RESEARCH ARTICLE

Plasma Vascular Endothelial Growth Factors A and C in Patients undergoing Prostatic Biopsy and TURP for Suspected Prostatic Neoplasia

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Abstract

Background: Formation of new blood vessels is necessary for the development and spread of neoplasms more than 1 mm^3 in volume, angiogenesis being responsible for formation of new from pre-existing blood vessels. Vascular endothelial growth factor (VEGF) is pivotal and the best studied angiogenic factor in all human cancers. Therefore we designed this study to investigate the role of VEGF-A and VEGF-C in prostate cancer in comparison with BPH controls in a north Indian population. Methods: In this case-control study a total of 100 subjects were included on the basis of confirmed histopathological reports, out of which 50 were prostate cancer patients and the other 50 were BPH patients with PSA levels >2 ng/ml and abnormal digital rectal examination (DRE) findings during September 2009 to August 2011 from the Department of Urology, KGMU, Lucknow, India. Plasma levels of VEGF were determined using quantitative immunoassay (ELISA- enzyme linked immunosorbent assay). Statistical analysis was carried out using SPSS 15.0 version. Results: The mean age of prostate cancer (67.6±5.72) patients was significantly higher (p=0.005) than BPH (63.6±7.92) patients. Expression of VEGF-A was not significantly higher in disease stage C1 than D1 or D2 and A or B (p=0.13) while the level of VEGF-A was significantly higher (p=0.04) in prostate cancer as compared to BPH subjects (PCa=13.0 pg/ml, BPH=6.8 pg/ml). Levels of VEGF-C were similar in both groups (PCa=832.6 pg/ml, BPH=823.7 pg/ml). In ROC curve, the area under curve (AUC) was 0.70 (95%CI: 0.60-0.80) and the cut-off value for which a higher proportion of patients was correctly classified (20%) was 26.0 pg/mL. Conclusion: Although VEGF-A is increased in cancer prostate patients a statistically significant correlation could not be established in this study. VEGF-C was not found to be a useful biomarker.

Keywords: Prostate cancer - BPH - angiogenesis - VEGF forms - prognostic marker

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Introduction

The most common pathological processes of prostate gland among aging men are benign prostatic hyperplasia (BPH) and prostate cancer (PCa). Although the specific reason for the initiation and progression of prostate cancer is not established.Prostate cancer is now recognised as a frequent cause of morbidity and mortality with 17,210 new cases registered in England and Wales in 1993 and 8570 deaths (Majeed et al., 2000). Benign prostatic hyperplasia (BPH) is a histological diagnosis that can produce lower urinary tract symptoms,which led to 1.9 million physician visits in the USA in 1991 (Mc Connell et al., 1994). Both conditions represent a major health care burden and a greater understanding of their pathophysiology and natural history is required. However, the etiology and pathogenesis of prostate cancer is poorly understood. Treatment strategies for these patients include active surveillance,radiation therapy and surgery (Zilinberg et al., 2012).

Risk factor for development of prostate cancer include demographical causes; aging, smoking, diet, obesity, alcohol consumption or genetic alteration. Prostate comprises three zones: anterior fibro-muscular zone, it contains 30% of prostate mass and no glandular element; peripheral zone, it contains 75% of prostate glandular element and is the site of prostate carcinoma; and central zone, it contains 25% of prostate glandular element and is the site of benign prostatic hyperplasia (Van der Heul-Nieuwenhuijsen et al., 2006). Abnormal prostate growth is responsible for aberrant cell proliferation, vascular permeability and angiogenesis.

Prostatic specific antigen (PSA) is widely used for prostate cancer diagnosis, even with its low accuracy

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across different cut-offs (Brawer, 2000). However, the need to avoid unnecessary biopsies and missed diagnosis has led to the study of several other biomarkers that could further help in deciding which of the patients should be referred for prostatic biopsy. Despite the combination of prostate specific antigen (PSA) molecular forms and other biomarkers have improved prostate cancer detection substantially, the survival rate of patients is still not optimistic.

