Evaluation of diagnostic value of the nucleolar organizer region comparing reactive cells and transitional cell carcinomas among Sudanese patients

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Abstract:

Background: bladder cancer is one of the commonest malignancies with high morbidity and mortality rates. The number of nucleolar organizer region per nucleus has been proved to correlate with proliferative activity of cells in addition nucleolar organizer region count appears to be of value in aiding the cytopathologic diagnosis in a variety of malignancies.

Objective: the purpose of this study was to evaluation of the nucleolar organizer region as diagnostic value comparing nonmalignant reactive cells and transitional cells carcinomas.

Method: 150 voided urine samples were collected and fixed in 95% alcohol and make smear on glass slide. The samples were stained using the AgNOR method and observed in immersion oil at 100X magnification. finally 10 cells were randomly selected, the AgNOR dots counted and their means recorded and used for data analysis, during the period from December 2015 to June 2016.
Results: in malignant transitional cells the mean AgNOR count was 4.4 and in Reactive transitional cells it was 2.04 and in Normal transitional cells it was 1.8 (sig. <0.000).

Conclusion: strong link between cells proliferation and cellular proliferative activity. Thus the investigated markers AgNOR can be used to comparing between nonmalignant reactive cells and transitional cells carcinomas.

Key words: nucleolar organizer region, AgNOR, NOR Proteins, Reactive cells, Urinary cytology, Transitional cells carcinoma (TCC), bladder cancer.

INTRODUCTION:

Bladder cancer represents a significant health problem, as it is the one of the most common cancers worldwide, bladder cancer is diagnosed in approximately 275,000 people each year, and about 108,000 die of this disease, most common cancer in males but only half as common in females.[2]

While it can occur at any age, even in children, it is rare under the age of 50 years and usually presents in old age, more than 90% of bladder cancers form in the lining of the bladder (the urothelium) and are known as urothelial carcinomas, or transitional cell carcinomas, other types of bladder cancer including squamous cell carcinomas and adenocarcinomas.[2,3] Nucleolar Organizer Regions(NORs) loops of ribosomal DNA on the short arms of five acrocentric chromosomes (chromosomes 13,14,15,21,22) in nucleoli of cells.[13] The agrophil NOR related proteins (RNA Polymerase I,B23protein,C23 protein or nucleolin) encoded by the genes located in the NORs, can be identified as small black dots in the nucleus under a light microscope using the rapid one-step agyrophilic NOR (AgNOR) staining technique the number of AgNORs per nucleus has been proved to correlation with proliferative activity of cells in
addition AgNOR count appears to be of value in the cytopathologic diagnosis in a variety of malignancies.[7,8].

Urinary cytology is useful as the primary screening and surveillance modality for the detection of urothelial neoplasia at most medical centers. Historically, neoplastic urothelial cells were first recognized in the urine in 1864, and confirmed in 1945 by Papanicolaou and Marshall as they confirmed the utility of urinary cytology in the diagnosis of urothelial malignancy. Further to this, there have been many studies supporting the diagnostic yield of this method, with cytohistologic correlation with reported as high as 95% and sensitivity (Positive /suspicious) for low grad neoplasms as high as 76%.[1].

Due to some limitations in the sensitivity and specificity of urinary cytology the silver staining technique that identifies NORs associated proteins was used for examining changes in AgNOR numbers in transitional cell carcinoma of the bladder. This method reveals AgNORs as black dots in the cell nuclei, and the number of AgNORs per cell (AgNORs/cell) has been taught to be related to cellular activation and to be a possible predictor of clinical outcome.[8,9].

PATIENTS AND METHOD:

One hundred and fifty patients were divided in 3 groups, in the first group there 50 patients (13 females, 37 males) having bladder cancer. In the second group there 50 patients (6 females, 44males) suspected with bladder cancer. In the third group there 50 patients (males) which were arrange as a normal control group. Data related to the studied subjects were retrieved from radiation and isotopes center –Khartoum (RICK) and Khartoum teaching hospital of Sudan urine samples were obtained from Sudanese patients during the period from December 2015 to Jun 2016.
Voided urine samples were collected and fixed in 95% alcohol and make smear on glass slide.

