EVALUATION OF NUCLEOLAR ORGANIZER REGIONS IN NON-HODGKIN'S LYMPHOMA

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INTRODUCTION
For accurate histopathological diagnosis of non-Hodgkin's lymphoma (NHL), recognition of nuclear details must be taken in account, (Lennert, 1985). These include characteristic nuclear profiles, distribution of chromatin and nucleoli, (Crocker and Nar, 1987). However, these features may not be clearly demonstrated in heamatoxylen and Eosin preparation, (Crocker et al., 1988). Moreover nucleoli themselves are often obscured by nuclear chromatin, (Stansfeld, 1985).

Recently nucleolar organizer regions (NORs) have attracted much attention as numerous papers have been published in the pathology literature which validate the usefulness of the enumeration of NORs in nuclei of certain neoplasms, (Ploton et al., 1986 and Crocker and Shilbeck, 1987).

NORs are loops of DNA which are present in the nucleoli of cells and which possess ribosomal RNA genes and thus involved in the synthesis of ribosomes. (Troster et al., 1985). Accordingly, these regions are of central importance in regulation of protein synthesis by the cells. Therefore they can be considered as a marker for the protein synthesis and thus the proliferation rate of a given cell that may be used as diagnostic and/or prognostic indices, (Ruschoff et al., 1989). NORs are readily visualized by means of Agryrophil silver staining technique that stain NORs - associated proteins and

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thus, the demonstrated structures are termed Ag-NORs, (Egan and Crocker, 1988).

The present study was, therefore, undertaken to assess the diagnostic value of Ag-NORs staining - a technique novel in histopathology - in NHL with special reference to evaluation of its applicability in relation to grading and prognosis.

**MATERIAL AND METHODS**

46 lymph node specimens of NHL from the same number of patients were included in the present study. The specimens had been fixed in 10% formalin and processed to paraffin wax. Sections were cut at 3 μm on glass slides, they were dewaxed in xylene, rehydrated through ethanol to deionized water. The sections were stained by:

1. Haematoxylen and Eosin for histopathological typing of NHL according to the working formulation classification.

2. Argyrophil silver staining technique for analysis of NORs. The staining procedure was essentially as described by (Crocker and Nar, 1987); Crooker et al., 1988 and Howat et al., 1988).

An attempt was made to quantify the numbers of Ag-NORs by counting individually discernable and separable black dots in each nucleus, Fields were selected at random and 200 cells were examined using a mechanical stage to prevent duplicate counting. The mean number of Ag-NORs per cell was then calculated and student t-test was applied to the numerical values obtained.

In addition to the specimens of NHL, another 5 lymph nodes showing follicular hyperplasia were examined and the interfollicular lymphocytes were served as control cells.

The informations obtained from Ag-NORs count in NHL specimens were correlated with those concerning the histopathological grading and survival of NHL patients.
RESULTS

(1) Histopathological Findings:

The lymph node specimens of NHL comprised the following types (According to the working formulation classification), table (1):

1. Low grade NHL: These included 12 cases. The saese were formed of 7 small lymphocytic, 2 follicular small cleaved, 3 follicular small and large cell lymphomas.

2. Intermediate grade NHL: This comprised 26 cases. They were classified into 3 follicular large cells, 4 diffuse small cleaved, 3 diffuse mixed small and large cells and 16 diffuse large cell lymphomas. The latter included 10 large cleaved and 6 large non-cleaved varieties.

3. High grade NHL: This included 2 immunoblastic and 6 Burkitt's lymphomas.

(2) NORs Count:

* Reactive hyperplasia of lymph nodes:

Nearly all cell types common to lymph node have the same NORs number ranging from 1 - 1.3 (mean 1.1 + 0.22) with the exception of the cells of the germinal center (transformed lymphocytes) which show the mean number of 2.2.

Non-Hodgkin’s Lymphoma:

The number of NORs was increased parallel with the increasing grade of malignancy. As shown in table (1), the high grade malignancy NHL possessed significantly more Ag-NORs than did the intermediate which possessed in turn significantly high Ag-NORs count than did the low grade tumours, (P<0.001) and all specimens had significantly high Ag NORs than the control cells (P<0.001). In the low grade group the range of Ag NORs count per cell was 1.6 - 2.8 (mean 2.13 ± 0.38) and 2.7 -
4.7 (mean 4.05 + 0.68) for the intermediate, while it was 4.9 - 5.9 (mean 5.03 + 0.80) for the high grade lymphomas.

