Some Observations on Spontaneous Sister Chromatid Exchange Frequencies and Cell Cycle Progression in Stimulated Lymphocytes of Patients With Different Malignancies

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ABSTRACT Total 23 patients with different malignancies viz. Ca. Lung (5), Ca. Uterine & Cervix (5), Ca. Head & Neck (5), Sarcomas (5) and Malignant Melanoma (3); were studied for spontaneous sister chromatid exchange frequencies (SCE) as well as cell cycle progression. All blood samples were collected prior to chemotherapy and/or radiotherapy to exclude the influence of these therapies, if any, on SCEs. Totally 15 healthy, age and sex matched individuals and belonging to the same socio-economic status, but no direct relatives of the patients were studied simultaneously as controls. The SCE rates, when compared to controls (4.00 ± 0.39) were found to be significantly high for patients with Ca. lung (9.42 ± 1.20), malignant melanoma (8.14 ± 0.21), Ca. head & neck (6.85 ± 0.89) as well as sarcomas (6.29 ± 0.79). However, no detectable difference was observed in the SCE rate for patients with Ca. uterine & cervix (5.02 ± 0.88). Cell cycle proliferation and thereby replicative index was significantly elevated in patients with carcinoma of head & neck as well as malignant melanoma. On the other hand, rest of the patients showed no much variation in cell cycle progression when compared to controls.

INTRODUCTION

The analysis of sister chromatid exchanges (SCEs) is a sensitive tool for evaluating DNA lesions of the kind that may lead to cancer development. Although the molecular mechanisms responsible for the production of SCEs are not still fully understood, yet SCE tests have been well accepted for studying carcinogenic and/or mutagenic potentials of chemical as well as physical agents (Privitera et al.1985). In human neoplasms SCE study has been used for various purposes and it may indicate a promising future for treatment monitoring (Sandberg 1980; Ohtsuru et al. 1980, 1982).

Retrospective and prospective reports on spontaneous SCE rates in lymphocytes of patients with different malignancies have so far yielded conflicting results. Few reports (Lukovje and Milson 1992; Xu 1993; Dhillon at al. 1996) have indicated higher spontaneous SCE rates in lymphocytes of cancer patients; while some (Brown et al. 1985; Adhvaryu et al. 1988; Nohutcu et al. 1991; Popp et al. 1994; Murthy et al. 1997) have indicated no changes in lymphocytic SCE rates for different cancer patients when compared to healthy controls. The results so far reported are therefore controversial.

Chromosome instability has long been suspected as a factor associated with neoplastic disorders. A relatively simple yet sensitive test that measures chromosome/DNA damage, is the quantitative analysis of SCE. This may signal changes associated with the pathologies of malignancy. The main aim of this study was therefore to determine whether peripheral lymphocytic SCE analysis of cancer patients has some potential to be of any value with respect to risk assessment, early detection and thereby possible prevention of cancer.

MATERIALS AND METHODS

Peripheral venous blood was collected aseptically in sodium heparinised vaccutainers from 15 controls and 23 patients with different malignancies viz. Ca. Lung (5), Ca. Uterine & Cervix (5), Ca. Head & Neck (5), Sarcomas (5) and Malignant Melanoma (3). These patients had not undergone any kind of chemotherapy and/or radiotherapy prior to collection of blood. Within 2-3 hrs of blood collection, the cultures were set up by adding 0.6 ml of blood to 6 ml HAM's F-10 medium (Sigma, USA), with 20% autologus serum and 2% reconstituted Phytohemagglutinin (PHA-M, Welcome Lab, UK). At 24 hr of initiation of culture, 10 µg/ml of Bromodeoxyuridine (5-BrdU,
Sigma, USA) was added. The cultures were protected from light to avoid BrdU degradation for all 72 hrs of incubation. Colcemid (Serva, Germany) was added at a final concentration of 2 mg/ml during last 2 hrs of incubation, to stop mitosis.

Harvesting of the cultures was done by routine protocols of hypotonic treatment (0.075 M KCl) and washes of freshly prepared fixative 3:1 methanol-acetic acid. The slides were prepared by conventional air-dried method.

Sister chromatid differential staining was performed using routine procedure of Perry and Wolff (1974). Coverslips were mounted on slides stained in Hoechst 33258 (30 minutes at room temperature in darkness) and then exposed to fluorescence light for 24hrs. The slides were then incubated in 2 X SSC (Standard Sodium Citrate) at 60°C for an hour. Finally they were stained with 3% Giemsa (Qualigens, India).

Total of 100 well spread metaphases with clearly visible chromosomes were scored for each individual (control as well as cancer patient). In order to measure SCEs, we have examined a minimum of 30 second division (M2) metaphases with least overlapping chromosomes.

