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VEGF Expression in Prostate Cancer

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Abstract

Background: Inspite of the various diagnostic markers for prostatic cancer, second ranking in men, it`s still difficult to find metastatic deposits elsewhere after radical prostatectomy. Gleason scoring and serum PSA alone are not helpful in assessing the metastatic tendency.VEGF, growth factor in the vascular endothelial proliferation and paving the spread of cancer cells, is also found to be increased in expression in cases with more aggressive prostate cancers.This study was done to identify prostatic carcinomas with increased VEGF expression and to correlate it with available Gleason scores and PSA levels.

Materials And Methods:25 cases of prostate cancer specimens were taken for histopathological examination and subjected for immunohistochemical study with VEGF marker, studied for the level and strength of expression on the cells, in comparison with the corresponding Gleason scoring and available serum PSA levels.

Results: VEGF strongly expressed in 48% cases and negative staining in 8% of cases. A p value <0.05, indicates as degree of malignancy increases, angiogenesis increases.

Conclusion: There is significant correlation in the expression of VEGF in comparison with Gleason score, confirming that with progression of the disease all these parameters are elevated(p value<0.05). The same does not go with PSA values and VEGF, as the PSA values are elevated in benign lesions also and there is no significant correlation, p value>0.05.

KEYWORDS: VEGF, PROSTATE, METASTASES

I. Introduction

A major public health problem in industrialized world, the prostate cancer, during the last decades of the past 20th century has been contributing to three fourth of the registered cases across the globe (Perin, 2001). Despite earlier diagnosis and smaller tumor volumes, many of the patients with clinically organ confined prostate cancer, found to have extra prostatic disease subsequent to radical prostatectomy. Advanced staging are determined by Gleason score, serum PSA, and clinical staging. Of them Gleason scoring and PSA levels are the most important.

Various studies done in western countries like, Relative expression of type IV collagenase, E-cadherin and VEGF in prostatectomy specimens distinguishes organ confined, from pathologically advanced cancers by HIROKI KUNIYASU et al[1] and Metastatic progression of prostate cancer and E-cadherin by AARON P.PUTZKE et al[2], show that levels of E-cadherin and VEGF are definitely related to aggressive and metastatic prostatic cancers.

The purpose of the study is to identify prostatic carcinoma with aberrant or increased expression of VEGF for treatment strategies and to find the degree of relation with PSA levels and Gleason scoring.

VEGF, a potent angiogenic factor has also been found to be overexpressed in prostate cancer in comparison with normal epithelium or benign prostatic hyperplasia. Thus, identifying such angiogenic factors involved in prostate cancer growth and understanding their regulation will lead to the development of anti-angiogenic strategies useful for diagnostic studies and therapeutic interventions.

AIM

- To study the expression of VEGF in prostatic carcinomas
- To study the Correlation of VEGF expressions with Gleason scoring and the serum PSA levels

II. Materials And Methods

A prospective study conducted in coimbatore medical college over a period of 1 year with the prostate biopsy specimens received from the hospital. All the necessary details were obtained regarding the MRI findings, serum levels and clinical details. Tissues were fixed by neutral buffered formalin 10% and paraffin embedded tissue sections from these specimens received were made and used for the study. Tissue sections of 4 to 5 micron thickness were cut manually using microtome and stained with haematoxylin and eosin stains for histological typing and grading of lesions and sections on slides coated with gelatin, chrome alum mixture, were subjected for immunohistochemical staining for VEGF expression by prostate cancer cells for the evaluation in the study.

The study is based on the immunohistochemistry of the cancer cells of the prostate.

Avidin biotin peroxidase method and 3,3-diaminobenzidene(DAB) Chromogen are applied for immunohistochemical analysis. Endogenous peroxidase activity blocked with 0.6% hydrogen peroxide.