Development and progression of prostate cancer (PCa) is associated with the growth of an adequate blood supply by means of angiogenesis (Nicholson et al., 2004) because blood circulation and a proper channel of blood vessels are essential for the development of cancer. Angiogenesis, formation of new blood vessels from the pre-existing blood vessel also known as neo-vascularisation, therefore may serve as diagnostic and prognostic marker for prostate cancer. Vascular endothelial growth factor (VEGF) is pivotal and best studied angiogenic factor. Its family includes VEGF-A, VEGF-B, VEGF-C, and VEGF-D. VEGF-A is the main pro-angiogenic factor and plays an important role in vascular permeability. VEGF-A binds and activates two receptors VEGFR1 and VEGFR2, and has varying role in the promotion of endothelial cell differentiation, cell growth, tubular formation, and migration (Ferrara et al., 2007). VEGF-C is essential for lymphangiogenesis by interacting with VEGFR-3 receptor (Yancopoulos et al., 2000) usually co-expressed at the site of lymphatic vessel sprouting, in embryo, and in various pathological conditions.

VEGF is necessary for the establishment of hematopoiesis (Kowanetz et al., 2006), while in pathological state, VEGF promote tumor angiogenesis and vascular permeability.All these evidences mean that VEGF plays a critical role in tumoregenesis and brings a prerequisite value for metastasis.

VEGF increase is the hallmark of all human cancer. Normal prostate tissue usually expresses no or low concentration of VEGF (Doll et al., 2001). Circulating levels of VEGF to predict disease staging and early identification of patients at higher risk are still under study and can provide important advances in prostate oncology. Circulating level of VEGF can be detectable in patients with PCa and BPH may be a marker of the degree and activity of cancer angiogenesis. Therefore we designed this study to investigate the role of VEGF-A and VEGF-C in prostate cancer and its correlation with BPH controls in north Indian population.

Materials and Methods

Study design and site

This was a case-control study. The PCa subjects were considered as cases and BPH subjects served as controls. The study was conducted at Department of Urology and Department of Pathology, King George Medical University (KGMU), Lucknow, U.P. India. In this case-control study, total of 100 subjects were included on the basis of confirmed Histopathological report, out of which 50 were prostate cancer patients and the other 50 were BPH patients with PSA level >2 ng/ml and abnormal digital rectal examination (DRE) investigation during September 2009 to August 2011. Blood sample were collected in plain vial for the analysis of total PSA and free PSA and in EDTA vial for the VEGF analysis and stored at -800C before TURP (Trans-Urethral Resection of Prostate) or any prostate oncology treatment. Clinical staging of the disease was done by Whittmore-Jewett method. Further, the biopsy tissues were sent to pathology department and Histopathological grading was done by consultant using Gleason's scoring system. The study was ethically approved by the Ethical Committee of the KGMU, Lucknow, UP, India. The informed consent was obtained from all the participants prior to sample collection.

Laboratory Assessment

Blood samples were collected from the subjects and allowed for clotting for 40 minutes and then centrifuged at 3000 rpm for 10 minutes for the separation of serum for PSA and plasma for VEGF analysis. Pre-diagnostic VEGF-A concentrations were assayed using the human VEGF-A enzyme-linked immunosorbent assay kit (ELISA) as given in protocol Catalog Number: DY293B (R & D systems Inc., Minneapolis, MN 55413, USA). Pre-diagnostic VEGF-C concentrations were determined using the human VEGF-C enzyme-linked immunosorbent assay kit (ELISA) as given in protocol Catalog Number: BMS297 (Bender Med Systems, Austria, Europe). All the samples were assayed in duplicate using microplate luminescence detection system. Solutions required other than the kit were TMB substrate DY999 (R & D systems, USA) and reagent diluents DY997(R & D systems, USA), PBS, wash buffer and stop solution.

Baseline total PSA and free PSA test of cases and controls, which had been done for the screening of PCa and BPH respectively in the Department of Pathology, CSM Medical University, Lucknow, UP, India, were recorded for study.

