High Frequency of Micronuclei in Peripheral Blood Lymphocytes as Index of Susceptibility to Pleural Malignant Mesothelioma

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Abstract

We evaluated the frequency of micronuclei (MN) in peripheral blood lymphocytes of patients with pleural malignant mesothelioma (MM), lung cancer, benign respiratory diseases, and healthy controls. A significant increased frequency of MN was observed in patients with MM in comparison with all the other groups (median, 11.4 binucleated MN/1000 binucleated cells versus 5.1, 6.1, and 6.2, respectively). No association was found between MN and asbestos exposure. Recently, genetic susceptibility associated with asbestos exposure has been recognized in the development of MM. The presence of high frequency of MN in peripheral blood lymphocytes of patients with MM could represent a useful index of individual susceptibility to this tumor.

Introduction

Pleural MM, a relatively rare neoplasm, is increasing in incidence in most countries (1). The main determinant of MM is asbestos exposure; nevertheless, the interaction between environmental factors and genetic susceptibility might play a crucial role in the etiology of this neoplasm (2). Heritable differences in host resistance to genetic changes may be identified at different phases of the carcinogenic process, i.e., DNA repair capacity, chromosome stability, and so forth. MM is associated with a high number of genetic alterations. It is unclear if these alterations are caused by asbestos, SV40, and other carcinogens or whether they reflect an intrinsic predisposition of the cells of these individuals to accumulate genetic damage. No data are available about cytogenetic damage in PBLs of patients affected by MM. Cytogenic damage, measured as chromosomal aberrations in PBLs, is a reliable biomarker for human cancer risk independently of the exposure to carcinogens (3). Micronucleus test in PBLs seems to be a useful method for monitoring individuals with genetic instability (4). Recent evidence suggests the usefulness of micronucleus test as a screening test for carriers of specific mutations in evaluating cancer susceptibility (5). This study was carried out to evaluate the MN frequency in PBLs of patients with pleural MM with respect to LC and two control groups in order to ascertain the relevance of this biomarker to express the susceptibility of individuals to develop pleuropulmonary tumors.

Materials and Methods

Subjects in this study were enrolled from March 1996 to August 2000 in three areas in northern Italy (Genova, Casale Monferrato, and La Spezia), characterized by asbestos-associated industrial activities. We included 21 patients (17 males and 4 females) with MM, 37 patients (35 males and 2 females) with LC, 33 patients (28 males and 5 females) with BRDs (mostly chronic obstructive pulmonary disease, asbestosis, and silicosis) as at-risk controls, and 62 healthy controls (44 males and 18 females). Peripheral blood samples were collected from controls and patients in heparinized vacutainers. Neoplastic patients were incident consecutive cases, with no previous chemotherapeutic and radiotherapeutic treatment. BRD patients were subjects admitted in the same hospitals of neoplastic patients, whereas the blood samples of HCs were recruited from a group of blood donors and from nonneoplastic patients admitted in ophthalmology departments. Informed consent was obtained from all subjects. Information was collected on smoking and lifestyle habits, occupational and environmental exposure, and clinical notes. The modified cytokinesis-blocked method of Fenech and Morley (6) was used to determine MN frequency. Analysis was performed blindly of subjects’ status only on lymphocytes with preserved cytoplasm. An average of 2000 cells were analyzed for each subject. All reported MN counts are the mean of duplicate determinations. Means, medians, and SDs were calculated in terms of BN cells with MN (BN-MN)/1000 BN cells. Nonparametric tests were used to check the differences among or between the groups. Relationships between categorical variables and correlations of MN with other variables were examined by means of the χ² test and the Spearman correlation method.