Antigen retrieval was done using microwave by incubating the slides in 10mmol/L TRIS EDTA buffer with a pH of 9, for VEGF antibodies. After blocking, sections are incubated at room temperature for 2 hours with antibodies to VEGF which are monoclonal mouse antibodies.

After treatment with antibodies, the slides were rinsed with tris buffer solution and treated with horse radish peroxidase label at room temperature for about 30minutes. Again the slides were rinsed with tris buffer solution and treated with DAB chromogen and hematoxylin for counter staining.

Gleason's scoring and grading was done with hematoxylin and eosin stained consecutive sections and staged accordingly with TNM staging.

The level of expression was assessed by evaluating the percentage, intensity and site of chromogen appearance and statistically evaluated along with Gleason`s scoring.

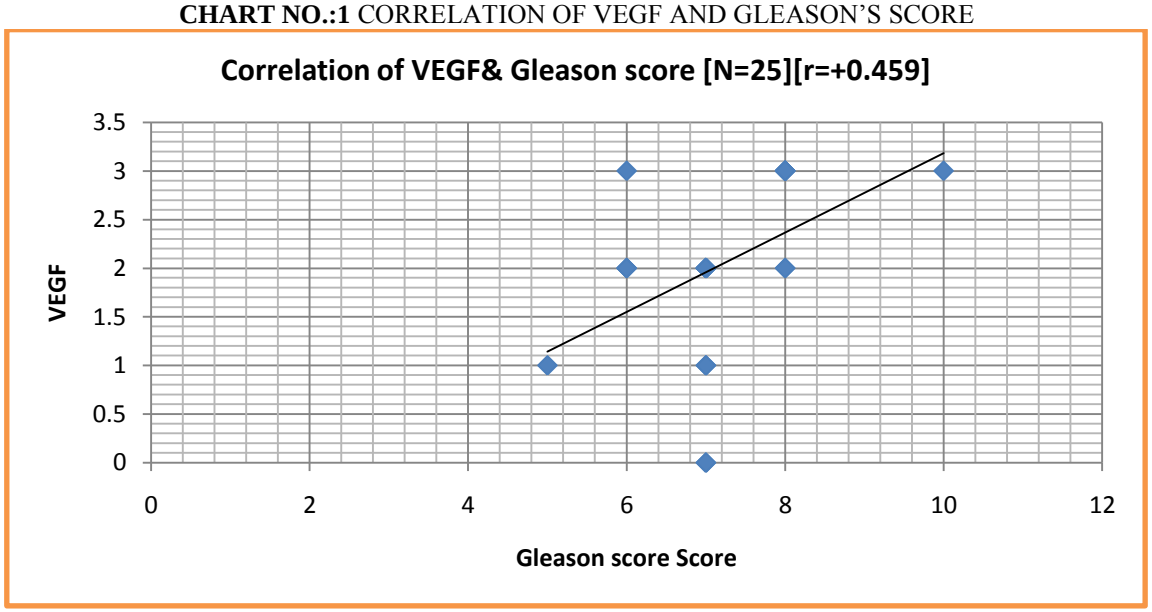
Inadequate and improperly fixed specimens were excluded from the study.

