Application of AgNOR (Argyrophilic Nucleolar Organizer Regions) Staining in Distinction of Non Neoplastic and Neoplastic Endometrial Lesions

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ABSTRACT
Study of AgNOR has been identified as a reliable indicator of cell proliferation and in turn, allows a clear distinction between benign, premalignant, and malignant epithelial changes. The aim of this study was to analyze and evaluate the application of AgNOR method as diagnostic tool in non-neoplastic and neoplastic lesions of endometrium. The study included evaluation of AgNOR staining on a total of 50 cases of endometrial specimens obtained by hysterectomy and dilatation and curettage sent for histopathology. Paraffin blocks of the above selected cases were taken for hematoxylin and eosin, and AgNOR staining. Out of total cases of endometrial specimens studied, sixteen cases were normal proliferative and secretory endometrium served as control. Twenty nine cases were non-neoplastic lesions and five cases were neoplastic. In present study, 8 cases of proliferative phase endometrium showed mean AgNOR count being 2.35 and 8 cases of secretory endometrium showed mean AgNOR count being 1.55 AgNOR/nucleus. In non-neoplastic lesion group, simple hyperplasia, complex hyperplasia and complex hyperplasia with atypia showed mean AgNOR count being 3.41, 3.71 and 4.3 AgNOR/nucleus respectively. In neoplastic lesions benign endometrial polyp showed mean AgNOR count 3.48 AgNOR/nucleus, whereas cases of endometrial carcinoma showed mean AgNOR count 5.62 AgNOR/nucleus. ANOVA t test was done and all the values were found to be statistically significant (p value< 0.0001). Present study suggests that AgNOR counts are reliable markers of endometrial proliferation and allow a clear distinction between non-neoplastic and neoplastic lesions of endometrium.

KEY WORDS: AgNOR, endometrium, histopathology.

INTRODUCTION:
The Nucleolar Organiser Regions (AgNOR) are loops of DNA projecting into the nucleoli of interphase nuclei.[1] AgNORs are chromosomal segments encoding for ribosomal ribonucleic acid, located on five acrocentric chromosomes numerically 13,14,15,21 and 22. These nucleolar regions are associated with acidic nonhistone proteins, which are argyrophilic.[2] Nucleolar organizer Regions (AgNORs) are situated within the nucleolus of a cell. The proteins are selectively stained by the silver colloid technique that is known as the AgNOR technique. AgNOR stain can be visualized as a black dot under the optical microscope.[3] The study of AgNOR has enjoyed a vogue in diagnostic tumour pathology as increased number of AgNOR correlates with increased cellular proliferation.[4] The principal advantage of the AgNOR technique is its relative simplicity of the staining method and the ease of its application to archival tissue. Disadvantages include the time consuming and tedious counting method of the little dots, often associated with the usual observer error.[5]

Endometrium is an important target for hormonal stimulation and affected by a variety of disease processes including endometritis, hyperplasias and carcinomas. Present study is undertaken to assess and evaluate the efficacy of AgNOR method for

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applying it as a simple, diagnostic tool to distinguish the non neoplastic and neoplastic lesions of endometrium. At will support the histopathological diagnosis of various non-neoplastic and neoplastic lesions of endometrium by AgNOR staining and also to observe the sensitivity of this technique in differentiating between grey zone lesions.

MATERIALS AND METHODS:

The study was performed on 50 uterine endometrial specimens in the age group 20-60yrs. We took 16 cases of normal, 22 cases of non neoplastic and 12 cases of neoplastic endometrium. Specimen of dilatation and curettage and hysterectomy were received in 10 percent formalin as fixative and subjected to histopathological processing using paraffin embedding techniques. Paraffin sections of the above selected cases were taken for hematoxylin and eosin staining and reviewed. After confirming and noting the diagnosis and microscopic details, sections were taken for AgNOR staining.

AgNOR STAINING:

Composition of silver colloid developer included Solution A- (Silver nitrate: 50gms, Deionised water: 100ml) and Solution B- (Gelatin: 2gms, Formic acid: 1ml, Deionised water: 100ml). Working solution is Solution A: 2 part and Solution B: 1 part.

