

# Argyrophilic Nuclear Organizer Regions (AgNORs) Pattern in Different Grades of Transitional Cell Carcinoma of Urinary Bladder

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**Objective:** To evaluate AgNOR count, size and pattern in different grades of transitional cell carcinoma.

**Design:** Descriptive.

**Material and Methods:** A total of fifty transurethally resected bladder tumour samples (TUR BT) were collected from Mayo Hospital, Lahore and Services Hospital, Lahore and processed for H & E and AgNOR stain. The grading of tumours were made on H&E stain. While AgNOR size, AgNOR count, pAgNOR and AgNOR dispersion were recorded for each case.

**Results:** The AgNOR size in grade II was predominantly of 2+ while AgNOR size in grade III was predominantly of 3+. A significant higher proportion of cases ( $p < 0.02$ ) with 3+ AgNOR dispersion were seen in grade III carcinoma.

**Conclusion:** AgNOR size and AgNOR dispersion were found to be an easy and reproducible alternative to traditional AgNOR counts in grading of transitional cell carcinoma. The demonstration of AgNOR dispersion is also a simple and cost effective procedure.

**Key words:** Nuclear organizer regions, Transitional cell carcinoma.

## Introduction

Malignancies of urinary bladder are one of the leading malignancies in the developed countries.<sup>1</sup> The incidence is also high in Northern Africa and Western Asia. The world wide estimate of carcinoma of urinary bladder of new cases is 261,000 annually.<sup>2</sup> Amongst these, more than 50,000 cases and an estimate of 9500 deaths have been predicted in United States alone.<sup>3</sup> The incidence is three times more in males as compared to females and twice as common in whites as compared to blacks.<sup>4</sup>

Carcinoma of urinary bladder accounts for 2% of all malignant tumors and approximately 7% of all urinary tract malignancies in males. Transitional cell carcinoma constitutes the vast majority of bladder cancers.<sup>5</sup> It is the second most common malignancy in the genitourinary tract.<sup>6</sup>

Carcinoma of urinary bladder is the 8<sup>th</sup> common malignancy in Pakistan. It is the fourth common malignancy in men and its incidence is 5.4% in North West Pakistan. It has frequency of 5.75% among men and 1.61% among women.<sup>7-9</sup>

To study the proliferative activity of bladder cancer, a number of parameters have been used like telomerase activity in patients with transitional cell carcinoma has been seen in early oncogenesis.<sup>10</sup> High pAgNOR and decreased E cadherin expression with stromal and muscle invasive tumour indicate aggressive behavior of the tumour.<sup>11</sup> It has also been observed that Cyclin D1 was closely related with tumour differentiation but not to tumour progression.<sup>12</sup> However in young patients, p53 gene product over expression is common in carcinoma of urinary bladder.<sup>13</sup> The proliferative activity has also been studied by AgNOR technique which has been extensively used in different malignancies.<sup>14</sup>

The argyrophilic nucleolar organizer regions (AgNORs) are loops of ribosomal DNA present on short arms of acrocentric chromosomes which are demonstrable as black dots in the silver stained tissue section.<sup>15</sup> AgNORs are loops of DNA in the nucleolus which code for ribosomal RNA and thus are of vital significance in the synthesis of proteins.<sup>16</sup>

The simple AgNOR method, together with its ready application to paraffin sections, has made it a potentially interesting method for tumour biology.<sup>17</sup> The AgNOR staining technique has been extensively studied to find alternate technique of cell kinetic analysis other than flow cytometry.

The number of AgNOR has been thought to be related to cellular activity. It is more in malignant as compared to its benign counterpart.<sup>18</sup> Malignant cells have larger AgNOR than benign cells.<sup>19</sup> The increased AgNOR count is due to increased transcriptionally active ribosomal sites. Small size, larger number and scattered distribution of AgNOR are characteristic of malignant cells.<sup>20</sup> AgNOR silver staining technique enables the distinction to be made between certain grades of malignancy and may enable prognostic assessment.<sup>21</sup>

This present study was carried out to study the AgNORs pattern.

In different grades of transitional cell carcinoma of urinary bladder.

## Materials and Methods

Fifty sample of transurethally resected urinary bladder tumour (TUR BT) were collected in 10% formalin solution from Mayo Hospital and Services Hospital Lahore. These formalin fixed tissue were processed in automatic processor for H&E staining<sup>22</sup> and AgNOR staining.<sup>23</sup>

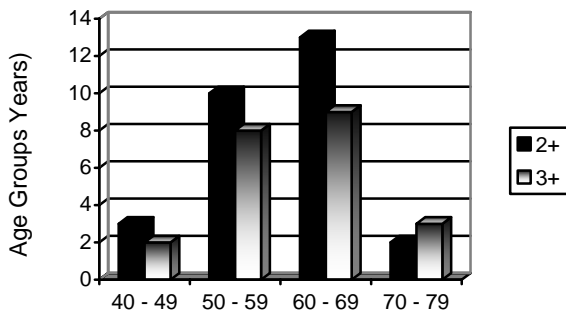
**Microscopic Interpretation**

The Mean AgNOR count was calculated. Moreover AgNOR size and dispersion were calculated according to Khan et al (2006)<sup>24</sup> AgNOR proliferative index was calculated according to Mourad et al (1992)<sup>25</sup>.

