50, and 100 µl of a standard solution of BaP. Each fortification was replicated 3 times. The average rate of recovery was 89%.

### Statistical Analysis

Food consumption and dietary BaP intake were compared between the 3 groups using one-way analysis of variance and least squares difference post hoc tests. Tobacco smoking, opium use, type of fuel used for heating and cooking, use of wood or charcoal for bread cooking and were exposed to stubble or wood burning. Cases consumed more bread and tea than both high-risk and low-risk controls, whereas rice consumption was similar in the 3 groups. Water intake was the lowest in cases, intermediate in high-risk controls, and the highest in low-risk controls.

BaP concentrations measured in the collected food and water samples are shown in Table 2. Mean BaP concentration in bread samples was similar (0.12 ppb) in the 3 groups. One of the samples collected from the low-risk controls had a very high BaP concentration (0.38 ppb); after exclusion of this sample, mean BaP concentration in this group decreased to 0.09 ppb, but the differences among the 3 groups remained nonsignificant. Mean BaP concentration in rice samples collected from high-risk controls (0.22 ppb) was higher than that for samples collected from the 2 other groups (0.11 and 0.14 ppb), but this difference was mainly because of 1 outlier; after excluding this sample, mean in samples from high-risk controls dropped to 0.15 ppb and was no longer significantly different from samples from the other 2 groups. After excluding 1 outlier, BaP concentration in rice samples from all 3 groups was close to 4 ppt, and differences were not statistically significant. In subsequent analyses, outliers were not included.

Table 3 shows the total daily BaP intake via rice, bread, and water. Total daily intake of BaP in ng/day was 99.0 for ESCC cases, 91.4 for high-risk controls, and 70.6 for low-risk controls.

In the multivariate regression analysis, having ESCC (vs being a control) had no independent effect on BaP, adjusted for age, sex, living place (urban/rural), and indoor pollutants from smoking, opium use, and burning fuel for cooking and heating. In the second model, residence in the low-risk area was associated with a significant decrease in total BaP intake (β = −24.9, 95% confidence interval = −36.0 to −13.9) adjusted for the same confounders.

### DISCUSSION

The results of a recent study (7), which showed high levels of urinary markers of BaP exposure in residents of Golestan Province, may imply that high exposure to PAHs is a cause of high ESCC rates in this province. That study (7), however, did not investigate the sources of PAH exposure. Therefore, we measured BaP concentration of rice, bread, and water in ESCC case and control subjects from Golestan and control subjects from Fars Province to examine if these food types can be a major source of exposure.

BaP concentrations in staple foods and water were relatively low and similar between samples collected from Golestan and Fars. These levels were also similar to those measured in rice
and bread samples in United States (12). These results confirm those of a study conducted in the 1970s, which showed low PAH concentrations in food samples collected from Northeastern Iran (13).

Total BaP intake from staple food and water, however, was higher in Golestan than in Fars Province. This difference reflects higher intake of bread and higher consumption of tea in Golestan rather than a difference in BaP concentration. These results are concordant with previous studies in Golestan that showed the staple food in this area was mainly bread and that tea is consumed at very high amounts (13,14). The relatively low concentration of BaP in bread, rice, and water samples from Golestan suggests that staple food and water are unlikely causes for the very high regional rates of ESCC. However, it is noteworthy that BaP intake through bread only was 60 ng/day in ESCC patients, equal to the mean daily intake of BaP in the US population (40–60 ng/day) (12), whereas the total daily intake from rice, bread, and water (90 ng/day) was considerably higher