III. Observation And Results

TABLE NO.:1 COMPARING THE IHC EXPRESSION OF VEGF MARKER

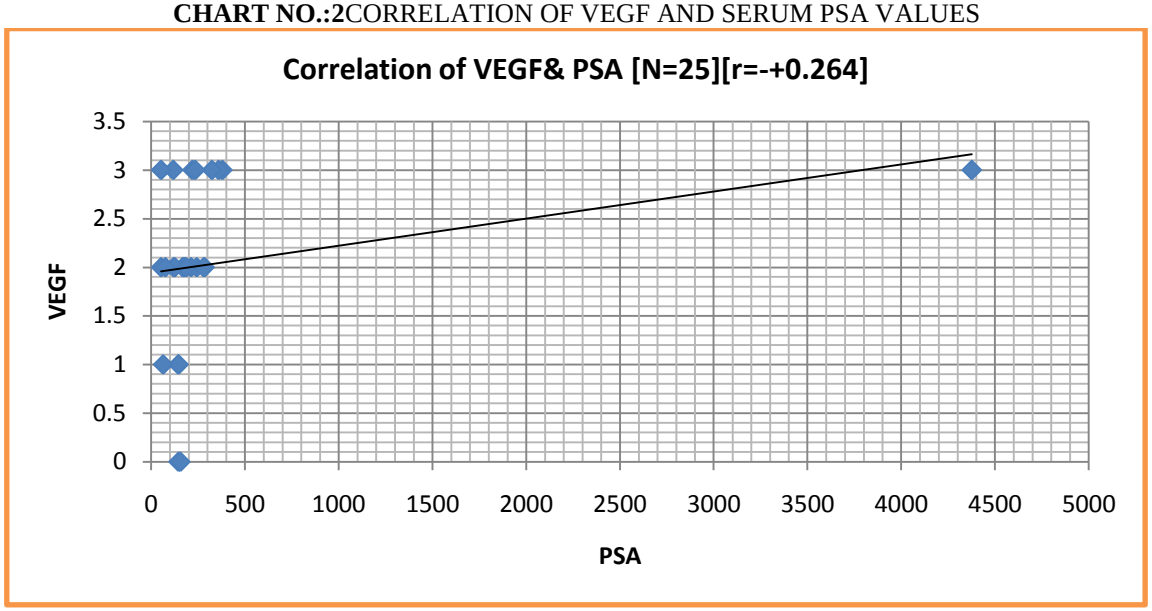
VEGF	n	(%)
1+	3	12%
2+	12	48%
3+	8	32%
Neg	2	8%
Total	25	100%

CHART NO.:1 CORRELATION OF VEGF AND GLEASON'S SCORE



There is almost a linear plot on comparing the expression of VEGF and Gleason's score, with a strong expression as the score increases to the maximum of 10(5+5). Vide chart no. 1

