Original Article

Expression of Vascular Endothelial Growth Factor in Prostatic Adenocarcinoma is associated with High Gleason Grade

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Abstract

Background: *Worldwide, prostate cancer is the second most common cancer in men and the fifth most common cause of mortality. Accurate diagnosis of prostate cancer is based on elevated serum specific prostatic antigen (sPSA) and subsequent histopathological examination with Gleason grading score. Vascular endothelial growth factor (VEGF) is one of the most potent mitogenic, highly specific tumor angiogenic factors, and has been found to be overexpressed in prostate cancer in comparison with normal epithelium or benign prostatic hyperplasia. High tumor expression of VEGF in prostate cancer is found to be associated with a high risk of failure after treatment with radiotherapy. Aim of this study was to determine the expression of VEGF and its association with serum PSA level and Gleason Grade in prostatic adenocarcinoma.* ***Materials & Methods:*** *This was a cross-sectional observational study. A total 81 cases were selected from the patients who were diagnosed as prostatic adenocarcinoma in the department of pathology at BSMMU from September 2021 to August 2023. Immunohistochemical staining for VEGF was performed along with appropriate positive control.* ***Results:*** *Among 81 selected cases, highest number (n=24, 29.6%) of the tumors were in grade group 5. In this study VEGF immune expression was positive in 35 (43.2%) cases and negative in 46 (56.8%) cases. Statistical analysis of present study cases showed a significant association between VEGF expression and Gleason grade group. This study also showed significant association between serum PSA values with VEGF expression.* ***Conclusion:*** *The use of VEGF immune stain, serum PSA value in addition to Gleason grade group may provide significant prognostic information for selected high-risk patients in prostatic adenocarcinoma.*

Keywords: Prostatic adenocarcinoma, Gleason grading system, VEGF, Serum PSA.

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Introduction

Prostate cancer is the second most prevalent type of cancer worldwide,(in terms of age-standardized incidence).[1] According to GLOBOCAN 2018, the prevalence of prostatic adenocarcinoma in Bangladesh is 4.63%. It accounts for 14.8% of male cancers (18.6% in developed countries and 8.4% in the least developed countries). About 6.6% of all deaths in men over 55 years old are attributable to prostate cancer.[2] It is the second-leading cause of men's cancer-related deaths and the leading cause of new cancers in men. In 2016, an estimate of 180,890 recently diagnosed cases of prostate adenocarcinoma were identified in USA. Prostatic carcinoma causes 4.0% of deaths in the Southeast Asian region.[2] In Bangladesh, age-specific incidence and mortality rate of prostatic carcinoma is 1.7 and 1.2 per 100000 people respectively.[3] The prevalence of clinically significant prostate cancer in Asia appears to be rising as the diet becomes more Westernized.[4] Prostatic carcinoma development is largely contributed by hormonal factors, and between 5 and 10 percent of cases also have a genetic component.[5] Pre treatment serum prostate-specific antigen (sPSA) levels and

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Gleason's grading system is crucial parameter in diagnosis and treatment selection.

Core needle biopsy procedure have become increasingly common in recent years. One of the most effective prognostic predictor for prostatic adenocarcinoma is the Gleason grading system, which was developed by Dr. Donald Gleason, chief of pathology at the Veteran's Hospital in Minnesota. From 1959 to 1964, conducted research and developed a prostatic carcinoma grading system.[6] Different histological patterns were the lay out this grading. Most tumors had two distinct histological patterns. Therefore, the two most predominant grade patterns, ranging from 2 to 10 were added. It is evidenced that mortality progressively increased with tumor grade.[7,8]

The new modified Gleason score, which is made up of five groups (groups 1 through 5), was created as a result of this system's extensive revisions. Grade stratification is made simpler and more accurate with the modified Gleason score. Additionally, the lowest grade in the recently changed system is 1 rather than 6.[9]

