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Chromosome Sensitivity to Bleomycin-induced Mutagenesis, an Independent Risk Factor for Upper Aerodigestive Tract Cancers

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ABSTRACT

Defective DNA repair capability, measured by enumerating mutagen-induced chromosomal lesions, might explain variable host susceptibility to the action of environmental carcinogens. We compared sensitivity to bleomycin-induced chromosome damage in 75 patients (53 men and 22 women) with previously untreated upper aerodigestive tract malignancies with that in 62 healthy control subjects. Data on tobacco and alcohol use were derived from a detailed, self-administered cancer risk factor questionnaire. Forty-five patients and 13 controls were sensitive to bleomycin-induced mutagenesis (average breaks/cell >0.8). Differential susceptibility was detected in patients categorized by primary tumor location. Odds ratios for chromosome sensitivity were significantly elevated for all sites (odds ratio = 10.3 for pharyngeal cancers, 8.0 for laryngeal cancers, and 3.8 for oral cavity cancers). On logistic regression analysis, chromosome sensitivity remained a strong and independent risk factor after adjustment for potential confounding from age, sex, and tobacco and alcohol use (odds ratio = 4.3, 95% confidence limits = 2.0, 10.2). Despite the small study size and design constraints, the strength of the association with chromosome sensitivity even after adjustment for potential confounders is impressive and suggests a promising avenue for further research. The preventive implications of a valid marker for carcinogen sensitivity are manifold.

INTRODUCTION

Host susceptibility to the genotoxic effects of environmental carcinogens varies; only a fraction of exposed individuals will develop cancer. This variable activation of carcinogens, which are usually mutagenic, may be due to genetic polymorphism. Cytogenetic experiments have shown quantitative variations in susceptibility to mutagen-induced genetic damage (1). Thus, enumerating-induced chromosomal lesions, which may reflect defective DNA repair systems, might be a biological marker of individual cancer risk.

Since upper aerodigestive tract cancers are sentinel diseases of exposure to tobacco and alcohol, they constitute an ideal group of diseases for an ecogenetic study, *i.e.*, for the evaluation of the interaction of chromosome sensitivity and carcinogenic exposures in risk of cancer. Few previous cytogenetic reports have attempted to control for potentially confounding variables in their evaluation of biological markers.

Preliminary analyses of patients with lung, colon, and upper aerodigestive tract cancers have revealed increased sensitivity to clastogen-induced chromosome damage (1, 2). The test assay used short-term cultures of peripheral blood samples incubated with the radiomimetic clastogen, bleomycin. The frequency of chromosome breaks was used as a measure of DNA repair capability to evaluate sensitivity to the assay. The purpose of this report is to expand upon these previously reported data, incorporating cytogenetic information with risk factor data to examine the association between mutagen-induced chromosome damage and cancer risk and the interaction of carcinogenic exposures and chromosome damage.

MATERIALS AND METHODS

Study Population. Cases were defined as patients with histologically confirmed and previously untreated squamous cell carcinoma of the upper aerodigestive tract, registered at The University of Texas M. D. Anderson Cancer Center from June 1987 to June 1988. These patients were required to have completed a self-administered cancer risk factor questionnaire and to have submitted to venipuncture prior to initiation of therapy.

The control population, for convenience, was made up of hospital employees (nurses and physicians) and spouses of patients who had similarly completed the risk factor questionnaire and from whom blood samples were also obtained. No formal matching for age, race, sex, or socioeconomic status was performed.

Chromosome Analysis. The test mutagen chosen was the radiomimetic clastogen bleomycin, which induces single-stranded and double-stranded DNA breaks. Bleomycin was selected as the clastogenic agent because its mechanism of action has been well elucidated, and it does not affect chromosome morphology to a great extent, thus enabling easier scoring of breaks (3).

Standard lymphocyte cultures were established as previously described (3). On the third day of incubation, cultures were treated with bleomycin (30 μg/ml) for 5 hours. During the last hour, the cells were treated with Colcemid (0.04 μg/ml) to accumulate mitoses before being harvested for conventional air-dried preparations. All prepared slides were coded and stained with Giemsa without banding.

