Mutagen Sensitivity in Upper Aerodigestive Tract Cancer: A Case-Control Analysis

Margaret R. Spitz, John J. Fueger, Susan Halabi, Stimson P. Schantz, Dory Sample, and T. C. Hsu

Departments of Epidemiology [M. R. S., J. J. F., S. H.], Head and Neck Surgery [D. S.], and Cell Biology [T. C. H.], The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030; and Department of Head and Neck Surgery [S. P. S.], Memorial Sloan Kettering Cancer Center, New York, New York 10021

Abstract
Variability in DNA repair capability may be a determinant of interindividual difference in susceptibility to carcinogenic exposures. A cytogenetic assay which quantifies chromosomal breakage induced by in vitro exposure to a clastogen provides an indirect measure of repair. We report the results of a case-control study of upper aerodigestive tract cancers assessing differences in mutagen sensitivity based on this assay. There were 108 cases with previously untreated squamous cell cancers and 108 age and sex frequency-matched controls selected from blood donors to the University of Texas M. D. Anderson Cancer Center. Sixty-nine percent of the cases, compared with 44% of the controls, were classified as mutagen sensitive (breaks per cell $\geq 0.8$). On multivariate analysis, mutagen sensitivity [odds ratio (OR), 2.5], heavy cigarette smoking (OR, 4.8), and heavy alcohol consumption (OR, 3.1) were associated with significantly increased risk. Stratified analyses showed that the combined effects of cigarette smoking (OR, 8.1) and mutagen sensitivity (OR, 3.2) were suggestive of a multiplicative effect (OR, 23.0). The combined estimate for alcohol use (OR, 3.0) and mutagen sensitivity (OR, 3.0) was 5.8. These data confirm those of a previously published preliminary study of upper aerodigestive cancers and underscore the importance of considering interindividual susceptibility in cancer risk characterization, even for those cancers with well-quantified exposures.

Introduction
Upper aerodigestive tract cancers are sentinel diseases of exposure to tobacco and alcohol insults, and, like lung cancer, could be considered the paradigm of environmentally induced diseases. However, since only a fraction of exposed individuals develop these cancers, interindividual differences in susceptibility to these environmental exposures must be postulated. Much attention has been focused on heritable polymorphisms in carcinogen metabolism. It is now being recognized that there is also population variance in DNA repair capability, of which the genetic instability syndromes exist at the extreme end of the spectrum (1).

These rare, recessively transmitted traits include three entities classified collectively as chromosome breakage syndromes (ataxia-telangiectasia, Fanconi's anemia, and Bloom's syndrome) which are characterized by chromosome instability and high risks of cancer development. A fourth, xeroderma pigmentosum, is notable for extreme sensitivity to UV radiation and susceptibility to skin cancer.

Skin fibroblasts and peripheral blood lymphocytes from patients with these disorders show increased sensitivity to mutagens, suggestive of defects in DNA repair (2). It has been demonstrated that skin fibroblasts from genetically predisposed individuals exhibit a common abnormality in their response to $G_2$ cell cycle phase irradiation. Frequencies of aberrations after irradiation were 2-fold higher than in comparable cells from clinically normal controls (2). These authors suggest, based on cytogenetic and biochemical studies with DNA repair inhibitors, that the enhanced irradiation damage results from deficient DNA repair processes during $G_2$. More recently, similar findings have been demonstrated in fibroblasts from patients with retinoblastoma and Wilms' tumor (3). Deficient DNA repair could increase the probability of mutations such as inactivation or deletions at a heterozygous cancer-predisposing locus (4).

To demonstrate the existence of interindividual differences in mutagen sensitivity, Hsu (5) developed a cytogenetic assay in which quantification of chromosomal breakage induced by in vitro exposure to the radiomimetic drug, bleomycin, has been used as an indirect measure of repair capability. Bleomycin-induced sensitivity and environmental exposures have been assessed in a case-control study of patients with previously untreated upper aerodigestive malignancies and in healthy control subjects (6). Mutagen sensitivity was shown to be a strong and significant risk factor (adjusted odds ratio, 4.3). However, this study was limited by size and design constraints. In particular, the control population for this preliminary study was less than optimal with respect to sex (overrepresentation of females), age (younger than cases), and selection (spouses and hospital employees only). We now report the results of a larger case-control study evaluating the risks of environmental exposures and mutagen sensitivity in cases and age- and sex-matched controls.
Mutagen Sensitivity in Upper Aerodigestive Tract Cancer

