Agnor Study in Gastro Intestinal Lymphoma

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Abstract: To apply Haematoxylin and Eosin stain and grading the gastrointestinal lymphomas, To assess the applicability of AGNOR stain in gastrointestinal lymphomas and for discrimination of high and low grade lymphoma. 21 cases from Institute of Pathology and Electron microscopy, Madras Medical College and Institute of Child Health, Egmore, Chennai were studied. These cases were diagnosed and sub typed with the help of H and E stain. For AgNOR staining, tissues fixed in 10% formalin processed to paraffin wax were cut at three microns thickness and stained. In our study 7 cases out of 21 were NHL-low grade and the mean AgNOR value is 14.6. Remaining 14 cases show high grade lymphoma and their mean AgNOR value is 55.5. In our study 3.3 is used as cut of point to discriminate between low and high grade lymphomas. Being a simple technique, easy to perform, AgNOR can be used as a routine stain for discriminating high and low grade lymphoma.

Key words: Gastro intestinal lymphoma • H and E • AgNOR stain • High and low grade NHL

INTRODUCTION

Nucleolar Organizer Regions (NORS) are chromosomal segments in which ribosomal RNA is encoded. During mitosis, ribosomal genes are associated with a set of silver stained nucleolar proteins, designated as Ag Nucleolar Organizer Region (NOR) proteins. The amount of AgNOR proteins estimated during interface may be used as marker of cell proliferation with a prognostic value for several human cancers [1]. Theoretically 20 AgNORs can be demonstrated in a normal human nucleus, whereas histo pathologists observe not more than 1 or 2 AgNORs in a benign cell, because they are congregating within a relatively small nucleus. But in malignancy they become dispersed throughout the nucleus enabling the histologists to count them readily [2]. Hence the histological AgNOR count does not reflect the real number of AgNORs, but a numerical index of dispersion. AgNOR counts are reported to assist in distinguishing between benign melanocytic lesions from malignant melanomas, reactive mesothelial proliferation from mesothelioma and low grade lymphomas from high grade. Since AgNOR count correlate with ploidy they may assist in grading of neoplasm. NOR numbers appear to reflect cell and nuclear activity. Since the classification of NHL takes nuclear detail into account [3] AgNOR staining will be useful in grading these tumours.

MATERIALS AND METHODS

The study included 21 cases of Gastro intestinal lymphomas previously diagnosed and sub typed with the help of H and E staining technique.

Staining Technique: Tissues fixed in 10% formalin processed to paraffin wax were cut at three microns thickness. These were de waxed in xylene and hydrated.
through alcohol to distilled water. AgNOR staining solutions were prepared by adding one volume of 2% gelatin in 1% formic acid to two volumes of 50% aqueous silver nitrate solution \[3\] and \[4\]. Tissue sections were exposed to freshly prepare working solution for 60 minutes in dark at room temperature. The silver colloid was then washed off with deionised water. The sections were dehydrated through alcohols, cleared in xylene and mounted in DPX mounting medium.

**Counting Procedure:** NORs in silver staining represents as black dots within nucleus, which may remain discrete or in aggregates. Rest of them stain pale yellow. 200 nuclei were assessed in each slide and then mean number of AgNORs per nucleus was determined. The sections were examined using a X100 oil immersion lens \[4\].

**RESULTS**

The mean AgNOR study in low grade lymphoma in our study is 14.6. Among these low grade lymphoma, three cases were in stomach, three in small intestine and one in colon. (Table 2). The mean AgNOR value of high grade lymphoma in our study is 55.5. In our study 3.3 is used as cut of point to discriminate between low and high grade lymphomas using AgNOR study. Regarding the distribution of low and high grade lymphomas in gastro intestinal tract in our study, in small intestine more high grade cases were found out and site wise also 11 cases of both low and high grade lymphomas in small intestine (Table 1). Age wise among 21 cases 7 cases in the age group of 0-10 years, 4 cases in 31-40 years age group, 3 cases each in 21-30 years, 41-50 years and 51-60 years and only one case in 11 – 20 years (Table 3). Sex wise distribution shows 12 cases in male and 9 cases in female.

<table>
<thead>
<tr>
<th>SITE OF LYMPHOMAS</th>
<th>NUMBER OF CASES</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>4</td>
<td>19.04</td>
</tr>
<tr>
<td>Small intestine</td>
<td>11</td>
<td>52.38</td>
</tr>
<tr>
<td>Ileocecal junction</td>
<td>3</td>
<td>14.29</td>
</tr>
<tr>
<td>Colon</td>
<td>3</td>
<td>14.29</td>
</tr>
<tr>
<td>TOTAL</td>
<td>21</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SITE</th>
<th>LOW GRADE</th>
<th>HIGH GRADE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small Intestine</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Stomach</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Ileocecal junction</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Large Intestine</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>TOTAL</td>
<td>7</td>
<td>14</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Jaki *et al.* \[5\] in their studies used application of colloidal siver method for demonstration of argyrophilic protein of nucleolar organizer regions in histological sections of NHL for discrimination of high and low grade NHL. In 1994 Kalir *et al.* \[6\] in their comparative study of NHL, by using AgNOR found that an increase in mean number and area of nucleolar organizers per nucleus in high grade lymphomas compared to low grade lymphomas. Hall *et al.* \[7\] has shown that AgNOR counts correlate with proliferative activity in malignant lymphomas as assessed by Ki 67 scores. According to Keil classification 2.9 NORs per nuclei was the cut off point for discriminating high and low grade lymphomas. In our studies 3.3 is the cut of point between these two grades. In comparison with the study of Jaki D *et al* we observed only minimal difference.

Tondini *et al.* \[8\] in their studies noted that the median age of gastro intestinal lymphomas is 50 years. In our study the median age of occurrence is 40.2 years. Peter and Issacson *et al.* \[9\] in their studies noted that in Middle East, most cases of gastro intestinal lymphomas arise in small intestine followed by stomach. In our study also the small intestine constitutes the most common site followed by stomach (Fig. 1) and in small intestine 52.38% and in stomach 19.04% (Table 1). Herman *et al.* \[10\] in their studies showed in children with definite male predominance occurs only in small intestine and ileocecal NHL. Our studies showed among 7 cases in children, 5 cases of small intestinal NHL showed female predominance and out of 2 ileocecal NHL, both the cases are female.

**CONCLUSIONS**

Being a simple technique, easy to perform AgNOR can be used as routine stain for gastro intestinal lymphomas. The increased number of AgNOR dots is, in many cases, considered to be of diagnostic and prognostic significance in tumor pathology, because of its direct relationship to the frequency of cell proliferation and other requirements for ribosome biogenesis \[11\]. It is easily understandable beyond doubt, that, AgNOR study is extremely useful in grading malignant lymphomas.
We have observed the definite value of AgNOR and its applicability in grading gastro intestinal lymphomas. In our study 33.33% of cases are of low grade and 66.6% are of high grade lymphomas (Fig. 2 and 3). However the Diagnosis of lymphoma has to be backed up by selection of immunological, molecular and cyto genetic research[12].

REFERENCES