Silver staining method for AgNOR: according to Ploton's method [8,11] the final working solution was freshly prepared by mixing one volume of 2% gelatin in 1% formic acid solution and two volumes of 50% aqueous silver nitrate solution. Slides were incubated in darkness with this silver solution for 30 minutes at 45°C. The AgNOR count was established in 10 cells for each cytologic smear. The cells were examined at 100X magnification under immersion oil.

Papanicolaou stain: smear hydrated through 100%, 90%, 70% and D.W

Treated by gill haematoxylin 30 sec and treated by orange G6 1min and treated by Eosin Polychrome 6 min dehydrate clear and mount.

STATISTICAL ANALYSIS:

SPSS Version 22 statistical software calculated was used for statistical analysis. the numerical results (AgNOR counts) were expressed as mean and SD, and the 95% of confidence intervals was used to compare the differences in categorical variables between the groups. Relationships between variables were analyzed using (ANOVA) correlation analysis. A sig. <0.05 was considered statistically significant.

RESULTS:

AgNOR dots were located strictly within the nuclei and were clearly visible as black dots. The mean and SD of AgNOR dots in three groups is shown in table.1 and figures.1.

The men and SD of AgNOR dots per nucleus in transitional cells carcinomas (TCC) group was statistically higher than the nonmalignant reactive cells group and normal
transitional cells group and (ANOVA) correlation analysis in table.2. (sig. <0.00).

Table.1.

Descriptive:

<table>
<thead>
<tr>
<th></th>
<th>Benign transitional cells</th>
<th>nonmalignant, reactive cells</th>
<th>TCC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>49</td>
<td>50</td>
<td>50</td>
<td>149</td>
</tr>
<tr>
<td>Mean</td>
<td>1.84</td>
<td>2.04</td>
<td>4.4</td>
<td>2.77</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>0.373</td>
<td>0.348</td>
<td>1.161</td>
<td>1.377</td>
</tr>
<tr>
<td>Std. Error</td>
<td>0.053</td>
<td>0.049</td>
<td>0.164</td>
<td>0.113</td>
</tr>
<tr>
<td>95% Confidence Interval for Mean</td>
<td>LBound 1.73</td>
<td>1.94</td>
<td>4.07</td>
<td>2.54</td>
</tr>
<tr>
<td></td>
<td>UBound 1.94</td>
<td>2.14</td>
<td>4.73</td>
<td>2.99</td>
</tr>
<tr>
<td>Minimum</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Maximum</td>
<td>2</td>
<td>3</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

Table.2.

ANOVA:

<table>
<thead>
<tr>
<th>Source of Diff</th>
<th>Sum of Squares</th>
<th>Df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>202.165</td>
<td>2</td>
<td>101.082</td>
<td>187.728</td>
<td>0.000</td>
</tr>
<tr>
<td>Within Groups</td>
<td>78.614</td>
<td>146</td>
<td>0.538</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>280.779</td>
<td>148</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Post Hoc Test (Scheffea):

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>N</th>
<th>Subset for alpha = 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Benign transitional cell</td>
<td>49</td>
<td>1.84</td>
</tr>
<tr>
<td>nonmalignant, reactive cell</td>
<td>50</td>
<td>2.04</td>
</tr>
<tr>
<td>TCC</td>
<td>50</td>
<td>4.4</td>
</tr>
</tbody>
</table>

Figure 1.