Dewax paraffin sections in xylene for 10 minutes. Hydrate through graded alcohols to deionized water for 10 mins. Incubate the sections in freshly prepared working solution which is silver colloidal developer solution, for 30-45 minutes in the dark at room temperature. Give three changes of sodium thiosulphate to reduce the non specific background silver staining and then wash in deionised water. Blot the sections lightly with blotting paper. Dehydrate through ascending grades of alcohol. Clear in xylene and mount in DPX. AgNOR sites are seen as intranuclear black to brownish black dots in pale yellow nuclear background.\(^2\)

Silver-stained slides were examined with the aid of a 1000x magnification with an oil-immersion lens. AgNORs appear as brown or black dots within a yellowish background of nucleus. The quantitations were performed in well preserved cells, excluding areas of tumor necrosis, staining artifacts and overlapped cells. First, the brown /black dots were counted in the nuclei of 100 cells/case and the mean number of dots were taken for each case (mAgNOR).

RESULTS:

The present study included evaluation of AgNOR staining on a total of 50 cases of endometrial specimens obtained by hysterectomy and dilatation and curettage (D and C) sent for histopathological examination to the Department of Pathology. Age wise distribution of cases showed 7 cases in age groups of 20-30 years, 26 cases in the age group of 31-40 years, 15 cases in the age group of 41-50 years and 2 cases in the age group of 51-60 years. Maximum number of cases (52%) were in the age group of 31-40 and least number of cases (4%) having seen in age group 51-60 years.

Out of total cases of endometrial specimens studied, sixteen cases (32%) having normal proliferative and secretory endometrium, served as control. Twenty two cases (44%) were non-neoplastic lesions and twelve cases (24%) were neoplastic. Twenty two cases of non-neoplastic lesions included 14 cases of simple endometrial hyperplasia (28%), 6 cases of complex hyperplasia (12%) and 2 cases of complex hyperplasia with atypia (4%). The neoplastic group of cases included 7 cases of endometrial polyp (14%), and 5 cases of endometrial carcinoma (10%).

In present study 8 cases of proliferative phase endometrium showed a range of 2.2-2.6 AgNOR/nucleus with mean Agnor count being 2.35 and 8 cases of secretory endometrium showed range of 1.2-1.9 AgNOR/nucleus with mean agnor count being 1.55 AgNOR/nucleus.

In non-neoplastic lesion group, simple hyperplasia, complex hyperplasia and complex hyperplasia with atypia showed mean AgNOR count being 3.41, 3.71 and 4.3 AgNOR/nucleus respectively. In neoplastic lesions benign endometrial polyp showed range of AgNOR count 3.6 to 4.4 and mean AgNOR count 3.48 AgNOR/ nucleus where as cases of endometrial carcinoma showed 5.62 mean AgNOR count with the range of 5.2-5.8 AgNOR/nucleus. ANOVA test was done and all the values were found to be statistically significant (p value< 0.0001; Table I).

DISCUSSION:

Quantitative study of AgNOR is valuable in field of histopathology as the size, number and position of AgNOR may provide useful morphometric data over and above morphological criteria. The advantage with the AgNOR applicability is that it can be easily applied over routinely fixed and paraffin wax embedded tissue.
Table 1: AgNOR range and mean AgNOR count of 50 cases.

<table>
<thead>
<tr>
<th>Histopathological Diagnosis</th>
<th>No of Cases</th>
<th>Agnor Count Range</th>
<th>Mean agnor count</th>
<th>Standard Deviation</th>
<th>Anova f Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proliferative</td>
<td>8</td>
<td>2.2-2.6</td>
<td>2.35</td>
<td>.13</td>
<td></td>
</tr>
<tr>
<td>Secretory</td>
<td>8</td>
<td>1.2-1.8</td>
<td>1.55</td>
<td>.2</td>
<td></td>
</tr>
<tr>
<td>Simple hyperplasia without atypia</td>
<td>14</td>
<td>3.1-3.8</td>
<td>3.41</td>
<td>.207</td>
<td></td>
</tr>
<tr>
<td>Complex hyperplasia without atypia</td>
<td>6</td>
<td>3.4-3.9</td>
<td>3.71</td>
<td>.194</td>
<td></td>
</tr>
<tr>
<td>Complex hyperplasia with atypia</td>
<td>2</td>
<td>4.2-4.4</td>
<td>4.3</td>
<td>.1414</td>
<td>242.483</td>
</tr>
<tr>
<td>Endometrial polyp</td>
<td>7</td>
<td>3.6-4.4</td>
<td>3.48</td>
<td>.2636</td>
<td></td>
</tr>
<tr>
<td>Endometrial carcinoma</td>
<td>5</td>
<td>5.2-5.8</td>
<td>5.62</td>
<td>.2489</td>
<td></td>
</tr>
</tbody>
</table>

p< 0.0001

Figure 1: Simple hyperplasia AgNOR stain: low power view and oil immersion view showing AgNOR dots.