CHART NO.:2CORRELATION OF VEGF AND SERUM PSA VALUES

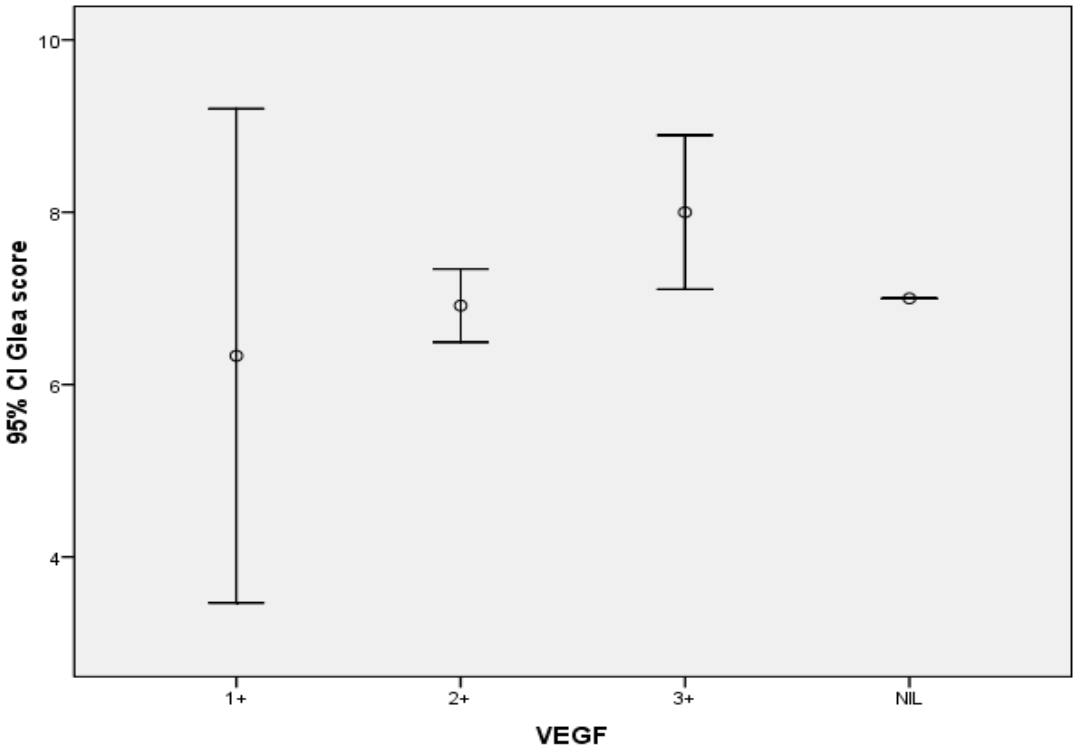


The scatter diagram shows clustering of the expression intensity values with increasing PSA values and lower number of negative cases. There is also strong expression of VEGF with the maximum PSA value of 4376ng/ml

TABLE NO.:2COMPARISON OF MEAN OF GLEASON WITH VEGF

				95% CI for Mean				
	VEGF	Mean	SD	Lower	Upper	Minimum	Maximum	Sig
Gleason score	1+	6.3	1.2	3.5	9.2	5	7	
	2+	6.9	0.7	6.5	7.3	6	8	
	3+	8.0	1.1	7.1	8.9	6	10	
	NIL	7.0	0.0	7.0	7.0	7	7	<0.05
	Total	7.2	1.0	6.8	7.6	5	10	

CHART NO.:3 COMPARISON OF MEAN OF GLEASON WITH VEGF

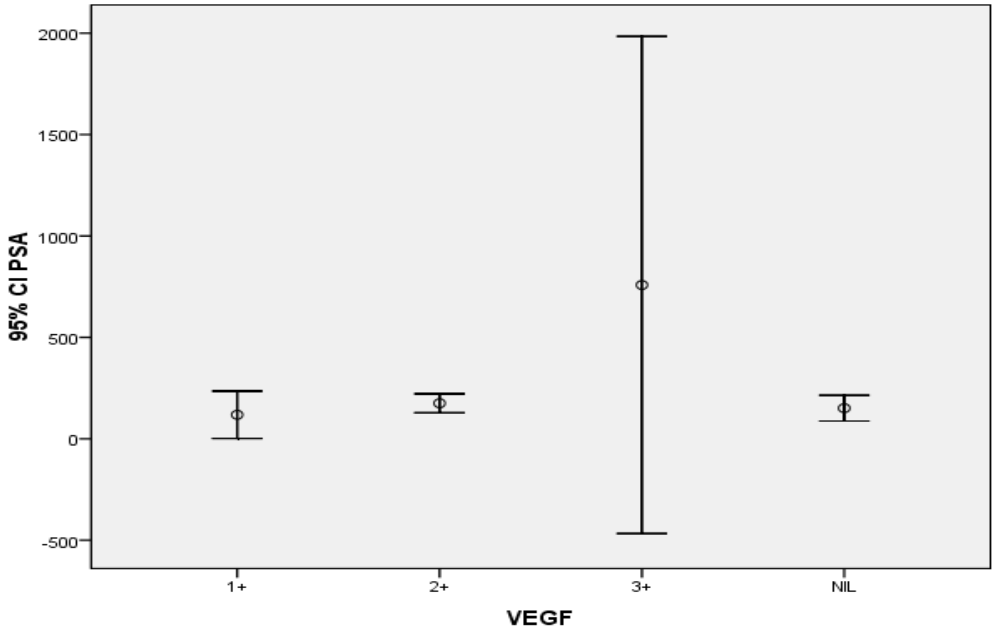


On comparing the mean value of Gleason score and the expression of VEGF, it was found that, there was increased and strong expression of VEGF with increasing scores. The maximum score of 10 showed intense 3+ positivity and the minimum score of 5 showed 1+ positive staining with a significant p value of<0.05 vide chart no. 3.