*Evaluation of diagnostic value of the nucleolar organizer region comparing reactive cells and transitional cell carcinomas among Sudanese patients*

**Figures 2, 3 and 4** AgNOR in normal transitional cells 100X

**Figure 2**

**Figure 3**

**Figure 4**
Shareef Khalid Gasmelsaid Abdelmaged, Ahmed Mohammed Ibrahim Hamed, Mutaz Mohammed Ibrahim Ali. **Evaluation of diagnostic value of the nucleolar organizer region comparing reactive cells and transitional cell carcinomas among Sudanese patients**

Figures 5 100X, 6 100X and 7 40X AgNOR in nonmalignant reactive cells

Figure 5

![Figure 5](image1.png)

Figure 6

![Figure 6](image2.png)

Figure 7

![Figure 7](image3.png)

Figure 8 AgNOR in transitional cells carcinomas 100X

Figure 8

![Figure 8](image4.png)
DISCUSSION:

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.[26].

Although the number of AgNORs is increased in malignancy, some workers considered it as not diagnostic due to overlap with benign proliferation.[27]. It seems that although the number of AgNORs per cell is not discriminatory enough on its own to determine malignancy, the addition of size or area measurements using image analysis gives improved diagnostic and prognostic specificity [11,28]. The present study was aimed to Evaluation of diagnostic value of the nucleolar organizer region comparing reactive cells and transitional cell carcinomas.

The present study revealed significant association between mean of AgNORs count and reactive cells and normal transitional cell group and transitional cell carcinomas TCC sig. <000.

The AgNOR technique in cytologic smears has not been used frequently. [14,15] used this Technique to distinguish reactive mesothelial cells from malignant cells in serous effusions applied to cytologic preparations. The author demonstrated that malignant cells presented large numbers per nucleus and irregularshaped AgNORs, while in benign cells the AgNOR number per nucleus was comparatively fewer, and the AgNORs had a regular shape.[ 8] also demonstrated a correlation between AgNORs in benign and malignant cells in fine needle aspiration cytology of salivary gland masses. In cytologic smears, the analysis of AgNORs is more accurate because the whole nucleus can be assessed as it occurs in tissue
sections. This present study successfully used the AgNOR technique in cytologic smears of voided urine samples.

In this study, low AgNOR numbers per nucleus, in both groups were characteristic of benign and reactive cells. However, the AgNOR number in smears of transitional cell carcinomas TCC was higher than those of benign and reactive transitional cells, indicating increased proliferative activity in cells of transitional cell carcinomas TCC.

CONCLUSION:

Overall this study provides evidence for a strong link between AgNOR count and malignant transitional cell carcinomas TCC in urine cytology smear Thus the AgNOR count can be used as pre-warning factors of bladder cancers among patients at risk of bladder cancer and diagnostic comparing reactive cells and transitional cell carcinomas.

REFERENCES:

1. Sheldon Bastacky, M.D. Stacey Ibrahim, M.D. Sharon P. Wilczynski, M.D. William M. Murphy, M.D. 4The Accuracy of Urinary Cytology in Daily Practice. CANCER (CANCER CYTOPATHOLOGY) June 25, 1999 / Volume 87 / Number 3.
4. Vikram Deshpande, M.D. Grace T. McKee, M.D. Analysis of Atypical Urine Cytology in a Tertiary Care
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Center. CANCER (CANCER CYTOPATHOLOGY) December 25, 2005 / Volume 105 / Number 6.


nucleolus organizer regions (NORs) by automatic image analysis: a contribution to standardization. J. Pathol.
organiser regions (AgNORs) in breast lesions, Journal of the Indian Medical Association, vol. 95, no. 9, pp. 492–
494, 1997;
1319,2004;
proliferation markers in squamous cell Carcinoma of head and neck; 108:859-64, 1994;
19. Go, Li, Yang, T., Yan, J. Lil, Zeng, Y., Yu, J.- Cyclooxygenase-2 Parallels invasive depth and increased


methods. Staining modifications and results in 126 invasive ductal breast carcinomas, Analytical and Quantitative Cytology and Histology, vol. 18, no. 1, pp. 9–18, 1996;