The number of Ag NORs were significantly higher in patients living less than 2 years than in patients living more than 2 years in all grades of NHL (table 2).

DISCUSSION

Since nuclear detail is an important consideration in the classification of lymphomas Lennert, 1985), any technique which might permit study of this nuclear detail is assissed. The Ag NORs, a technique which has been largely used on chromosomal preparation to study the chromosomes in genetic disorders, (Giri et al., 1989), has only recently applied to routine paraffin sections. In this respect, there are reports showing that it distinguishes benign from malignant melanocytic lesions, (Crocker and Shilbeck, 1987) and hyperplastic glands from malignant ones, (Ploton et al., 1986). Even, in reports that validate partial usefulness of the Ag NORs in such distinction, (Giri et al., 1988 and Giri et al., 1989), they conclude that the method is a reliable discriminant for malignancy. In the present study we have investigated further the diagnostic usefulness of the Ag NORs technique in the distinction of the different grades of malignancy in NAL, and its prognostic impact on survival.

Our results demonstrated that in reactive lymph nodes, most component cells - with the exception of the follicular center cells - possessed approximately one Ag NORs per nucleus. The follicular center cells are transformed lymphocytes and they showed significantly high Ag-NORs count. This finding agree that observed by Wachtler et al., (1986) who used phytohaemagglutinin stimulation on peripheral blood lymphocytes and revealed multiple dispersed Ag-NORs in the transformed cells, while they are one per nucleus in the unstimulated cells. A suitable explanation is that in a resting or relatively inactive cells the acrocentric chromosomes bearing Ag-NORs orientate in close apposition to each other to form a central, smoothly
outlined nucleolus, (Crocker and Nar, 1987), on the other hand, the increased Ag-NORs count in both follicular center cells and the in vitro transformed lymphocytes reflects the high mitotic potential of these cells. (Wachtler et al., 1986).

As regard to the lymph node specimens with NHL, it must be stressed that in the present study there is well-defined differences between low, intermediate and high grade malignancy, this finding is in agreement with that is observed by Crocker and Nar (1987) and Crocker et al. (1988) who reported - in a study performed on NHL diagnosed according to kiel classification - that there is a statistically significant difference between low grade and high grade lymphomas. Several possible explanations for the increased numbers of Ag-NORs in NHL were put forward. These included increased number of NORs bearing chromosomes, (Crooker et al., 1988 and Underwood and Giri, 1988) and increased cell proliferation with increased transcriptionally active sites, (Hall et al., 1988). the present study clarified that the Ag-NORs count was steadily increased parallel to the increased grade of malignancy. This finding is logic as the previous explanations given for the increased Ag-NORs in NHL are supposed to be more evident with increasing grades.

The prognostic significance of Ag-NORs in malignancies are controversial. While Egan et al., (1988) proved a correlation between Ag-NORs and survival of patient with neuroblastomas, Howat et al., (1988) observed that Ag-NORs are of no prognostic value in patients with cutaneous melanoma. No reports directly studied the prognostic significance of Ag-NORs in NHL in the available literature. On the basis of the informations of the present study, there is a trend of increased survival with progressively less Ag NORs count confirming the finding reported by Egan et al. (1988), while contradict that observed by Howat et al. (1988).

From the above mentioned data we concluded that the present study documented the considerable value of
the AgNORs count for both grading and prognosis in NHL. Therefore, it is suggested that this method previously localised to the province of the cytogeneticist, should find widespread applications in the field of tumour histopathology especially it is easy and non-expensive method.

**SUMMARY**

46 lymph nodes specimens of N.H.L were included in the present study.

Paraffin processed sections were prepared and stained with the followings:

1. Hx. & E. for histopathological typing of N.H.L. according to the working formulation classification.

2. Argyrophil silver staining for analysis of NORs.

Our results demonstrated that there is well defined differences between low, intermediate and high grade lymphomas on the basis of NORs count of their cells. Also NORs count increased in patient living less than 2 years.

Our findings documented the considerable value of the Ag-NORs count for both grading and prognosis in NHL.
Table (1): The range of Ag-NORs with the Mean count and standard deviation (S. D.) in different types and grades of NHL.