Prostate-specific antigen (PSA) is still regarded as the most essential prostatic carcinoma biomarker. The normal PSA level in the serum is between 0 and 4 ng/ml. In the early detection and screening of prostatic cancer, serum PSA levels are crucial. It has been found that the PSA level and the microscopic grade of prostatic adenocarcinoma are well correlated.[10,11] There is a strong association between Gleason's score and PSA value. It is inferred that as the Gleason's score raises, the serum PSA level also raises accordingly.[12]

Perineural invasion in biopsies is a significant prognostic marker. Presence of perineural invasion is an independent predictor for survival and therefore a recommended parameter to add in standardized pathology reporting.[13,14] Compared to patients without perineural invasion, those with perineural invasion at biopsy are twice as likely to progress.[15]

Angiogenesis, the synthesis of blood vessels from already existent vessel, is a key event in solid tumor growth, invasion and metastasis.[16] Vascular endothelial growth factor (VEGF) is among the most potent angiogenic factors thus far detected and has been found to be highly specific for endothelial cells in vitro and in vivo, promoting endothelial cell proliferation and increasing vascular permeability of the cell division cycle as well as in mitosis.[17] VEGF is a 45 kDa heparin-binding polypeptide of the platelet-derived growth factor family and is secreted by a variety of malignant cells. It has been shown to be expressed in many different types of tumors, including renal cell carcinoma, breast carcinoma, gliomas, and hepatocellular carcinoma.[18,19] Additionally, angiogenesis is a crucial event in tumorigenicity and metastasis and it is necessary for the development of tumor vasculature and the progression of prostate cancer.[20] Meta-analysis had shown that increased VEGF expression in prostate malignant cells may indicate poor prognosis. Moreover, VEGF levels in the plasma and urine of patients with metastatic castration-resistant prostate cancer are independent predictors of overall survival.[21]

Materials and Methods

This study was a cross-sectional observational study conducted in the Department of Pathology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, after taking institutional review board clearance (BSMMU/2022/6257). 81 cases of prostatic adenocarcinoma were taken. Carcinomas other than prostatic adenocarcinomas, insufficient biopsies and autolyzed specimens were excluded. Demographical and relevant clinical information such as age, pretreatment serum PSA levels, histopathological diagnosis with Gleason's Grade of the tumor at the time of diagnosis were collected from the departmental records. Paraffin blocks of all selected cases were retrieved from department and checked and reviewed. After confirming the diagnosis immunohistochemical staining for VEGF

was performed along with appropriate positive control. 3-4 mm thick sections were cut and gently lowered on surface of water bath at 45˚ C and were spread wrinkle free on to the slides coated with 0.1% poly L-lysine for 15 minutes at 37°C and air dried. Then the slides were kept on hot plate at 60˚C for baking for 30 minutes. Dewaxing was done by treating the slides in xylene followed by rehydration in absolute alcohol, 90% alcohol and 70% alcohol. For antigen retrieval slides were put in preheated pressure cooker having citrate buffer, then boiled and allowed to cool naturally. To block the endogenous enzyme activity hydrogen peroxide was added in a moist chamber at room temperature. Monoclonal mouse anti-human VEGF (INVITROGEN, Thermo Fisher Scientific, UK) was used as primary antibody. Then primary antibody was added for 1-2 hrs in moist chamber at room temperature.

Enhancement of primary antibody was done by adding antibody enhancer (super enhancer) and incubated in moist chamber for 20min. The peroxidase antiperoxidase method was followed for secondary staining. DAB was used for coloring the antigen-antibody complex. This was followed by counterstaining with hematoxylin. VEGF expression patterns was scored based on the intensity and extent of staining. The intensity of cytoplasmic and/or membranous VEGF staining in the tumor tissue was scored as: 0 (Negative), 1 (weak), 2 (moderate), 3 (strong).

