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ROLE OF MICROVESSEL DENSITY IN TRANSITIONAL CELL CARCINOMAS OF URINARY BLADDER

Pathology

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ABSTRACT

INTRODUCTION: Bladder carcinoma is a neoplasm arising from the lining transitional epithelium. More than 90% of bladder cancer cases are urothelial (transitional cell) carcinomas. Out of various prognostic factors, grade and stage are important. The non-muscle-invasive disease in spite of lower stage has a high recurrence of about 50–70% of the patients. Thus additional Prognostic factors play a role in determining the recurrence. Microvessel density is one such factor which is associated with recurrence. In this study, microvessel density is calculated using CD 34 and compared with standard clinicopathological variables.

MATERIALS AND METHODS: This study is an observational study of randomly selected 50 cases of Transitional cell carcinomas of urinary bladder that were submitted for histopathological evaluation during the period of 2 years in our institution. The slides were reviewed from the archive and histopathological parameters assessed. Immunohistochemical analysis was done with CD 34 and microvessel density (MVD) was calculated by counting the hot spots in 200X power. The results were compared with clinicopathological parameters.

RESULTS: The mean MVD values showed statistically significant increase in male gender and non-papillary tumours. The increase shows no statistical significance in multiple tumours, high grade and in recurrent tumours. The mean MVD values showed a decrease with increasing stage, size and infiltration and this association was statistically insignificant.

CONCLUSION: Though MVD values were reducing with stage, patients with high microvessel density (>50) presented with recurrence. Thus evaluating microvessel density can be an adjuvant factor in determining the prognosis of the patients.

KEYWORDS

Transitional Cell Carcinoma, Urinary Bladder, Cd 34, Microvessel Density

INTRODUCTION

Bladder cancer is tenth most common cancer in the world. In men and women it ranks sixth and seventh in incidence respectively. In 2018, approximately 550,000 new cases were diagnosed. The highest incidence rate was observed in Europe, North America and also in Syria, Israeli, Egyptian and Turkish males. Lowest rate was observed in Southeast Asia, Subsaharan Africa, Mexico, some countries in Middle East and central Asia [1]. In India it ranks eighteenth with incidence of 1.6% and Mortality of 1.3% [2]. This marked difference is due to exposure to risk factors like cigarette smoking, chemical carcinogens, arsenic and urinary bladder infection like Schistosoma haematobium[1]. The industrialised countries show a heavy burden of bladder cancer due to increase in smoking, especially in female[3]. Bladder carcinoma arise from the transitional lining epithelium. Urothelial cell carcinoma(transitional) is the most commonest histological type ,followed by squamous cell carcinoma, Adenocarcinoma and small cell carcinoma[4,5]. Based on histological appearance, bladder carcinomas can be classified into super ficial(80%) and invasive disease(20%). The non muscle invasive tumour shows a high recurrence rate of 50–70% [3,6]

Various factors such as age, recurrence, size, number, stage, grade, insitu changes, location and lymphovascular invasion play an important role in determining outcome [7-9,10-14]. Out of these factors, Grade and stage of tumour are the most important prognostic factors that affect survival. The tumour with similar stage and grade shows variable outcome, suggesting other prognostic factors may also play a role. Many studies found positive association of tumour angiogenesis with worse prognosis. Tumour angiogenesis is quantified by measurement of microvascular density. The Microvessel density (MVD) has been considered as an important prognostic factor in many solid tumours and also in carcinomas of bladder. Increased MVD has been found to be associated with increased stage, grade and increased chances of recurrence[15].

MVD can be measured Immunohistochemically using pan endothelial markers like CD 31 and CD 34 or by activated endothelial markers like CD 105. The CD 34 and CD 31 stains inflammatory cells also, but can be reduced by pre-treatment with microwave oven. Counting of microvessels can be done either manually or by using computer image analysis. The positivity of the markers can also be used for targeted therapy[15]. Weidner et al first defined high vascular spot area (high vascular dense area within tumour) in breast cancer and showed that metastatic breast as high MVD compare to non metastatic cancer with low MVD. In 1993 Weidner et al confirmed similar correlation and its prognostic significance in prostatic carcinoma[16].