<table>
<thead>
<tr>
<th>Grade and type</th>
<th>Number of Cases</th>
<th>Ag - NORs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean ± S. D.</td>
</tr>
<tr>
<td>Low grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- S. L.</td>
<td>12</td>
<td>1.6 - 2.8</td>
</tr>
<tr>
<td>- F. S. C.</td>
<td>7</td>
<td>1.6 - 2.7</td>
</tr>
<tr>
<td>- F. S. C &amp; large cells</td>
<td>2</td>
<td>1.85 - 2.5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.9 - 2.8</td>
</tr>
<tr>
<td>Intermediate grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- F. L. cells</td>
<td>25</td>
<td>2.7 - 4.7</td>
</tr>
<tr>
<td>- Diffuse S. C.</td>
<td>3</td>
<td>2.7 - 3.6</td>
</tr>
<tr>
<td>- Diffuse mixed small</td>
<td>4</td>
<td>3.3 - 4.4</td>
</tr>
<tr>
<td>&amp; large cells</td>
<td>3</td>
<td>3.2 - 4.7</td>
</tr>
<tr>
<td>- Diffuse large cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a - L. C.</td>
<td>10</td>
<td>3.2 - 5.3</td>
</tr>
<tr>
<td>b - L. non C.</td>
<td>6</td>
<td>3.9 - 5</td>
</tr>
<tr>
<td>High grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Immunoblastic</td>
<td>8</td>
<td>4.9 - 5.9</td>
</tr>
<tr>
<td>- Burkitt's</td>
<td>2</td>
<td>4.9 - 5.7</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>3.8 - 5.9</td>
</tr>
</tbody>
</table>

Control cells contained mean Nors Count of 1.1 ± 0.22 P < 0.001
S. L.: Small lymphocytic
F. S. C.: Follicular small cleaved
F. L.: Follicular large
L. C.: Large cleaved
L. non C.: Large non cleaved
S. C.: Small cleaved

Table (2): Overall survival rate in relation to Ag-NORs in NHL patients.

<table>
<thead>
<tr>
<th>NHL Grade</th>
<th>Total</th>
<th>&lt;2 Years</th>
<th>&gt;2 Years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO. Of Cases</td>
<td>%</td>
<td>NORs ± S. D.</td>
</tr>
<tr>
<td>Low grade</td>
<td>12</td>
<td>4</td>
<td>33.3</td>
</tr>
<tr>
<td>Intermediate grade</td>
<td>26</td>
<td>14</td>
<td>53.8</td>
</tr>
<tr>
<td>High grade</td>
<td>8</td>
<td>7</td>
<td>87.5</td>
</tr>
</tbody>
</table>
**Fig. 1:** Interfollicular lymphocytes in hyperplastic lymph node (control cells) showing 1-2 Ag-NORs/nucleus (X 40).

**Fig. 2:** The same previous section but with high magnification (X 100).

**Fig. 3:** Small lymphocytic lymphoma with low Ag-NORs count (X 100).

**Fig. 4:** Lymphocytic large non-cleaved lymphoma with relatively high Ag-NORs count (X 100).

**Fig. 5:** Buirkitts lymphoma showing the highest Ag-NORs count (X 100).
REFERENCES


تقييم أهمية المناطق المنظمة لبناء البروتينات والموجودة في نوتيات خلايا الليفوما غير الهودجكين.

أجرى هذا البحث على عينة غدة ليسمارية من المرضى المصابة بالليفوما غير الهودجكين بالإضافة إلى 5 حالات من الفرد النشطة.

وقد تم تجهيز شرائح بطريقة البرافين وتم صيغتها كالآتي:

1- هيماتوكسيلين وأيوسين لتشخيص نوع الليفوما غير الهودجكين وبيان درجة التباين النوعي فيه.
2- صبغة الفضه الغروي (الأرجيروفل) لدراسة المناطق المنظمة لبناء البروتينات والموجودة في نوتيات خلايا الليفوما غير الهودجكين.

ومع ذلك يهدف تقييم أهمية هذه المناطق إلى تشخيص أنواع مختلفة للليفوما غير الهودجكين وكذلك علاقتها بدرجة التباين النوعي وأيضاً التنبؤ بمستقبل المرض.

وقد نروقت النتائج وثبت أن دراسة المناطق المنظمة لبناء البروتينات والموجودة في نوتيات الخلايا يمكن بها التفرقة بين درجات التباين النوعي في الليفوما غير الهودجكين وكذلك وجد أن لها دلالة بالنسبة لعمر المرضى حيث ترتفع فترات الحياة تقل كلما زاد متوسط عدد هذه المناطق بالتعرض.

ومن هذا يمكننا أن دراسة هذه المناطق في حالات الليفوما غير الهودجكين لها فائدة مزدوجة حيث أنها تساعد في التشخيص أو أيضاً في التنبؤ بمستقبل المرض.

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