The extent of staining was scored as: 0 (0%), 1 (1-25%), 2 (26-50%), 3 (51-75%), 4 (76-100%) according to the percentage of the positively stained areas in relation to the total carcinoma area. The results of the study were statistically analyzed using the Statistical Package for the Social Sciences (SPSS) version 20 (IBM Corp. SPSS statistics, Chicago, Illinois, USA) for windows. Data were expressed as mean ± SD for the quantitative variables, numbers, and percentage. Comparison between multiple groups were made using Chi square test for qualitative data. A value of $P < 0.05$ was taken as significant.

Results

The present study was a cross-sectional observational study. It was conducted in the Department of Pathology, BSMMU. The study population were the patients diagnosed as prostatic adenocarcinoma in the department of pathology at BSMMU during the study period. Patients of all ages were included in the study. A total of 81 cases were selected and demographic and histopathological variables (age, grading of tumor, etc.) were assessed and immunohistochemically expression of VEGF was observed.

Figure 1 shows age distribution among the study population. The present study reveals that mean age of the study population was 69.03±9.20 (SD) years (Range: 40-100 years). Majority of the patients were in age group 61-70 years (44.40%).

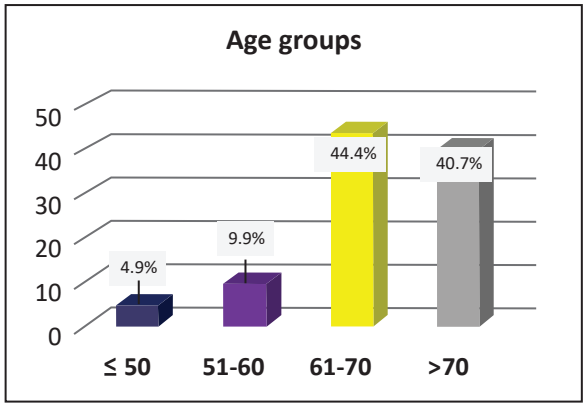

Figure 1: Distribution of study population according to age (n=81).

From the present study we observe that out of the 81 specimens, 90.1% (n=73) were core needle biopsy and 6.2% (n=5) were TURP chips (Figure 2).

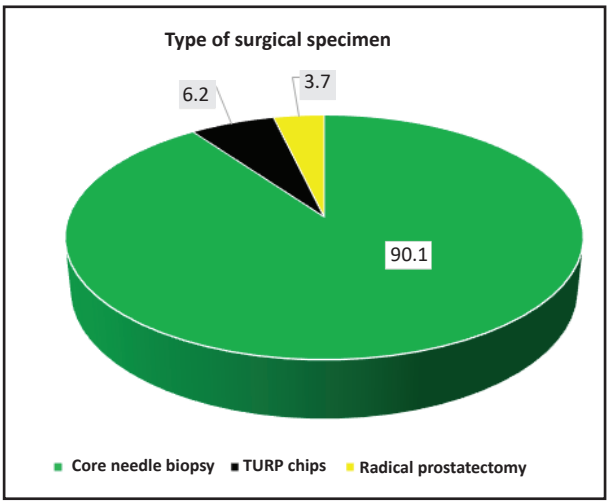

Figure 2: Distribution of study population according to surgical specimens.

In this study 9.9% of patients presented with PSA level 2-10 ng/ml. However, majority of the patients (56.8%) had PSA levels more than 50 ng/ml, while 19.8% and 14.8% of patients presented with PSA levels of 11-25 ng/ml and 26-50 ng/ml respectively (Table I).

Table I: Distribution of the study subjects according to PSA values (n=81)

PSA (ng/ml) Level	Frequency	Percent (%)
2-10 ng/ml	7	9.9
11-25 ng/ml	16	19.8
26-50 ng/ml	12	14.8
>50 ng/ml	46	56.8
Total	81	100.0

Among 81 selected cases, majority (n=24, 29.6%) of the cases were in grade group 5 tumors. Two (2.5%) and thirteen (16%) cases were in grade group 1 and 4 respectively. Remaining 42 cases were equally distributed (25.9% each) into grade group 2 and 3 (Figure 3).