Materials and Methods

Study Population. Cases were 108 white patients with histologically confirmed and previously untreated squamous cell carcinomas of the upper aerodigestive tract registered at The University of Texas M. D. Anderson Cancer Center from June 1987 to June 1991. Patients were required to have completed a self-administered cancer risk factor questionnaire and to have submitted to venipuncture before therapy. None of these patients had been included in the study cited above (6). The control population (with no cancer history) was frequency matched to the cases according to age (±5 years), sex, and ethnicity. We had intended to select peer controls for this study. This type of control selection would have assured us that they were of comparable socioeconomic status with the cases and had similar lifestyle habits, including smoking and dietary exposures. However, as has also been observed by other investigators, patients with potentially disfiguring or lethal diseases are generally reluctant to provide names of controls (7). We were thus limited to selecting controls from blood and platelet donors identified by the M. D. Anderson Cancer Center Blood Bank. Controls likewise completed a risk factor questionnaire and provided blood samples.

Mutagen Sensitivity Assay. The assay has been described in detail previously (8). In brief, on the third day of incubation, standard lymphocyte cultures were treated with bleomycin (0.03 units/mL) for 5 h. During the last hour, the cells were treated with Colcemid (0.04 μg/mL) to accumulate mitoses before harvesting for conventional air-dried preparations. All prepared slides were coded and stained with Giemsa without banding.

Using coded slides, breaks were scored on 50 metaphases per sample by T. C. H. who had no knowledge of the case-control and exposure status of the individual. Only frank chromatid breaks or exchanges were recorded; chromatid gaps or attenuated regions were disregarded. Since the spontaneous b/c1 rate was less than 0.02, it was not deemed necessary to deduct these values from those of the bleomycin-treated samples. Any individual expressing more than 0.8 b/c was considered sensitive.

Data Collection. Risk factor data were derived from a modified and validated self-administered comprehensive cancer risk factor questionnaire that is distributed to all newly registered adult patients at our institution. This instrument is the central component of our Patient Risk Evaluation Program, described in detail previously (9). The questionnaire is comprehensive with respect to established and putative risk factors and includes detailed questions on tobacco, alcohol, and family history. The latter data have been reported separately (10).

A smoker was defined as someone who had smoked at least 100 cigarettes in his or her lifetime. A former smoker was defined as one who had smoked cigarettes in the past but had stopped smoking more than 1 year previously.

Statistical Analysis. To test for significant associations between tobacco, alcohol, mutagen sensitivity, and cancer risk, univariate OR were calculated as estimates of the relative risks. Ninety-five % confidence limits were computed according to the method of Woolf (11). Logistic regression using SAS statistical software (SAS Institute Inc., Cary, NC) was used to estimate risks, which were adjusted for multiple factors. All variables that were statistically significant in the bivariate analysis were included in the first logistic model. Bleomycin-induced sensitivity and educational level were dichotomized, and the respective referent categories were the expression of less than 0.8 breaks/cell and a college or higher education. Cigarette smoking was grouped into 4 categories (non-smoker as the referent; 1–14, 15–24, and ≥25 cigarettes/day). Alcohol consumption (beer, wine, or hard liquor) was grouped into 3 categories (nondrinker or less than 1 drink/day as the referent group; 1–2 and >2 drinks/day).

Results

Patient Characteristics. There were 44 (41%) patients with oral cavity cancers, 31 (29%) with pharyngeal cancers, and 33 (30%) with laryngeal malignancies. Seventy-six (65%) of the cases were male. The mean age of the cases was 53.8 years, compared with 51.1 years for the comparison group. The cases were significantly less educated than the controls (33% had only a high school education, compared with 9% of controls). Similarly, one-half of the cases had a gross annual family income of less than $23,000 compared with 6% of the controls (P < 0.001). Univariate Analysis. Cigarette smoking (Table 1) predictably was associated with significantly elevated risk with evidence of a linear dose-response effect. Alcohol consumption was also associated with increased risk, but only the highest category was statistically significant. Tobacco chewing (OR, 1.2) and cigar use (OR, 1.7) (data not shown) were associated with moderately elevated but not statistically significant risk.

