Evaluation of Sister Chromatid Exchange and Chromosome Breaks in a Cohort of Untreated Hodgkin’s Disease Patients

Sara S. Strom, Ken R. Hess, Alice J. Sigurdson, Margaret R. Spitz, and Jan C. Liang

Departments of Epidemiology [S. S. S., A. J. S., M. R. S.], Biomathematics [K. R. H.], Laboratory Medicine [J. C. L.], The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

Abstract

Cytogenetic biomarkers, chromosomal breaks [spontaneous breaks (SB) and bleomycin-induced breaks (BIB)], and sister chromatid exchange (SCE) have been shown to be sensitive cytological assays to detect susceptibility to DNA-damaging effects. However, little information is available on how environmental factors and demographic and clinical characteristics influence variation among individuals. We sought to characterize interindividual variability in a cohort of 105 untreated adult Hodgkin’s disease patients. SB, BIB, and SCE data were integrated with epidemiological data by using linear regression analysis. Age, sex, ethnicity, education, histology, history of mononucleosis, and family history of cancer showed no association with any biomarker. In univariate analysis, alcohol intake was significantly associated with high SCEs (P = 0.005) and SBs (P = 0.02). Current smoking was associated only with high frequencies of SCE (P = 0.05). Advanced stage of disease was related with high SBs (P = 0.01). BIBs were not associated with any of the variables studied. In multivariate modeling, current alcohol intake was associated with high SCEs (P = 0.04) and SBs (P = 0.01). Former smokers had higher SBs than nonsmokers did (P = 0.02). A small positive correlation was found among each pair of markers. The higher SCEs and SBs in patients who smoke and consume alcohol indicate the need for evaluating these exposures when interpreting these biomarkers.

Introduction

Chromosomal breaks (SBs and BIBs) and SCE in peripheral blood lymphocytes are cytogenetic biomarkers that have been shown to detect susceptibility to DNA damage. However, very little is known about how interindividual variability is influenced by environmental factors and demographics and clinical characteristics. A variety of factors (age, sex, smoking, alcohol and coffee consumption, and WBC count) may affect baseline SCE levels (1–4). However, with the exception of smoking, the results have not been consistent. Available information on the influence of various exposures and SCE frequency in HD patients has been based on small studies (5–7). No data are available on chromosome breaks. As for BIBs, in studies of lung and head and neck cancers, age, sex, and tumor stage showed no effect on break frequency (8, 9).

In the present study, we determined whether the frequency of SCE and chromosome breaks in 105 untreated HD patients was associated with age, sex, ethnic group, environmental exposures (smoking and alcohol intake), positive family history of cancer, and clinical characteristics (histology, stage, and history of mononucleosis). We also assessed the relationship between SCE, SBs, and BIBs.

Patients and Methods

Study Population. The study group consisted of 105 patients with histologically confirmed and previously untreated HD seen at The University of Texas M. D. Anderson Cancer Center between 1986 and 1992. These patients are part of a follow-up study of therapy-induced genetic damage for whom cytogenetic data were available. Patients with HD diagnosed in other institutions, who had received radiation treatment for any condition, or had a previous cancer were not eligible for this study.

Data Collection. Data were collected from a self-administered questionnaire distributed to all new adult patients at initial hospital registration. This instrument includes detailed questions on tobacco and alcohol use; family history of cancer; health history; and demographics. For alcohol use, the subjects were classified as follows: nondrinkers or former drinkers who had quit at least a year before diagnosis; occasional drinkers who drank less than one drink a day; moderate drinkers who drank one or two drinks a day; and heavy drinkers, who drank three or more drinks a day. Smoking status (current, previous, or never), years smoked, and number of cigarettes smoked daily were also determined. Medical records were abstracted to obtain histopathological and staging information.

Cytogenetic Analysis. A 10-ml sample of peripheral blood was obtained from each patient before starting therapy, and standard lymphocyte cultures were established (7). For analysis of BIBs, each culture was treated with 0.03 unit/ml bleomycin for 5 h before mitosis was arrested. SB and BIB slides were stained with Giemsa as described elsewhere (7, 8). The frequency of SBs and BIBs was calculated by averaging the number of breaks counted in 50 cells.

For analyses of SCE, standard techniques were followed as described previously (7). The slides were stained with Hoechst 33258 and Giemsa, and then 50 metaphases were evaluated to determine the frequency of SCE. All three assays were performed by one experienced technician.

Statistical Analysis. Due to outliers, distributional robust linear regression was used for univariate and multivariate analysis (10). Biomarker frequencies were measured on a continuous...
Family history of invasive cancer in univariate analysis. The results of the univariate and multivariate analyses, regression coefficients, and P values are shown in Table 3. Stage II or IV was strongly associated with any of the markers analyzed. For current alcohol intake, the effect of the average number of drinks consumed daily showed that the 13 heavy drinkers (those consuming an average of three or more drinks a day) had significantly more BIBs and SCEs than nondrinkers (P = 0.002 and P = 0.001, respectively).