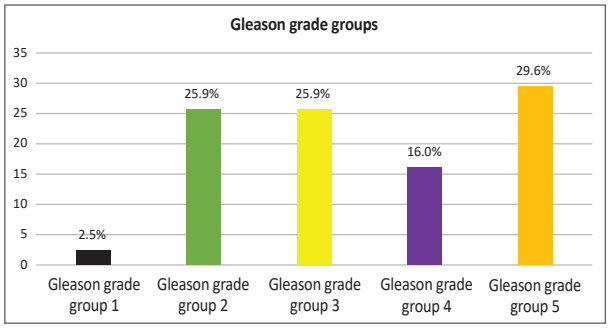

Figure 3: Distribution of Patients According to Grade Group (n=81)

The current study reveals that among the 81 cases, VEGF was positive in 35 cases (n=35; 43.2%). This is shown in Table II.

Table II: VEGF expression in study subjects

VEGF expression*	Frequency	Percent (%)
Positive	35	43.2
Negative	46	56.8

From the current study, a significant association ($p<0.05$) was found between VEGF expression and the grade group of tumors (Table III). The majority of the VEGF-positive patients had Gleason grade group 5 (54.3%, N=19).

Table III: Association between VEGF expression and Gleason Grade Group.

	Categories	VEGF status		X^2 (*p* value)
		Positive	Negative	
Gleason grade groups	Gleason grade group 1	0	2	30.686 (<0.001)
	Gleason grade group 2	2	19	
	Gleason grade group 3	5	16	
	Gleason grade group 4	9	4	
	Gleason grade group 5	19	5	
Total		35	46	81

s = significant ($p<0.05$)

The study shows that a significant association ($p<0.05$) was found between PSA (ng/ml) level and grade group of the tumor (Table IV). PSA was progressively raised with increasing grade groups of the tumor.

A significant association was found between VEGF expression and serum PSA (ng/ml) level ($P< 0.05$) (Table V).

Table IV: Association between Gleason grade group and PSA level

Gleason Grade categories	PSA (ng/ml) Level				Total	X^2 (P-value)
	2-10 ng/ml	11-25 ng/ml	26-50 ng/ml	>50 ng/ml		
Grade group 1	2	0	0	0	2	36.380(0.003^s)
Grade group 2	3	5	3	10	21	
Grade group 3	1	7	2	11	21	
Grade group 4	0	1	3	9	13	
Grade group 5	1	3	4	16	24	

s = significant ($p<0.05$)

Table V: Association between VEGF expression and serum PSA level

	Categories	VEGF status		X^2 (*p* value)
		Positive	Negative	
Serum PSA level in ng/ml	2-10 ng/ml	2	5	8.311 (0.040^s)
	11-25 ng/ml	3	13	
	26-50 ng/ml	4	8	
	>50 ng/ml	26	20	

s = significant ($p<0.05$)

No statistically significant association was found between Gleason grade group and age groups (Table VI).

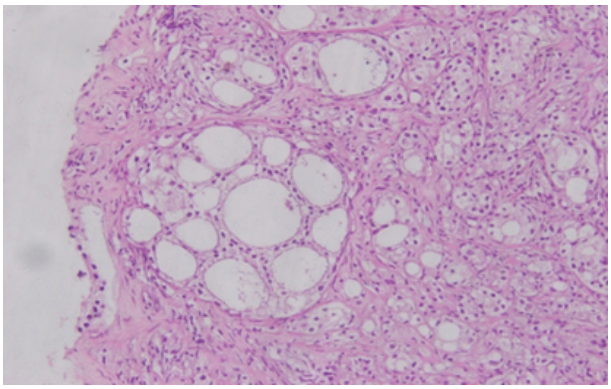

Figure 4: Photomicrograph showing histology of Gleason pattern 4 (poorly formed fused glands forming cribriform architecture) (H&E,200X).