Breaks were scored from coded slides on 50 metaphases per sample by one of us (T. C. H.) who was blinded as to the case-control and exposure status of the individual. Only frank chromatid breaks or exchanges were recorded; chromatid gaps or attenuated regions were disregarded (4). Bleomycin increases the frequency of gaps, as well as frank breaks. However, Hsu has presented comparative data documenting that adding chromatin gaps to the score increases the total frequency of lesions, but not the overall sensitivity profile (4). Because of the subjective element in scoring, conservatism is used in recording aberrations, and all ambiguous lesions are discounted.

In the final computation, each chromatid break was recorded as one break, and each chromatid exchange was recorded as two breaks. The frequency of breakage was expressed as b/c[2] for comparison. Previous experience with 335 normal controls had demonstrated that the 25th percentile value above the median was 0.8 b/c (1). Any individual expressing >0.8 b/c was therefore considered sensitive to bleomycin-induced chromosome damage; above 1.0 b/c, the individual was considered hypersensitive.

Data Collection. Risk factor data were derived from a self-administered comprehensive cancer risk factor questionnaire that is distributed to all newly registered adult patients at the institution. This instrument is the central component of our Patient Risk Evaluation Program, described in detail previously (5). The questionnaire is comprehensive with respect to established and putative cancer risk factors and includes detailed questions on tobacco (smoking and smokeless) and alcohol use. The questionnaire has previously been evaluated for validity and reliability (6).

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[2] The abbreviations used are: b/c, breaks per cell; OR, odds ratio; CL, confidence limits.

Exposures to cigarettes were categorized as never smoked, current smoker (including recent quitters of less than 1 yr), and former smoker. A smoker was defined as someone who had smoked cigarettes on a daily basis for at least 6 months. Former smokers were defined as those who had smoked cigarettes in the past, but had stopped smoking more than 1 yr previously. Alcohol use was defined as consumption of more than one alcoholic beverage (beer, wine, or spirits) per day.

Data Analysis. To test for significant associations between tobacco, alcohol, chromosome sensitivity, and cancer risk, univariate ORs were calculated as estimates of the relative risks. Ninety-five percent confidence limits were computed according to the method of Woolf (7). Logistic regression using BMDP statistical software (Los Angeles, CA) was used to estimate risks, with adjustment for multiple factors. Differences in the age and sex composition of the case and control groups were adjusted for in the analysis. Test-based confidence intervals for the adjusted ORs were calculated using the estimated logistic coefficient and corresponding standard error. Multivariate analysis was restricted to responders who provided information on all the relevant variables.

RESULTS

Seventy-five patients (53 men and 22 women) met the criteria for inclusion into the study. Of these, 22 (29.3%) had carcinoma of the larynx, 29 (38.7%) had oral cavity lesions, and 23 (30.7%) had pharyngeal malignant tumors. One man had a primary tumor of undetermined origin. The control group, because of the absence of clearly defined matching criteria, consisted of 44 women and 18 men. They were also younger (mean age, 48 yr for men and 42 yr for women) than the patients (mean age, 56 and 58 yr, respectively).

The distribution of patients and controls by three categories of chromosome breakage scores is summarized in Table 1. The patients with upper aerodigestive tract malignancies expressed significantly increased numbers of chromosome breaks following bleomycin exposure, as compared with the control group. The number of b/c ranged from 0.22 to 2.76 for patients and 0.22 to 1.34 for controls. Over half (52.2%) of the patients were hypersensitive (b/c >1.0), compared with only 18.2% of the controls. Conversely, over three-quarters of the control scores fell within the normal range (b/c <0.8) of chromosome sensitivity, compared with only 34.8% of the patient b/c scores. Altogether, 45 patients (65.2%) and 13 controls (23.6%) could be considered sensitive to bleomycin-induced mutagenesis.

Chromosome sensitivity on univariate analysis was a strong predictor of cancer risk for all three upper aerodigestive tract cancer sites (Table 2). Patients with pharyngeal malignancies had the highest risk (OR = 10.3), compared with ORs of 8.0 and 3.8 for patients with laryngeal and oral cavity cancers, respectively. There were also site-specific differences in the elevated risks associated with cigarette smoking (current smokers and recent quitters) and alcohol consumption (>1 alcoholic beverage daily). Risk from cigarette smoking was highest (OR = 10.8) in patients with laryngeal malignancies. For each variable, oral cavity cancers exhibited the lowest risks.