TABLE NO.:3 COMPARISON OF MEAN OF PSA WITH VEGF EXPRESSION

				95% CI for Mean				
	VEGF	Mean	SD	Lower	Upper	Minimum	Maximum	Sig
PSA	1+	118.3	47.1	1.4	235.2	64	146	
	2+	175.3	73.5	128.6	222.1	53	285	
	3+	758.3	1466.2	-467.6	1984.1	53	4376	
	NIL	151.0	7.1	87.5	214.5	146	156	>0.05
	Total	353.1	842.9	5.1	701.0	53	4376	

CHART NO.: 4 COMPARISON OF MEAN OF PSA WITH VEGF EXPRESSION



The analysis of the comparison between PSA values and that of VEGF expression show that, though there was intense 3+ positivity with the maximum value of 4376ng/ml, there was also intense 3+ positivity with a minimum value of 53ng/ml. this gives us a nil significant p value of >0.05, vide table no.3 and chart no.4.

Statistical Analysis:
The data are reported as the mean +/- SD or the median, depending on their distribution.
Frequencies are expressed in percentages.
The differences in quantitative variables between groups were assessed by means of the unpaired t test.
Comparsion between groups was made by the Non parameteric Mann - whitney test.
ANOVA was performed.
The chi square test was used to assess differences in the categoric variables between groups.
A p value of <0.05 using a two-tailed test was taken as being of significance for all statistical tests.
All data were analysed with a statistical software package .(SPSS, version 16.0 for windows)

IV. Discussion

Despite earlier diagnosis and smaller tumor volumes, many of the patients with clinically organ confined prostate cancer, found to have extra prostatic disease subsequent to radical prostatectomy. Advanced staging are determined by Gleason score, serum PSA, and clinical staging. Of them Gleason scoring and PSA levels are the most important.

Both serum PSA and Gleason scoring provide significant prognostic information as individual variables when their values are at the extremes. But, they cannot be used to assess the risk of progression and metastatic potential. Serum PSA levels have to be correlated with histopathological diagnosis, as it may be elevated in benign conditions and monitoring PSA levels help in identifying early secondaries in already diagnosed cases. It cannot help in assessing the risk of progression and metastatic potential of prostate cancer.

Though Gleason score helps in grading and differentiating the tumor, exact assessment of risk for progression and metastatic potential cannot be made into well, moderate and poorly differentiated types. Molecular studies have to be correlated with elevated scores, like the present study.

This study was done to identify prostatic carcinoma with aberrant or increased expression of VEGF for treatment strategies and to correlate these findings with PSA levels and Gleason scoring.

VEGF, a potent angiogenic factor has also been found to be overexpressed in prostate cancer in comparison with normal epithelium or benign prostatic hyperplasia. Thus, identifying such angiogenic factors involved in prostate cancer growth and understanding their regulation will lead to the development of anti-angiogenic strategies useful for diagnostic studies and therapeutic interventions.

Currently, new pathologically important prognostic factors are being investigated like, Ki67, p53, microvessel density, etc. but better biomarkers of disease progression like angiogenesis factors, will be useful in predicting the progression or prognosis[10,11].

Tumor cells are stimulated by angiogenic factors to result in angiogenesis and nodal metastasis associated with bone marrow metastasis. Tumor cells without nutritional factors, oxygen or angiogenic factors cannot survive beyond 2-3 mm of dimension, anywhere.

One of the factors induced by hypoxia, leading to the formation of neovascularisation is VEGF-Vascular Endothelial Growth Factor, whose expression closely relates with advanced progression of the disease state- particularly malignancy.[10] VEGF stimulates angiogenesis and increases microvessel density, indirectly stimulating tumor growth and prostate cancer elsewhere from the primary tumor site.