Statistical analysis

The data collected was entered in Microsoft Excel sheet and checked for any inconsistency. The results are presented in mean (±sd) and percentages with its 95% confidence intervals (CI). The categorical/dichotomous variables are compared by using Chi-square and continuous variables are compared with independent t-test. The data is tested for normally by using Kolmogorov-Smirnov test. The Spearman's correlation coefficient is used to find out correlation between two variables. A receiver operating characteristic (ROC) analysis is used to compute the area under the ROC curve (AUC) and to identify the VEGF level cut-off for which a higher proportion of patients are correctly classified when distinguishing prostatic cancer from BPH. Unconditional logistic regression analysis was carried out to find out the risk factors for prostate cancer. The p-value<0.05 was considered as significant. All the analyses were carried out by using SPSS 15.0 version.

Results

The mean age of prostate cancer (67.56±5.72)

patients was significantly higher (p=0.005) than BPH (63.56±7.92) patients. There was no difference in the history of diabetes and hypertension between prostate cancer patients and BPH patients. Habit of smoking, use of tobacco/pan Masala and alcohol was similar in both the groups. However, vegetarian dietary habit was significantly (p=0.04) lower in prostate cancer (46%) patients as compared to BPH (66%) patients. More than half of the prostate cancer patients were in stage C1 or C2 (56%) followed by A or B (24%) and D1 or D2 (20%). The median tPSA (PCa=19.7ng/ml, BPH=4.2 ng/ml), fPSA (PCa=4.8 ng/ml, BPH=1.2 ng/ml) and VEGF-A (PCa=13.0 pg/mL, BPH=6.8 pg/mL) was significantly higher in prostate cancer patients as compared to BPH patients. However, VEGF-C was almost similar in both the groups (PCa=832.6 pg/mL, BPH=823.7 pg/mL) (Table 1).

The age (r=0.29, p>0.05), BMI (r=0.34, p>0.05), tPSA (r=0.32, p>0.05), fPSA (r=0.09, p>0.05) and f/t PSA ratio (r=-0.23, p>0.05) were poorly correlated with VEGF-A in prostate cancer patients. However, tPSA was strongly correlated with VEGF-A (r=0.98, p<0.01) in BPH patients. Age, BMI, fPSA and f/tPSA ratio were not correlated with VEGF-A in BPH patients (Table 2).

The multivariate logistic regression analysis revealed that only age (adjusted OR=1.10, 95%CI=1.01-1.18) was significantly associated with the risk of prostate cancer. The higher the VEGF-A (adjusted OR=1.02, 95%CI=0.97-1.06), the risk of prostate cancer was higher, however, this was statistically insignificant (p=0.49) (Table 3).

The ROC curve of VEGF-A plasma levels for the detection of prostate cancer is presented in Figure 1. The area under curve (AUC) was 0.70 (95%CI: 0.60-0.80) and the cut-off value for which a higher proportion of patients was correctly classified (20%) was 25.96 pg/mL.

In prostate cancer patients, the mean VEGF-A levels

Table 1. Baseline Characteristics of BPH and Prostate Cancer Patients

	BPH (n=50)	Prostate Cancer (PCa) (n=50)	p-value
Age in years			
Mean±sd	63.56±7.92	67.56±5.72	0.005*
<60	17 (34.0%)	3 (6.0%)	0.002*
60-70	25 (50.0%)	35 (70.0%)	
>70	8 (16.0%)	12 (24.0%)	
Diabetic	3 (6.0%)	4 (8.0%)	0.7
Hypertensive	4 (8.0%)	7 (14.0%)	0.34
Vegetarian dietary habit	33 (66.0%)	23 (46.0%)	0.04*
Smoker	13 (26.0%)	7 (14.0%)	0.13
Use of tobacco/pan masala	15 (30.0%)	14 (28.0%)	0.83
Alcoholic	1 (2.0%)	2 (4.0%)	0.56
BMI	23.55±2.53	25.48±3.82	0.003*
Disease stage			
A or B		12 (24%)	
C1 or C2		28 (56%)	
D1 or D2		10 (20%)	
Median Total PSA (ng/ml)	4.2	19.7	<0.0001*
<4	24 (48.0%)	2 (4.0%)	<0.0001*
4-10	15 (30.0%)	14 (28.0%)	
>10	11 (22.0%)	34 (68.0%)	
Median F-PSA (ng/ml)	1.2	4.8	<0.0001*
<1	24 (48.0%)	3 (6.0%)	<0.0001*
1-2	6 (12.0%)	17 (17.0%)	
>2	20 (40.0%)	36 (72%)	
f/t PSA ratio	0.38±0.50	0.35±0.27	0.71
VEGF-A (pg/ml)	6.8	13	0.04*
<5	18 (36.0%)	15 (30.0%)	0.02*
5-10	13 (26.0%)	4 (8.0%)	
>10	19 (38.0%)	31 (62.0%)	
VEGF-C (pg/ml)	823.7	832.6	0.62
<800	21 (42.0%)	19 (38.0%)	0.89
800-900	15 (30.0%)	17 (34.0%)	
>900	14 (28.0%)	14 (28.0%)	