Stratified analysis of cigarette smoking and alcohol use by consumption frequency showed predictable increased risks with increasing exposure (Table 3). The lowest category (1 to 14/day) of cigarette smoking was not associated with elevated risk. However, heavy smoking (25+ cigarettes/day) and heavy alcohol consumption (>6 drinks/day) both exerted strong effects (OR = 12.7 and 44.5, respectively). Although cigar use (OR = 1.9), snuff dipping (OR = 3.0), and tobacco chewing (OR = 1.5) were all associated with increased risk, none of these estimates achieved statistical significance, and the results are not tabulated.

To evaluate the independent effect of chromosome sensitivity and the interaction of chromosome sensitivity with cigarette smoking and alcohol consumption, risk estimates for various combinations of sensitivity and smoking or alcohol use were computed in stratified analyses (Table 4). These analyses were restricted to study participants for whom all relevant information was available. Referent categories were study participants who were not chromosome sensitive and were nonusers of either cigarettes or alcohol. The data were sparse in some categories, and the resultant measures of effect are therefore somewhat unstable. Chromosome sensitivity was a risk factor in the absence both of smoking (OR = 5.8) or of alcohol use (OR = 3.6), and there were significantly elevated risks associated with

Table 1 *Distribution of bleomycin-induced chromosome sensitivity*

	Patients			Controls		
Breaks/cell	Male	Female	Total	Male	Female	Total
<0.8	18	6	24 (34.8)[a]	11	31	42 (76.4)
0.8–1.0	7	2	9 (13.0)	0	3	3 (5.4)
>1.0	24	12	36 (52.2)	4	6	10 (18.2)
Total[b]	49	20	69	15	40	55

[a] Numbers in parentheses, percentage.
[b] Scores were unavailable for 6 patients and 7 controls.

Table 2 *Site-specific risks for chromosome sensitivity, cigarette smoking and alcohol use*

	Odds ratio		
Variable	Pharynx	Larynx	Oral cavity
Chromosome sensitivity[a]	10.3 (3.2, 33.7)[b]	8.0 (3.6, 25.0)	3.8 (1.4, 10.2)
Cigarette smoking[c]	4.5 (1.3, 15.3)	10.8 (2.3, 51.1)	2.0 (0.8, 5.3)
Alcohol[d]	6.7 (2.1, 21.6)	4.3 (1.3, 13.9)	3.3 (1.2, 9.1)

[a] Chromosome sensitivity = score of >0.8 breaks/cell.
[b] Numbers in parentheses, 95% confidence limits.
[c] Cigarette smoking includes current smokers and recent (<1 yr) quitters.
[d] Alcohol use = consumption of >1 alcoholic beverage/day (beer, wine, or hard liquor).

Table 3 *Univariate risk estimates for categories of cigarette smoking and alcohol use*

Variable	Patients/controls	Odds ratio	95% Confidence limits
Cigarette smoking (cigarettes/day)[a]			
None	17/32	1.0	
1–14	5/14	0.7	0.2, 2.2
15–24	19/13	2.8	1.1, 6.9
25+	27/4	12.7	3.8, 42.3
Alcohol use (drinks/day)			
None	18/30	1.0	
1–2	9/8	1.9	0.6, 5.7
3–6	12/4	5.0	1.4, 17.9
>6	13/0	44.5	2.5, 793.9

[a] Smokers include recent quitters (<1 yr).

Table 4 *Risk estimates for combinations of cigarette smoking, alcohol use, and chromosome sensitivity*

Analysis was restricted to responders who provided full information on all risk factor variables (n = 98).

	Cases	Controls	Odds ratio	95% Confidence limits
Smoking/chromosome sensitivity				
No/no	4	18	1.0	
No/yes	9	7	5.8	1.3, 25.1
Yes/no	18	15	5.4	1.5, 19.5
Yes/yes	22	5	19.8	4.6, 84.8
Alcohol use/chromosome sensitivity				
No/no	10	27	1.0	
No/yes	12	9	3.6	1.2, 11.1
Yes/no	12	6	5.4	1.6, 18.3
Yes/yes	19	3	17.1	4.1, 70.6

smoking and tobacco use in chromosome-stable persons (OR = 5.4). The combined effects of chromosome sensitivity and smoking (OR = 19.8) or alcohol use (OR = 17.1) were consistent with a multiplicative scale of no interaction.