Based on the differential staining categories, the number of cells in first division (M1), second division (M2) and the third division (M3) stages were counted for cell cycle progression study. The replicative index (RI) was calculated employing following formula:

\[
RI = \left( \frac{1 \times \% M_1 + 2 \times \% M_2 + 3 \times \% M_3}{100} \right)
\]

For statistical analysis, two tailed Student's 't' test was employed and a P value of 5% or less was considered statistically significant as per the standard scientific practice.

RESULTS AND DISCUSSION

In the present study we have tried to analyze the different malignancies for their spontaneous baseline SCE frequency rates, as well as cell cycle progression. Mean SCE/cell and RI for controls as well as various cancer patients have been presented in Table 1. The relative frequency in percentage of cells in M1, M2 and M3 division stages among various cancer patients and controls are shown in Figure 1. Sister chromatid exchanges among controls and cancer patients have been depicted in Figure 2, a and b, respectively.

Total of 15 healthy individuals who comprised the control study group had no history of familial malignancy and were not direct relatives, but belonged to the same socio-economic status of the cancer patients. The mean SCE level in them was found to be 4.00 ± 0.39, which served as a control guide-line for the malignant patients. Their RI was found to be 1.92. This background frequency of SCEs match very well with those reported for controls in our other studies (Gadhia and Joseph 1997; Joseph and Gadhia 2000).

Five patients with the carcinoma of lung (in the initial stage of disease) were selected for SCE examination. Significant increase (P<0.001) in mean SCE was found in these patients as compared to controls. However, they showed no significant difference in the RI.

The mean SCE/cell was 6.85 ± 0.89 for cancer of head & neck patients which stood significantly high (P<0.05) as compared to controls. At the same time, RI was significantly increased among the patients with this malignancy for the number of M1 cells was decreased while that of M3 was increased as compared to controls (Fig. 1). Our results are in agreement with those of Nohutcu et al. (1991) and Murthy et al. (1997) who have shown higher SCE rates in patients with carcinoma of head & neck. Similar results have been suggested by Shankarnarayan et al. (1989). However, earlier Bazopoulou-Kyrkanidou et al. (1986) reported

<table>
<thead>
<tr>
<th></th>
<th>No. of samples</th>
<th>No. of cells scored</th>
<th>Percentage of cells</th>
<th>Mean RI</th>
<th>Mean SCE/cell ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>M1</td>
<td>M2</td>
<td>M3</td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
<td>1785</td>
<td>39</td>
<td>30</td>
<td>31</td>
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<td>Ca. Lung</td>
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<td>580</td>
<td>44</td>
<td>27</td>
<td>29</td>
</tr>
<tr>
<td>Ca. Uterine &amp; Cervix</td>
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<td>635</td>
<td>36</td>
<td>29</td>
<td>35</td>
</tr>
<tr>
<td>Ca. Head &amp; Neck</td>
<td>5</td>
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<td>31</td>
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<tr>
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<td>5</td>
<td>584</td>
<td>43</td>
<td>28</td>
<td>29</td>
</tr>
<tr>
<td>Malignant Melanoma</td>
<td>3</td>
<td>370</td>
<td>30</td>
<td>26</td>
<td>44</td>
</tr>
</tbody>
</table>

* - Significant at (P<0.05) from the control  † - Significant at (P<0.01) from the control  ‡ - Significant at (P<0.001) from the control
normal baseline SCE levels in the patients with this malignancy. One of the unusual findings of the present study was for 5 females with carcinoma of uterine & cervix, who showed normal SCE rates. Further in them RI was also normal with no remarkable difference. Majority of the results regarding baseline SCEs in Ca. uterine & cervix are inconsistent. Xu (1993), Lukovic and Milsain (1992) and Dhillon et al. (1996) have reported increased SCE frequencies for this malignancy. While, Adhvaryu et al. (1985) have found normal baseline SCE in patients with Ca. uterine & cervix as compared to controls. Similarly, our results also

![Cell cycle proliferation in cancer patients](image1.png)

**Fig. 1.** Cell cycle proliferation in cancer patients

![Sister chromatid exchanges from control](image2.png)

**Fig. 2(a).** Sister chromatid exchanges from control (X 7500).
failed to show higher baseline SCEs in patients with Ca. uterine & cervix.

As far as we are aware, there is paucity of information available for baseline SCE in sarcoma patients. We found significantly higher (P<0.05) SCE rates among sarcoma patients (3 Osteosarcoma and 2 rhabdomyosarcoma) as compared to controls. Still RI in these patients was nearly normal.

Finally in the present study, 3 patients with malignant melanoma also revealed significantly higher (P<0.01) SCE rates. They also exhibited high RI.

Overall, results of the present study indicate higher spontaneous SCE rates for all malignancies studied except for carcinoma of uterine & cervix; in comparison to controls. With regards to RI study patients with carcinomas of head & neck and malignant melanoma showed increased RI, while the rest showed normal to near normal results.

CONCLUSION

Thus, looking to overall results it seems logical that SCE study has some significance while RI study is of little importance in identifying an individual’s predisposal to malignancy. However, no totalitarian consideration be attributed to SCE in cancer diagnosis.

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