Figure 2: Endometrial carcinoma AgNOR stain: low power view and oil immersion view showing AgNOR dots.
blocks. AgNOR has been applied in cytology as well as in histopathology to assess the proliferative activity. It has been applied over thyroid lesions[13] normal bone marrow cells[14], CNS tumors like meningiomas,[15] melanotic and cutaneous tumors,[16] ovary[17] cervix[18] breast tumors[19], bladder cancer[20] resected non small lung cancer[21] and prostate.[22] AgNOR staining has shown to be of great value in differentiating benign from malignant lesions of breast, cervix, oral cavity, skin, soft tissue tumours, lymphomas and melanomas. Niwa K et al[23] Brustmann H et al [24] and Rajani kaushik et al[25] studied 11, 9 and 8 cases of proliferative phase respectively and 10, 5 and 7 cases of secretory phase endometrium. They noted mean AgNOR counts of 3.8, 3.2, and 2.12 in proliferative phase respectively and 2.7, 2.7 and 1.88 in secretory phase respectively with a significant p values. The present study showed mean AgNOR counts of 2.35 in 8 cases of proliferative phase and 1.5 in 8 cases of secretory phase (p < 0.0001) which is in concordance with previous studies with significant differences between proliferative and secretory phase. Our counts were found to be little lower in comparison to other studies. This could be explained as visualisation of AgNORs is subjective and depends on temperature and temporal length of staining reaction.

Studies conducted by Niwa K et al[23] Brustmann H et al[24] and kaushik R et al[25] showed that mean AgNOR counts in endometrial adenocarcinoma were significantly higher than that of complex hyperplasia with atypia, complex hyperplasia without atypia and simple hyperplasia without atypia. (p values < 0.05). Also, mean AgNOR counts in complex hyperplasia with atypia were significantly higher than that of complex hyperplasia without atypia and simple hyperplasia without atypia (p values < 0.05). These findings in all studies concorded well with the present study. Brustmann H et al in1995 got relatively increased number of AgNORs in comparison to our study. Reasons for varying AgNOR counts include different section thickness, different staining procedures and different counting methods. Prolonged fixation also appears to cause the AgNORs to coalesce, thus resulting in a low count.

As mean AgNOR counts were positively correlated with degree of hyperplasia, ranging from simple to complex to atypical hyperplasia, our observations are thus in accordance with the concept that the different degrees of endometrial hyperplasia correlate with increasing proliferative activity. Mean AgNOR count in endometrial carcinoma in study by Brustmann et al[24] was 10, kaushik R et al[25] 5.29 and Niwa K et al[23] was 5.5. The present study shows concordance with previous 3 studies with mean scores of 5.62 in endometrial adenocarcinomas (n=5) and the differences were statistically significant (p <0.0001). Thus, the number of AgNORs tended to increase with the advancement of neoplastic changes.

Despite the limitations, mainly related to section thickness, tediousness of procedure and time consuming, these findings in our study indicate that AgNOR technique can be used as a useful adjunct to routine histopathology to evaluate endometrial lesions as it is simple and useful for evaluation of proliferative activity in human endometrium.

CONCLUSION:

In the present study we concluded that mean AgNOR scores were found to increase steadily from normal to non neoplastic lesions comprising of hyperplasias and maximum in neoplastic lesions. The AgNOR count is found to be an important index for assessment of proliferating cells. AgNOR scores can be used to differentiate between different types non neoplastic and neoplastic endometrial lesions and can be applied in conjunction with routine histopathology as a diagnostic tool.

REFERENCES:

7. Ahmadi SA, Samadi N. Evaluation of argyrophilic nucleolar organizer region staining in predicting the


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