In this study, an attempt is made to study the micro vessel density to assess their prognostic significance in association with other clinicopathological parameters and recurrence in transitional cell carcinoma of bladder.

AIMS AND OBJECTIVES

To study Microvascular Density using CD34 in Transitional cell carcinoma of bladder.

To study prognostic significance of MVD in association with clinico pathological parameters like gender, Number, size, Histological type, Grade, infiltration, Stage and Recurrence.

MATERIALS AND METHODS

This study is a prospective and retrospective study of 50 randomly selected cases over a period of 2 years. Transitional cell carcinomas of bladder specimens irrespective of the age, sex and the procedure done were included in the study. Carcinomas other than transitional cell type, cases with inadequate material and preoperative chemotherapy or radiotherapy cases were excluded from the study.

Detailed history of the cases regarding age, sex, type of procedure, site, size and previous surgery details were obtained from surgical pathology records. Hematoxylin and Eosin slides were reviewed and the tumour typing as per WHO 2016 was made and TNM staging done as per 8th edition American Joint Committee on cancer[17-18].

They were subjected to immunohistochemistry for CD 34 in paraffin embedded tissue samples using Super-sensitive polymer HRP system based on non-biotin polymeric technology. The antigen retrieval is done by microoven heat Pre-treatment method. The antigen was bound with mouse monoclonal antibody (Biogenex) and detected by the addition of secondary antibody conjugated with horse radish peroxidise-polymer and diaminobenzidine substrate.

The immunohistochemically stained slides were analyzed for the presence of cytoplasmic staining for CD 34. Vascular Hot spots (region for high vascular density within tumour) were identified and at least 3 such hot spots were identified under 200X power and the vessels were counted. The maximum of these was taken as microvessel density. The mean microvessel density was calculated in relation to the prognostic

factors.

The statistical analysis was performed using statistical package for social science software version 11.5 which consisted computing the frequency counts and percentages for qualitative variables and mean for the quantitative variables.

The expression of microvessel density was correlated with clinico-pathological factors like gender, tumour site, tumour configuration, size, histological types, histological grade, depth of infiltration, stage and recurrence. Statistical analysis was done using the Student t-test and Anova t-test.

OBSERVATION AND RESULTS

Among the study group, 30 cases (60%) were low grade and 20 cases (40%) were high grade. 45 cases (90%) showed papillary morphology and 5 cases (10%) showed flat morphology. 8 cases (16%) belonged to stage Ta (non-invasive), 23 cases (46%) belonged to T1, 16 cases (32%) belonged to T2a, 1 case (2%) belonged to T2b, 1 case (2%) belonged to T3b and 1 case (2%) belonged to T4a. 8 cases (16%) belonged to stage 0, 23 cases (46%) belonged to stage 1, 17 cases (34%) belonged to stage 2 and 2 cases(4%) belonged to stage 3. Among the 50 cases, 19 cases (38%) showed evidence of recurrence. The microvessel density ranged from 8 to 102 with a mean value of 36.44. In this study, various prognostic factors were compared with mean microvessel density.

CORRELATION OF MICROVESSEL DENSITY WITH VARIOUS CLINICO – PATHOLOGICAL PARAMETERS

The comparison of microvessel density with various clinicopat h ol og ical variables is given in table/figure 1.

In this study, the mean microvessel density for male patients was found to be 38.45 with the standard deviation of 18.0. The mean MVD for female patients was found to be 25.88 with the standard deviation of 6.3. Since the standard deviation was found to be very high, the logarithmic transformations was carried out before applying the student's t-test. The significant P-value infers that male patients have higher MVD than female patients.

This study showed increased mean MVD in patients with multiple tumours (42.2±17.8) when compared to patients having single tumours (35.8±17.8). However, this increase was found to be statistically insignificant.

In this study, out of 50 patients, 29 of them had tumour size less than or equal to 4 cm. Their mean MVD level was 38.1 with the standard deviation of 17.0. Remaining 21 patients had tumour size above 4 cm and their mean MVD level was 34.05. The non-significant p-value infers that MVD is independent of the tumour size.