We further analyzed the effect of smoking by including in the model the number of cigarettes smoked daily and the number of years smoked (data not shown). No association was found with any of the markers analyzed. For current alcohol intake, the effect of the average number of drinks consumed daily showed that the heavy drinkers (those consuming an average of three or more drinks a day) had significantly more BIBs and SCEs than nondrinkers (P = 0.002 and P = 0.001, respectively).

Spearman’s rank correlation coefficients were calculated to determine the strength of the association among these biomarkers in the 87 patients for whom data were complete. A positive association was found among each pair of markers. As expected, BIB and SB showed the strongest correlation (ρ = 0.29; P = 0.04). The correlation coefficients for BIB and SCE were ρ = 0.24 (P = 0.03) and for SB and SCE, ρ = 0.17 (P = 0.12).

Discussion
The purpose of this study was to evaluate pretreatment interindividual variability in susceptibility to genetic damage, as measured by SCE and chromosome breaks, in a cohort of 105 HD patients. SCE, the most widely used cytogenetic marker, showed the highest variability. Our mean SCE value was lower than the values reported for HD (11, 12), based on fewer than 15 subjects, but was similar to those reported for leukemia patients (13). Of all the factors analyzed, the strongest association was found between alcohol intake and high SCEs, particularly when heavy drinkers were compared to nondrinkers. These results agree with those from other studies (11, 14, 15); however, further studies are necessary to determine the role of alcohol in the induction of SCEs and in the inhibition of DNA repair.

We found no association between SCE and smoking status, number of daily cigarettes smoked, or years of tobacco use in the multivariate analysis, although current smoking and high SCEs were strongly associated in the univariate analysis. These results differ from those of other studies, which showed increased SCE values in smokers, with some showing a dose effect (1, 14, 16, 17). Still other studies showed no association (18–20). The few HD studies were based on a small number of patients, and to our knowledge no multivariate analyses were performed. The fact that we did not find an association with smoking could indicate that cigarette smoking may have complex effects on SCE induction (at least in HD patients) that need to be further evaluated. Our data did not confirm that women have higher SCEs than men do, as has been reported previously for healthy individuals (1, 21).

No association was found between SCEs and age, education, disease stage, histology, history of infectious mononucleosis, or positive family history of cancer in first-degree relatives.

In our study, high frequencies of SBs were associated with alcohol intake, possibly because alcohol inhibits DNA repair (16), and with advanced stage of disease. BIB frequencies were not correlated with any of the variables analyzed, which was similar to previous reports (17, 18). Our results do not agree with two studies that suggested an association between a positive family history of cancer and hypersensitivity to BIB (22, 23). The weak correlations found among these biomarkers point
to the strong possibility that SBs, BIBs, and SCEs are the result of different types of genetic damage.

In conclusion, we showed in a large sample of HD patients that different exposures and clinical characteristics have different effects on the frequency of these biomarkers. In studies whose goals are etiological associations between biomarkers and disease, information on possible confounders such as alcohol and smoking should be included and controlled in the analysis.

Acknowledgments
We thank Yun Gu for assistance in the preparation of the manuscript.

References

<table>
<thead>
<tr>
<th>Marker</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>Lower 25th percentile</th>
<th>No. of patients</th>
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</thead>
<tbody>
<tr>
<td>SCB</td>
<td>0.04 ± 0.09</td>
<td>0.00–0.88</td>
<td>0.04</td>
<td>36</td>
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<tr>
<td>BIB</td>
<td>0.53 ± 0.49</td>
<td>0.00–2.30</td>
<td>0.68</td>
<td>23</td>
</tr>
<tr>
<td>SCE</td>
<td>5.83 ± 2.07</td>
<td>2.30–11.92</td>
<td>7.15</td>
<td>23</td>
</tr>
</tbody>
</table>

Table 2 Mean and upper 25th percentile of baseline SCB, BIB and SCE frequencies among 105 HD patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>Univariate</th>
<th>Multivariatea</th>
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<tbody>
<tr>
<td>SCB</td>
<td>Coef P</td>
<td>Coef P</td>
</tr>
<tr>
<td>Stage (III, IV versus I, II)</td>
<td>0.02 0.01</td>
<td>0.02 0.01</td>
</tr>
<tr>
<td>Alcohol</td>
<td>Previous versus never</td>
<td>0.02 0.03</td>
</tr>
<tr>
<td></td>
<td>Current versus never</td>
<td>0.02 0.01</td>
</tr>
<tr>
<td>Smoking</td>
<td>Previous versus never</td>
<td>0.01 0.04</td>
</tr>
<tr>
<td></td>
<td>Current versus never</td>
<td>0.01 0.04</td>
</tr>
</tbody>
</table>

Table 3 Univariate and multivariate regression coefficients for chromosome breaks and SCEs

a Adjusted for age, sex, and race.

Regression coefficient.

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