Sixty-nine % of the cases expressed a b/c value greater than 0.8 compared with 44% of the controls. The smoking-adjusted risk estimate for mutagen sensitivity

<table>
<thead>
<tr>
<th>Variables</th>
<th>Risk estimates</th>
<th>95% confidence limits</th>
</tr>
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<tbody>
<tr>
<td>Cigarette smoking (number/day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–14</td>
<td>4.2</td>
<td>1.4–12.8</td>
</tr>
<tr>
<td>15–24</td>
<td>7.9</td>
<td>3.2–19.1 (Trend)</td>
</tr>
<tr>
<td>≥25</td>
<td>11.0</td>
<td>4.4–27.4 (P &lt; 0.001)</td>
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<tr>
<td>Alcohol (drinks per day)</td>
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<tr>
<td>1–2</td>
<td>1.0</td>
<td>0.7–1.6</td>
</tr>
<tr>
<td>3–6</td>
<td>1.7</td>
<td>0.7–4.1 (Trend)</td>
</tr>
<tr>
<td>&gt;6</td>
<td>14.0</td>
<td>4.2–47.0 (P &lt; 0.001)</td>
</tr>
<tr>
<td>Mutagen sensitivity (b/c)</td>
<td></td>
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<tr>
<td>≥0.8*</td>
<td>2.9</td>
<td>1.5–5.4</td>
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</tbody>
</table>

* Adjusted for cigarette smoking.
(≥0.8 b/c) was 2.9 (1.5–5.4). Risk estimates were elevated at all sites, with the highest OR being noted for pharyngeal cancers (data not shown).

The distribution of mutagen sensitivity within the case group and the control group did not differ significantly by smoking status (Table 2). Sixty-three % of currently smoking cases were classified as mutagen sensitive, compared with 71% of nonsmoking cases. The comparable percentages for controls were 52 and 44%, respectively (Table 2). The prevalence of mutagen sensitivity was even higher among former (75%) and non-smoking cases (71.4%) than among current smokers (63.5%). This pattern was not discernible for controls.

We also evaluated the distribution of mutagen sensitivity by the self-reported degree of smoking (Table 2). Among the smoking cases, the prevalence of mutagen sensitivity increased with increasing number of cigarettes smoked, although this trend was not statistically significant. There was no such relationship among the smoking controls. Among the controls, more of the heaviest smokers (62.5%) were nonsensitive than were sensitive. There were no differences in mutagen sensitivity by either of two measures of socioeconomic status, income and educational attainment (data not shown). For example, 65.6% of cases who achieved only a high school education were mutagen sensitive, compared with 69.4% of cases who were better educated. The comparable percentages for controls were 50 and 43.2%, respectively.

None of the distribution differences were statistically significant.

To evaluate the independent effect of mutagen sensitivity and its interaction with cigarette smoking and alcohol consumption, risk estimates for various combinations of sensitivity and smoking or alcohol use were computed in stratified analyses (Table 3). These analyses were restricted to study participants for whom all relevant information was available. Referent categories were study participants who were not mutagen sensitive and were nonusers of either cigarettes or alcohol. The data were sparse in some categories and the resultant measures of effect were therefore somewhat unstable. Mutagen sensitivity was a risk factor in the absence both of smoking or alcohol use (OR, 3.2 and 3.0, respectively), and there were significant elevated risks associated with smoking in mutagen-stable persons (OR, 8.1). The combined effect of cigarette smoking and mutagen sensitivity (OR, 23.0) was suggestive of a multiplicative effect. The combined OR for alcohol use and mutagen sensitivity (5.8) was suggestive of an additive effect.

The results of the logistic regression (Table 4) showed that heavy smoking (≥25 cigarettes/day; OR, 4.8), heavy drinking (≥3 drinks/day; OR, 3.1), and mutagen sensitivity (OR, 2.5) were all associated with significantly elevated risk. Higher education was negatively associated with cancer risk. None of the interaction terms entered into the model achieved statistical significance.

### Discussion

These data accord well with our previous findings (3). In the first study, 65% of the cases were classified as mutagen sensitive. The comparable percentage for this study was 69%. Thirty-five % of the cases in the previous study were documented as having b/c values <0.8 compared with 31% in this study. The concordance among the two comparison groups was not as close. Fifty-six % of the new comparison group were considered nonsensitive, compared with 82% of the previous study. These differences had the expected effect of reducing the risk estimates, although they were still statistically significant. The adjusted odds ratios for mutagen sensitivity (defined as b/c ≥0.8) were 4.3 and 2.5 in the original and subsequent studies, respectively. As with the previous study,
we noted the highest estimate for mutagen sensitivity to be associated with pharyngeal cancers and lowest for oral cavity malignancies.