Table VI: Association between age groups and Gleason grade group

		Age Groups				Total	*p* value
		50 or less	51-60	61-70	>70		
Grade Group	1	1	0	0	1	2	0.443^{ns}
	2	1	2	10	8	21	
	3	1	2	8	10	21	
	4	0	2	5	6	13	
	5	1	2	13	8	24	
Total		4	8	36	33	81	

ns= Not significant ($p>0.05$)

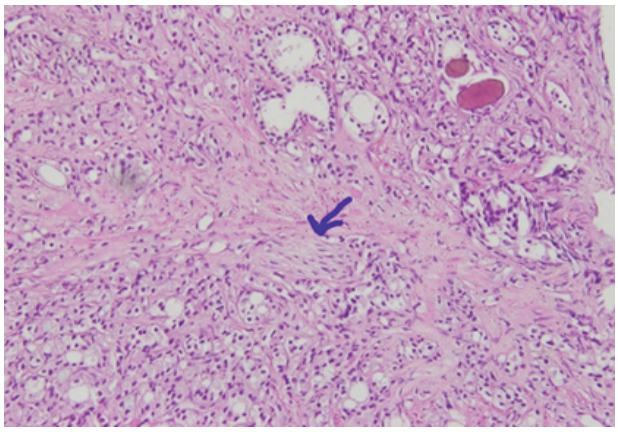

Figure 5: Photomicrograph showing poorly formed glands with focus of perineural invasion (Blue arrow), Gleason pattern 4 (H&E, 200X)

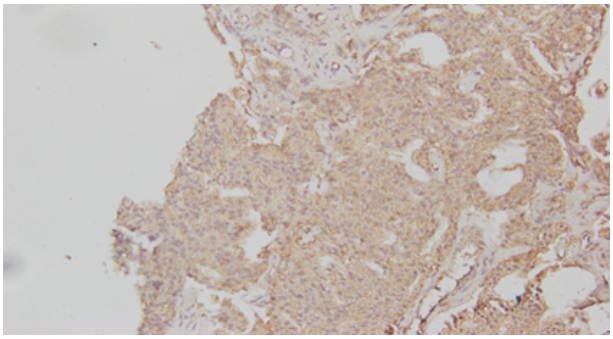

Figure 6: Photomicrograph showing positive VEGF expression in the cytoplasm and cell membrane of a Gleason grade group 3 tumor (Case no 01, 200X).

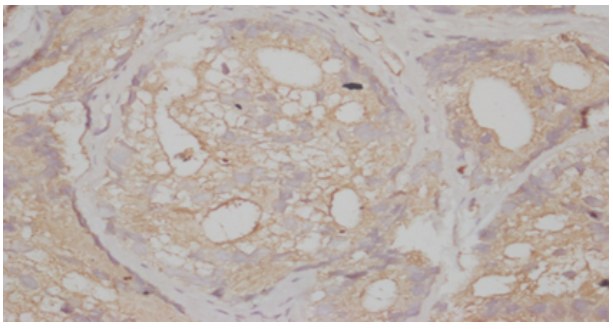

Figure 7: Photomicrograph showing positive VEGF expression in the cytoplasm and the cell membrane of a Gleason grade group 4 tumor (Case no 12, 400X).

Discussion

Prostate cancer is the second most common cancer in men and the fifth most common cause of mortality worldwide. The majority of prostate carcinomas may not progress to clinically significant disease. A minor fraction of the clinical cases remains confined to the prostate for many years and other carcinomas progress rapidly to a life-threatening disease. How to distinguish these three biologically different types of prostate cancer is a question of great importance.[22] In view of the above, early diagnosis and effective treatment of the disease are immensely important. The increasing number of options for the treatment of prostate cancer has made the prognostic evaluation of the disease even more important. Histological grading is a very important factor for the assessment of prognosis. The Gleason's grading system is the most favored prognostic factor, and significantly associated with survival and/or progression. Angiogenesis is found to play an important role in tumorigenesis, proliferation, and metastasis in prostate cancer.[23] VEGF is one of the most potent mitogenic, highly specific tumor angiogenic factors and has been found to be overexpressed in prostate cancer.[24] High tumor expression of VEGF in prostate cancer is found to be associated with poor prognosis in those receiving both surgery & radiotherapy as primary treatment.[25] In this study, 81 cases were included. The age of the patient ranged from 40 years to 100 years and the mean age of the patients was 69.03±9.20 (SD) years. The majority of patients were in the age group 61-70 years (44.4%). These results were consistent with studies that found the mean age of the patients was 67.9 years. A cohort study conducted in the USA reported that older men were more likely to have high-grade prostate cancer.[26] Another study found that the probability of a high Gleason score and high-risk disease increased with age, nearly tripling from ages 50-54 years to 80-84 years.[27] It was also observed that older men were more likely to be diagnosed with