Results and Discussion

Table 1 reports MN frequency in all groups. A significantly higher median frequency was recorded for MM patients: 11.4 BN-MN/1000 BN cells in comparison with LC (5.1BN-MN/1000 BN; P < 0.0001); BRD (6.2 BN-MN/1000 BN; P = 0.002); and HC (6.2 BN-MN/1000 BN; P < 0.0001). No significant difference in MN frequency was found between LC or MM histological types or LC stages. No association was observed between MN frequency and sex, age, smoking status, cigarette pack/year, or exposure, nor duration of exposure (data not shown) to asbestos in any group (Table 2). Two MM patients (females, ages 63 and 68 years, respectively) did not report any

<table>
<thead>
<tr>
<th>Table 1</th>
<th>BN cells with MN in study subjects</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td>MM</td>
<td>21</td>
</tr>
<tr>
<td>Epithelioid</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Biphasic</td>
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</tr>
<tr>
<td>NOSb</td>
<td>5</td>
</tr>
<tr>
<td>LC</td>
<td>37</td>
</tr>
<tr>
<td>Small cell</td>
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</tr>
<tr>
<td>Adenocarcinoma</td>
<td>10</td>
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<td>18</td>
</tr>
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<td>NSCLC</td>
<td>4</td>
</tr>
<tr>
<td>NOS</td>
<td>3</td>
</tr>
<tr>
<td>BRDs</td>
<td>33</td>
</tr>
<tr>
<td>HCb</td>
<td>62</td>
</tr>
</tbody>
</table>

a NOS, not otherwise specified; NSCLC, non-small cell lung cancer.

b Differences between HCs and the other groups did not change when considering only subjects (24 males and 5 females) matched to MM by age and sex (mean MN: 6.3 ± 2.7; median, 5.8) MM/LC: P < 0.0001; MM/BRD: P = 0.002; MM/HC: P < 0.0001.

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The abbreviations used are: MM, malignant mesothelioma; PBL, peripheral blood lymphocyte; LC, lung cancer; BRD, benign respiratory disease; HC, healthy control; MN, micronuclei; BN, binucleated.
asbestos exposure. MN frequency in these subjects was 13.6 and 14.5 BN-MN/1000 BN, respectively. Also the third (out of four) female affected by mesothelioma despite a low level of exposure to asbestos fibers, as wife of a dockyard worker, showed a very high frequency of MN (21.4 BN-MN/1000). The fourth female with a low number of MN (4.3 BN-MN/1000) is already alive 6 years since the diagnosis of a tubular-papillary plural mesothelioma.

In conclusion, a significant increased MN frequency in PBLs was observed only in patients with MM in comparison with all other groups. Neither difference was observed between LC patients and HCs, nor among the different types of BRD subjects. Asbestos exposure was not associated with high frequency of MN. DNA alterations and chromosomal damage have been described in asbestos-treated mammalian cells (7). Numerical and structural chromosomal aberrations and an increase in MN frequency were also reported in human cells in a number of studies (8–10). PBLs are not the direct target for asbestos fibers, and an increase in MN frequency in this surrogate tissue supplies an index of the accumulated genetic damage occurring during life span.

Intriguingly, MN induction in PBLs, recently, has been indicated as a biomarker for cancer predisposition in breast cancer families (11, 12). Because ~20% of MM cases occur in individuals without asbestos exposure (13) and only a small percentage of exposed individuals develop the disease, factors other than asbestos are likely to play a crucial role in tumor development.

The presence of two cases with no clear exposure to asbestos in combination with high frequency of MN strengthens the role of individual susceptibility in determining the risk of MM. Moreover, reports of familial aggregation of MM (2) attest to the relevance of genetic susceptibility in this neoplasm. Our findings show that asbestos exposure does not determine per se genetic instability leading to MN formation and support our hypothesis that the increase of micronucleated PBLs in MM patients is consistent with the possibility of genetic factors predisposing to the development of MM.

Alternatively, the disease itself may somehow play a role in determining the high frequency of MN in PBLs of MM patients. However, the low frequency of MN in PBL of patients with BRDs and with LC (Table 2) and the lack of a relationship between MN in PBLs and disease progression (see MM survival in Table 2) strengthen the hypothesis that the high frequency of MN is a predisposing factor.

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References

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