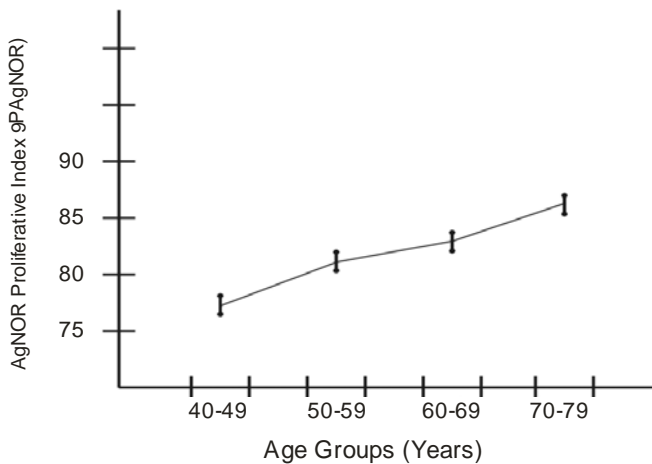
Student’s test was used to compare mean AgNOR Count and Chi Square test was used for evaluating size and dispersion.

**Results**

The ages of the patients ranged from 43 to 72 years with a mean of 61 years. Maximum number of cases were in 60 to 69 years age group (Fig. 1). All the patients presented with haematuria. Other complaints included fever and urinary retention (Fig. 2).



**Fig. 1:** AgNOR size in different age groups in transitional carcinoma.



**Fig. 6:** Distribution of pAgNOR in different age groups.

At the time of diagnosis, 34 cases (68%) were diagnosed as grade II tumour while 16 cases (32%) were those of grade III. The grade II tumour was dominant in all age groups except the age group of 70—79 year where the grade III was predominant (Fig. 3 ).The AgNOR count ranged from 6.25 to 12.14 with mean +SD value of 8.65+2.40.

Mean AgNOR count of the patients increased in relatively higher age group. However the difference was not statistically significant in different age group (Table 1).

Comparison of mean AgNOR count in patients with duration of symptoms of 3 months or less is compared with those of longer duration of symptoms in table 2. The AgNOR size of 2+ was predominant in all age groups except age group of 70—79 years where AgNOR size of 3+ was predominant (Fig. 4). The AgNOR dispersion of 3+was predominant in all age groups (Fig. 5).

**Table 1:** Mean AgNOR Count in different age groups.

Age Groups (years)	No of Cases	AgNOR count Mean+SD
40 — 49	5	8.25 + 3.74
50 — 59	18	8.40 + 2.47
60 — 69	22	8.28 + 1.75
70 — 79	5	10.15 + 3.63

p=NS: Between different groups

**Table 2:** Comparison of grade of tumour with AgNOR count.

Grade of tumour	No of cases	Mean AgNOR count +SD
II	34	8.16 + 2.50
III	16	10.06 + 1.39

P=NS

**Table 3:** Comparison of Grade of tumour with AgNOR size.

Grade	AgNOR size		No of cases
	2+	3+	
II	21	13	34
III	7	9	16
Total	28	22	50

P=NS

**Table 4:** Comparison of grade of transitional cell carcinoma with pAgNOR.

Grade	No of Cases	pAgNOR Mean+ SD
II	34	78.88 + 4.82
III	16	88.62 + 5.80

P&lt; 0.05

**Table 5:** Comparison of Grade of tumour with AgNOR dispersion.

Grade	AgNOR dispersion		No of cases
	2+	3+	
II	15	19	34
III	1	15	16
Total	16	34	50

P&lt; 0.02

### Discussion

Carcinoma of urinary bladder accounts for 2% of all malignant tumours and approximately 7% of all urinary tract malignancies in males.<sup>5</sup> It is the second most common malignancy in the genitourinary tract.<sup>6</sup> Smoking and exposure to a variety of chemical compounds are main known factors promoting the onset of carcinoma of urinary bladder.<sup>26</sup> The average age at diagnosis of transitional cell carcinoma has been reported to be 65 years.<sup>4</sup> While the present study shows the average age of 61 years which is in accordance with the finding of regional study of Faisalabad in Pakistan.<sup>9</sup>

Mean AgNOR count + SD has been evaluated in grading of transitional cell carcinoma. In present study mean AgNOR count increased from 8.16+2.50 to 10.06+1.39 as the grade of tumour increased (Table 2). Similarly an increase in AgNOR count from 5.94+1.42 to 8.54+1.01 had been reported by Pisac et al.<sup>27</sup> Likewise an increase in AgNOR count from 6.59 to 8.37 was also reported by Misra et al.<sup>28</sup>

However the AgNOR proliferative index showed significantly higher ( $p<0.05$ ) in grade III tumours when compared with tumours of grade II and these findings are consistent with those of Shina et al.<sup>29</sup> Our study has shown that variation in AgNOR size in grade II tumours was predominantly of 2+ while it was predominantly of 3+ in grade III tumours (Table 4).

In our study, the AgNOR dispersion in higher grade tumours showed a significantly higher ( $p<0.02$ ) when compared with lower grade tumours. This indicated that AgNOR dispersion is more useful parameter than AgNOR size in assessing the aggressiveness of transitional cell carcinoma (Table 5).

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