high-grade or high-risk disease than younger men. The patients were grouped into five grade group. Grading was done on the basis of recent WHO grade group (Grade group 1 - Gleason's score ≤6; Grade group 2 - score 3+4=7; Grade group 3 - score 4+3=7; Grade group 4 - score 8; Grade group 5 - score 9 to 10).[28] Out of 81 cases, 24 (29.6%) cases were in grade group 5, 2 (2.5%) and 13 (16%) cases were in grade groups 1 and 4 respectively. The remaining 42 cases were equally distributed (21% each) into grade groups 2 and 3 respectively. This finding was similar to a study where 33% of cases were in grade group 5.[28]

In the past, digital rectal examination was a screening tool for the detection of prostate cancers but currently, emphasis has been shifted to measurement of PSA levels. However, the confirmation of prostate cancer is carried out only through histopathological analysis of the biopsy sample. The demographic profile and clinical details of the cases of the current study were similar to many studies conducted in relation to prostatic carcinoma. In this study, PSA levels in prostate carcinoma were divided into four groups: 2-10, 11-25, 26-50, and >50 ng/ml.[29] Out of 81 cases, the majority of patients (56.8%) had PSA levels of more than 50 ng/ml, while 19.8% and 14.8% of patients presented with PSA levels of 11-25 ng/ml and 26-50 ng/ml, respectively. Similar results were observed in other studies.[30]

Pretreatment serum PSA levels are a prognostic marker and stratify patients into different prognostic categories. This study's results were in concordance with other studies that found a statistically significant association between higher Gleason score and increased PSA levels. A significant association ($p<0.05$) was found between PSA (ng/ml) level and the Gleason grade group of tumors (shown in Table IV. It was observed that PSA progressively increased with the increasing grade group of the tumor. Similar results were obtained in other studies.[31,32] The data regarding these variables varied among different authors.[33] Regarding the evaluation of vascular growth factor (VEGF) expression, the final immunohistochemical staining score (0–7) was obtained by the sum of the intensity (0-3) and the extent of staining (0-4), with tumors having a final staining score of 3 or more being considered as positive.[34]

In this study, among 81 cases, positive VEGF staining was found in 35 (43.2%) cases and negative in 46 (56.8%) cases. In a previous study, VEGF expression was positive in 53.4% of patients with prostate cancer.[35] Another study found that 58% of patients with prostatic adenocarcinoma had high VEGF expression. These findings are consistent with prior studies in the literature that report VEGF expression in approximately 40% to 100% of prostate cancer cases.[36] From the current study, a significant association was found between VEGF expression and Gleason grade group as well as serum PSA.[37] This finding is consistent with previous studies. Similar results were observed in other studies.[38,39]

Conflict of Interest

The authors have no conflict of interest to declare.

Ethical Approval

This study was approved by the Institutional Review Board of Bangabandhu Sheikh Mujib Medical University (BSMMU/2022/6257).

Conclusion

Gleason grade of prostatic adenocarcinoma showed statistically significant association with immunoexpression of VEGF and serum PSA. Gleason scoring pattern is the overall useful predictor of prostatic carcinoma and assessment of angiogenesis status by VEGF immunoexpression may add value to the Gleason scoring pattern in selected patients.

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