TABLE 1  
Distribution of cases and controls for selected variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Case&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Golestan controls&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Fars controls&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>62.0 ± 11.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>62.8 ± 11.2</td>
<td>62.3 ± 11.2</td>
</tr>
<tr>
<td>Female</td>
<td>21 (52.5)</td>
<td>21 (52.5)</td>
<td>21 (52.5)</td>
</tr>
<tr>
<td>Bread consumption (g/day)</td>
<td>503.3 ± 265.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>401.0 ± 169.9</td>
<td>326.3 ± 171.7</td>
</tr>
<tr>
<td>Rice consumption (g/day)</td>
<td>224.5 ± 153.1</td>
<td>228.1 ± 144.0</td>
<td>240.5 ± 131.3</td>
</tr>
<tr>
<td>Tea consumption (cup/day)</td>
<td>11.9 ± 6.6&lt;sup&gt;e&lt;/sup&gt;</td>
<td>8.1 ± 4.9</td>
<td>3.3 ± 1.5&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Water consumption (cup/day)</td>
<td>1.8 ± 1.4</td>
<td>2.5 ± 1.7</td>
<td>3.9 ± 2.5&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ever smoking</td>
<td>11 (27.5)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3 (7.5)</td>
<td>1 (2.5)</td>
</tr>
<tr>
<td>Ever opium use</td>
<td>13 (32.5)</td>
<td>9 (22.5)</td>
<td>0 (0)&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Use of natural gas for heating</td>
<td>10 (25)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>27 (67.5)</td>
<td>40 (100)&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Use of natural gas for cooking</td>
<td>39 (97.5)</td>
<td>37 (92.5)</td>
<td>40 (100)</td>
</tr>
<tr>
<td>Wood, charcoal as bread cooking fuel</td>
<td>18 (45)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>6 (15)</td>
<td>0 (0)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Any other exposure to wood or stubble burning</td>
<td>33 (82.5)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>20 (50)</td>
<td>0 (0)&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values in parentheses are percentages.  
<sup>b</sup> n = 40.  
<sup>c</sup> Mean ± SD.  
<sup>d</sup> P < 0.05.  
<sup>e</sup> P ≤ 0.001.  
<sup>f</sup> P < 0.01, between cases and Golestan controls.  
<sup>g</sup> P ≤ 0.005.  
<sup>h</sup> P < 0.005, between the 2 controls.

TABLE 2  
BaP concentration of food and water samples collected from Golestan and Fars provinces.

<table>
<thead>
<tr>
<th>BaP concentration</th>
<th>Samples</th>
<th>Mean ± SD</th>
<th>Min-Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bread BaP (ppb)</td>
<td>Cases (n = 10)</td>
<td>0.12 ± 0.07</td>
<td>0.06–0.26</td>
</tr>
<tr>
<td></td>
<td>Golestan controls (n = 10)</td>
<td>0.12 ± 0.06</td>
<td>0.05–0.21</td>
</tr>
<tr>
<td></td>
<td>Fars controls (n = 10)</td>
<td>0.12 ± 0.10</td>
<td>0.03–0.38&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rice BaP (ppb)</td>
<td>Cases (n = 10)</td>
<td>0.11 ± 0.05</td>
<td>0.01–0.20</td>
</tr>
<tr>
<td></td>
<td>Golestan controls (n = 10)</td>
<td>0.22 ± 0.08</td>
<td>0.03–0.92&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Fars controls (n = 10)</td>
<td>0.14 ± 0.04</td>
<td>0.06–0.22</td>
</tr>
<tr>
<td>Water BaP (ppt)</td>
<td>Cases (n = 10)</td>
<td>3.90 ± 0.67</td>
<td>3.2–5.1</td>
</tr>
<tr>
<td></td>
<td>Golestan controls (n = 9)&lt;sup&gt;3&lt;/sup&gt;</td>
<td>3.80 ± 0.58</td>
<td>3.0–4.9</td>
</tr>
<tr>
<td></td>
<td>Fars controls (n = 10)</td>
<td>4.18 ± 0.67</td>
<td>3.4–5.8</td>
</tr>
</tbody>
</table>

BaP: benzo(a)pyrene mean ± SD and min-max after exclusion of outliers with no impact on significance  
<sup>1</sup> (0.09 ± 0.03), (0.03–0.13)  
<sup>2</sup> (0.15 ± 0.05), (0.03–0.22).  
<sup>3</sup> Outlier sample (2536 ppt) which not included in the mean BaP concentration.
than mean BaP intake of the US population. If we add to this BaP from other food sources that were not included in the present study, daily intake levels would most likely be higher. Therefore, the results of this study may partly explain why urinary markers of PAH exposure were high in Golestan Province (7).

Because there is a high correlation between BaP concentration and the total carcinogenic PAHs in food, BaP is used as a marker for the carcinogenic PAH. Based on the hazard characterization for carcinogenic PAH in food suggested by European Commission 2002 (EC 2002), the virtually safe dose, that is, dose that is associated with 1/10^6 exposed subjects for a lifetime, is between 0.6 and 5 ng/kg body weight/day. The carcinogenic potency of total PAH in food would be 10 times higher than expected from BaP alone. Therefore, a virtually safe dose of BaP as a marker of the mixture of carcinogenic PAH in food would be in the range 0.06 to 0.5 ng/kg body weight/day. The lowest virtually safe dose of daily intake of BaP via food for an adult weighing 70 kg would be 4.2 ng/day, and the upper limit would be 35 ng/day (15). We did not determine the total daily BaP intake, but our participants obtained more BaP only from staple food and water than people in the low-risk area, and the actual exposure levels are likely to be higher. These higher BaP levels might contribute to, but do not completely explain, the high rates of ESCC in northeastern Iran.

### REFERENCES