Logistic regression analyses were restricted to responders who provided full information on all relevant factors entered into the model (n = 98 cases and controls). In order to adjust simultaneously for design constraints (age and sex), chromosome sensitivity, and smoking and alcohol use, a five-factor model was used. The crude OR for chromosome sensitivity was 3.9 (95% CL, 1.6, 9.1). In the five-factor logistic model, the adjusted OR was 4.3 (95% CL, 2.0, 10.2).

DISCUSSION

These results are consistent with the hypothesis, previously proposed, that upper aerodigestive tract cancer patients express increased chromosome sensitivity when their cells are exposed *in vitro* to a clastogen (2). In our data, chromosome sensitivity remained a strong and significant risk factor for head and neck cancer after adjustment for potential confounding from age, sex, cigarette smoking, and alcohol consumption.

Cytogenetic monitoring is a relatively inexpensive approach to evaluating individual carcinogen susceptibility. The number of induced chromosomal breaks is a reasonably good estimate of defective DNA repair capability. Lymphocyte samples are readily accessible for study, and chromatid breaks are relatively easy to observe and quantitate. The assay results have been shown to be reproducible on repeated tests of four separate samples at least 1 wk apart, with a relatively narrow range of variation (1). Furthermore, 70% of patients with lung cancer, and a similar percentage with colon cancer, also exhibited scores >0.8 b/c, compared with 65.2% of our patients with aerodigestive tract malignancies (1).

Finally, our findings are biologically plausible. Impaired excision repair systems of patients with xeroderma pigmentosum clearly predispose them to cancer development. However, the sensitivity, specificity, and predictive value of this marker clearly require further elucidation (8).

Several methodologic issues regarding the design of this study must be considered. Selection bias is one such concern. The patient group is derived from a tertiary care institution with its differential referral patterns. Nonrepresentativeness of the control group is also a potential problem. However, external comparisons show that the background level of chromosome sensitivity in our control population was as expected. In a prior analysis of 335 control individuals, 23% exhibited mean b/c values >0.8 (compared with 23.6% in our data) (1). Twelve percent of these controls exhibited a response of >1.0 b/c; our comparative percentage was 18.2%.

Of greater concern is the age and gender composition of the control group, reflecting the use of a convenience sample. Any of these differences could have resulted in an exaggerated disparity of alcohol or tobacco use between cases and controls with a resultant overestimation of risk for these exposures. These factors were adjusted for in the analyses, rather than the design. Although inefficient in terms of sample size, the results should not be biased.

The age differential should not affect the prevalence of bleomycin sensitivity. While older persons show a higher frequency of spontaneous chromosome aberrations, including aneuploidy and chromosome rearrangements, spontaneous breakage and sensitivity to bleomycin are not associated with advancing age. In fact, Hsu (1) showed that the sensitivity profile of heavy smokers over 50 yr of age was significantly skewed toward the resistant stratum, so that decreasing, rather than increasing prevalence of sensitivity with age (due to differential survival) would be predicted.

Site-specific differences in mutagen sensitivity are of interest and suggest that, although surface epithelia are susceptible to the same carcinogens, their degrees of susceptibility may vary. Variable susceptibility to the effects of exposure to alcohol and tobacco within the upper aerodigestive tract has been demonstrated previously, with higher smoking-related risks associated with laryngeal and pharyngeal cancers, and lowest risks for oral cavity lesions (8).

An interaction between smoking and chromosome sensitivity is biologically plausible. According to Nakayama *et al.*, cigarette smoke, like bleomycin, induces single-stranded DNA breaks in humans (9). The interaction of alcohol with chromosome sensitivity is a more surprising finding.

The use of genetic markers clearly enhances epidemiological research. Few previous cytogenetic reports have evaluated confounding exposures (10). The preventive implications of a precise and valid marker for carcinogen sensitivity are obvious. We are aware of the need for extensive validation of the assay and for rigorously designed and conducted epidemiological studies. The strength of the association between cancer risk and chromosome sensitivity, despite the inherent problems in the size and design of the studies, is impressive. The thesis that chromosome instability and or defective DNA repair may underlie susceptibility to environmental carcinogenesis is plausible and may present a promising avenue for further research.

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