Earlier studies were controversial that benign conditions had more expression of VEGF. But current and recent studies by Wu et al, in their work on Benign and malignant prostatic epithelium, they detected a vast difference in the VEGF immunoreactivity by tumor cells of prostate- which is contrary to the older studies.[12]

Also, in the study by Jose luis et al, on plasma levels of VEGF in patients with metastatic prostate cancer, it was confirmed that the median level of plasma VEGF was 28.5pg/ml in patients with metastasis.[12] While the same was about 7pg/ml in patients with localised disease and 0pg/ml in normal disease free controls. This statistically significant difference gave the conclusion that plasma VEGF levels are higher in patients with metastatic prostate cancer, than those with localised disease or healthy individuals.

Similarly the study on VEGF expression in human prostate cancer: in situ and in vitro expression of VEGF by human prostate cancer cells by Fernando A.Ferrer "demonstrated that in 20 of 25 specimens, prostate cancer cells stained positively for VEGF. BPH and normal prostate cells displayed little staining for VEGF".[15]So, they concluded that significant expression was present with VEGF in prostate cancer, but not with BPH or normal prostate

So, as per the study by Clara Hwang and Elisabeth I Heath in their review article titled, "Angiogenesis inhibitors in the treatment of prostate cancer", inhibition of angiogenesis can be made one of the relatively novel anti –neoplastic approaches, which can target the dependance of tumor growth on the formation of newer blood vessels. The same strategy has been in use successfully in other cases of solid tumor types like breast, lung, colon,etc.[17] A similar situation is not far, for treating prostate cancers with extra prostatic extension, as studies are already on with antiangiogenic therapies, targeting the organs.

The conclusion of the research article titled, "Prognostic value of Vascular Endothelial Growth Factor expression in patients with prostate cancer: a systematic review with meta-analysis, by Kai Wang, Hong-Ling Peng and Long-Kun Li, states that VEGF can be regarded as a better prognostic marker for prostate cancer as per their meta-analysis.[20] "But to achieve a more definitive conclusion enabling the clinical use of VEGF in prostate cancer, we need more high quality interventional original studies following agreed research approaches or standards" and the same is emphasised.cellsinvivo. The invitro studies showed a differential regulation of angiogenesis factor expression in prostate malignancy. Hence identifying these angiogenic factors that are involved in cancer growth and a better understanding of their regulation will lead us to the development of necessary anti-angiogenic modalities useful for diagnostic and therapeutic interventions.

Another study titled, Metastatic properties of prostate cancer cells are controlled by VEGF done by Chen J1, De S, Brainard J, Byzova TV concluded that prostate cancer cells expressed Vascular endothelial growth factor (VEGF), its receptors (VEGFRs) and alpha5beta1 integrin, in vitro and by the same prostatetumors in vivo.[18] The expressionof these factors were elevated at the sites of bone metastasis when compared to the original prostate tumor itself. VEGF, by means of interaction with its receptors, it regulated the adhesive and migratory properties of these cancer cells. Also in the study "Vascular Endothelial Growth Factor (VEGF) Expression in Prostate Cancer and Benign Prostatic Hyperplasia byMichael W. Jackson, Jacqueline M. Bentel, Wayne D. Tilley", where they studied the immunohistochemical localisation of VEGF in both malignant and non-malignant conditions of prostate.[9] They finalised that there was widespread distribution of VEGF receptors in the prostate cancers and also the BPH specimens suggesting that the VEGF165, VEGF189 isoforms and the novel 90 and 110 kD forms , when detected may contribute to the authentic establishment or progression of these conditions.

V. Conclusion

The expression of VEGF was strong in a majority of 48% of cases and negative staining was observed in a minimum of 8% of cases.

There is significant correlation in the expression of VEGF in comparison with Gleason score, confirming that with progression of the disease all these parameters are elevated(p value<0.05).

There is no correlation with PSA values and VEGF, as the PSA values are elevated in benign lesions also and there is no significant correlation, p value>0.05.

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