This study showed mean MVD of 34.89±15.7 in case of papillary tumours and 50.4±25.9 in case of non-papillary tumours. This increase was found to be statistically significant.

This study showed an increase in MVD in high grade carcinoma patients (38.6±24.4) in comparison with low grade patients (35±10.5). However, this difference was not found to be statistically significant and hence MVD was not influenced by the grade of the tumour.

This study showed increased MVD in cases of superficial carcinomas (44.75±25.3) in comparison with infiltrative carcinomas (34.86±15.2) and the increase in MVD in superficial carcinomas was not statistically significant.

In this study, the patients with recurrence had an increased mean MVD value (39.9±18.4) than those without recurrence (33.9±16.3). However, this increase in MVD values was not statistically significant.

TABLE/FIGURE: 1- CORRELATION OF MICROVESSEL DENSITY WITH CLINICOPATHOLOGICAL PARAMETERS

Clinicopathological parameter		N	Mean MVD	Standard deviation(SD)	t-value*	P-value
Gender	Male	42	38.45	18.002	2.047	0.046
	Female	8	25.88	6.379		
Number	Single	45	35.80	17.801	1.143	0.259
	Multiple	5	42.20	11.256		
Size	<4 cm	29	38.17	17.046	0.787	0.435
	>4 cm	21	34.05	17.735		
Histological type	Papillary	45	34.89	15.712	1.845	0.071
	Non-papillary	5	50.40	25.929		
Grade	Low grade	30	35.00	10.518	0.184	0.855
	High grade	20	38.60	24.356		
Infiltration	Superficial	8	44.75	25.336	1.351	0.183
	Infiltrative	42	34.86	15.208		
Recurrence	Present	19	41.73	18.409	1.333	0.189
	Absent	31	33.19	16.281		

***For the logarithmic values of MVD the t-test has been applied.**

This study showed that there is no statistically significant correlation between MVD and site of tumour. Since only one patient had tumour at the posterior wall, for statistical purpose its MVD value was combined with that of the base. The mean values have been compared using ANOVA test. The non-significant p-value infers that the site of the occurrence of the tumour has no influence on the MVD level. (Table/figure 2).

TABLE/FIGURE: 2 - CORRELATION OF TUMOUR SITE WI TH MICROVESSEL DENSITY

Site	N	Mean MVD	SD	ANOVA F-value*	P-value
Right lateral wall	16	32.69	9.884	0.472	0.795
Left lateral wall	13	37.54	20.630		
Base & Posterior wall	5	40.40	18.756		
Dome	5	30.60	13.722		
Entire bladder	6	40.83	30.407		
Multiple	5	42.20	11.256		

***For the logarithmic values of MVD the ANOVA test has been applied.**

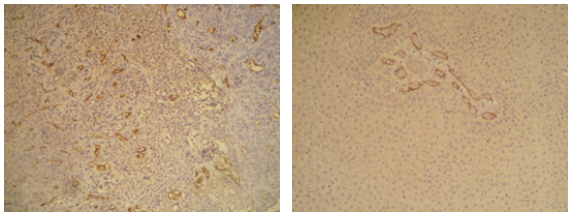
This study showed mean MVD of 44.75±25.3 in stage 0 (non-infiltrative) patients, 36.88±17.9 in stage I patients, 32.44±10.9 in stage II patients and 30±11.3 in stage III patients. Thus there was no increase in MVD in patients of increasing stage and this had no statistical significance. (Table/figure 3)

TABLE/FIGURE: 3 - CORRELATION OF TNM STAGE WITH MICROVESSEL DENSITY

Stage	N	Mean MVD	SD	ANOVA –F value*	P-value
0	8	44.75	25.336	0.700	0.557
I	23	36.88	17.858		
II	17	32.44	10.899		
III	2	30.00	11.314		

***For the logarithmic values of MVD the ANOVA test has been applied.**

The present study showed that there was statistically significant association between microvessel density and histological type, male gender and necrosis. Microvessel density was seen to increase with increasing number, grade and in recurrent tumours. But when subjected to statistical analysis this association was not found to be significant. There was an increased expression in large tumours involving the entire bladder.