This study has controlled for the effects of age and gender biases by matching controls and cases according to these variables, which might be expected to modify mutagen sensitivity status. However, there were significant differences between the cases and controls in terms of socioeconomic status. Socioeconomic status would, per se, be unlikely to modify inherent mutagen sensitivity status. In fact, we showed no differences in the distribution of sensitivity among cases and controls by various measures of socioeconomic status. On the other hand, upward bias in the estimates of risk for smoking and alcohol use could be attributed to this disparity between cases and controls. We have demonstrated that cigarette smoking, per se, did not shift the sensitivity profile in either cases or comparison subjects, although intensity of smoking did appear to be positively associated with sensitivity in cases but not controls. In both studies, the combined effect of mutagen sensitivity and cigarette smoking was associated with an OR consistent with a multiplicative effect. The results for alcohol and mutagen sensitivity differ in that this study suggests that the combined effect of mutagen sensitivity and alcohol was consistent with an additive rather than multiplicative effect. The stratified analyses also showed that both cigarette smoking and alcohol use were determinants of risk even in the absence of mutagen sensitivity. In nonsmokers and nondrinkers, mutagen sensitivity was still associated with increased risk.

The finding of a suggestive synergistic effect of alcohol and mutagen sensitivity has stimulated further in vitro studies of the effect of alcohol in this test assay system (12). Cultured human cells were treated with bleomycin at a fixed concentration together with ethanol at concentrations varying from 0.1 to 4%. The frequency of chromosome breaks, compared with that of the bleomycin control, was unchanged at 0.1 and 0.5% ethanol, but beginning at 1% ethanol the frequency was dramatically elevated (12). This series of experiments indicated that alcohol, although itself not a clastogen, could potentiate the genotoxic property of bleomycin with a dose-dependent effect.

The potentiation property of ethanol on mutagens with different molecular mechanisms has also been demonstrated (12, 13). Hsu used the base analogue cytosine arabinoside, the UV-mimetic carcinogen 4-nitroquinoline-1-oxide, the alkylating agent triethylene melamine, and a sample of cigarette smoke condensate (12). In all cases, ethanol enhanced their genotoxicity. Hsu (14) has also demonstrated that 20% ethanol in vitro inhibits DNA repair following bleomycin exposure. Thus our findings of an interaction of mutagen sensitivity with alcohol have biological plausibility. An interaction between cigarette smoking and mutagen sensitivity is also plausible considering the capability of in vitro exposure of both bleomycin and cigarette smoke to induce single-stranded DNA breaks (15).

These results confirm our previous observation that persons with untreated upper aerodigestive tract cancer express greater mutagen sensitivity than do controls when their cells are exposed in vitro to clastogens. These findings provide added plausibility to our observation that this assay also has predictive value in identifying those patients with upper aerodigestive tract cancers at higher risk for developing second malignant neoplasms (16). They have highlighted the prevention potential in predicting high-risk subgroups who can be targeted for the most intensive behavior modification and screening surveillance programs. These data also underscore the importance of considering interindividual susceptibility differences in risk characterization of cancers, even those with well quantified and characterized exposures.

Acknowledgments

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References


Table 4 Logistic regression analysis of variables associated with upper aerodigestive tract cancer risk

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio</th>
<th>95% confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol consumption</td>
<td>3.14</td>
<td>1.32-7.44</td>
</tr>
<tr>
<td>(≥3 drinks/day versus nondrinker)</td>
<td>4.79</td>
<td>2.31-9.95</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>2.45</td>
<td>1.23-4.90</td>
</tr>
<tr>
<td>(≥25 cigarettes/day versus nonsmokers)</td>
<td>0.30</td>
<td>0.15-0.60</td>
</tr>
<tr>
<td>Educational level</td>
<td>0.58</td>
<td>0.28-1.20</td>
</tr>
<tr>
<td>(College versus lower)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Likelihood ratio, 15.23; df (degrees of freedom) = 11; P = 0.17.
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