TABLE/FIGURE 4: Transitional cell carcinoma of bladder- high grade cases showing high microvessel density- 102 vessels and low microvessel density- 15 vessels. (200X)

DISCUSSION

In the present study, immunohistochemical evaluation was done in 50 cases of transitional cell carcinomas of bladder and an attempt was made to correlate the microvessel density with the known prognostic factors of bladder cancers and with recurrence.(Table/Figure 5)

TABLE/FIGURE: 5 - COMPARISON OF MEAN MICROVESSEL DENSITY WITH WORLD STATISTICS

Studies	Grade		Infiltration		Stage			Recurrence/progression	
	Low	High	Present	Absent	1	2	3	Present	Absent
Eun Yong Choi et al	94.5	109.5	-	-	-	-	-	131.1	94.5
Khaled El Gehani et al	65.8	106.3	-	-	72.1	104.8	86.6	-	-
N.E.Stavropoulos et al	20.8	16.4	20.73	17.58	-	-	-	19.61	19.1
E.A. philp et al	47.7	51.8	-	-	46.19	53.90	52.2	-	-
C.K.Hawke et al	77.4	90.8	-	-	-	-	-	-	-
Jonathan C. Goddard et al	75.8	81.3	65.6	85.5	-	-	-	-	-
AliCanoglu et al	90.4	95.7	88.9	94.0	-	-	-	92.7	87.4
Current study	35.0	38.6	44.8	34.9	36.9	32.4	30.0	39.9	33.9

Khaled EL Gehani et al [19] and AliCanoglu et al [20] studies showed increased MVD with increasing Grade and its correlation was significant statistically.

Other Studies conducted by Eun Yong Choi et al [21], E.A.philp et al [22], C.K.Hawke et al [23] and Jonathan C.Goddard et al [24] showed increased MVD with increasing grade, but no significance statistically. Similarly, Present study showed Increased MVD with increasing grade with no statistical significance.

Jonathan C.Goddard et al [24] and AliCanoglu et al [20] studies showed significant correlation between MVD and non-infiltrating carcinoma. Our study showed increased MVD with invasive carcinoma with no significant correlation, similar to the studied done by N.E . Stavropoulos et al[25]

Comparing stage and MVD showed Increased MVD with stage II with significant correlation in Khaled El Gehani et al [19] and E.A.philp et al [22]. Present study showed MVD 36.9, 30, 39.9 respectively in stage 1, II, III with no significance.

Eun Yong Choi et al [21] and AliCanoglu et al [20] Studies in table/figure 5 showed increased MVD with recurrence and its significant correlation. Present study showed MVD 39.9 in recurrence compared to 33.9 in non-recurrent cancer and but it is insignificant statistically, which was similar to study done by N.E.Stavropoulos et al [25].

L. Santos et al (2003) studied 66 superficial papillary carcinomas and found that MVD was an independent prognostic factor for recurrence [26].

Our study showed increased MVD (50.40) with nonpapillary carcinoma compared to papillary carcinoma (34.89) with statistical significance. Similarly, Male gender showed significant correlation .

Though this study showed a decrease in MVD with increased stage, 5 Cases with 3 Ta and 2 T1 stage with non invasion with high MVD of 102,68,60,58,62 respectively (i.e > 50) showed recurrence similar to the study done by Jonathan et al. Hence more cases in early stage should be evaluated for MVD and closely followed for possible recurrence. More similar studies with large sample size may be helpful to assume its clinical significance.

CONCLUSION

Microvessel density had a statistically significant association with non-papillary tumours and in male gender. An increased microvessel density was noted in multiple, high grade, recurrent tumours which were statistically insignificant. The values were reduced with increasing stage, size and infiltration which was not found to be statistically significant.

In this study, though MVD was found to decrease with stage, the patients with high MVD values i.e >50 were presenting with recurrence. Also many of the recurrent tumours presented in early stage. Hence more patients presenting with early stage tumour for the first time can be evaluated for MVD and closely followed up for possible recurrence. More such studies with large samples size will help in identifying the possible association between the stage, MVD status and recurrence to formulate treatment strategies and possible targeted therapy.

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