*Significant

Table 2. Correlation (Spearman correlation coefficient) between VEGF-A and Other Factors

Factors	BPH	Prostate cancer
Age	-0.16	0.29
BMI	0.05	0.34
tPSA	0.95**	0.32
fPSA	0.48*	0.09
f/t PSA ratio	-0.18*	-0.23

*p<0.05, **p<0.01

Table 3. Factors Associated with the Risk of Prostate Cancer-Unconditional Multivariate Logistic Regression

Factors	Beta coefficient	S.E.	Adjusted odds ratio	95%CI of OR	p-value
VEGF-A	0.015	0.021	1.02	0.97 1.06	0.488
tPSA	0.061	0.035	1.06	0.99 1.14	0.08
fPSA	0.142	0.108	1.15	0.93 1.43	0.19
f/tPSA Ratio	-0.224	0.822	0.8	0.16 4.01	0.78
Age	0.09	0.039	1.1	1.01 1.18	0.02*
BMI	0.117	0.084	1.13	0.95 1.33	0.16
Constant	-10.354				

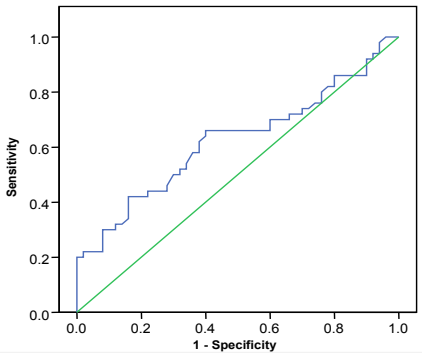

Figure 1. Receiver Operating Characteristic (ROC) Curves for Vascular Endothelial Growth Factor (VEGF) Serum Levels as a Test for Diagnosis Prostate Carcinoma Using the Biopsy Results as the Gold Standard (Area under ROC curve=0.70 (0.60-0.80)

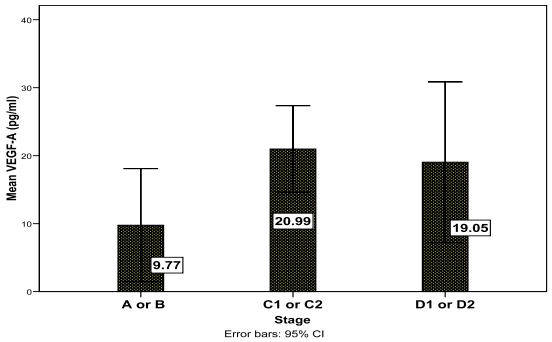

Figure 2. VEGF-A (pg/ml) Level by Stage of Prostate Cancer (p=0.13)

were insignificantly higher in disease stage of C1 or C2 (20.99, 95%CI = 14.63-27.35) than D1 or D2 (19.05, 95%CI=7.24-30.85) and A or B (9.77, 95%CI=1.46-18.00) (Figure 2). The VEGF levels were not significantly different across Gleason score groups. The median values were 14.0 pg/ml for patients with histological Gleason score <6, 3.14 pg/mL for those with Gleason score 6, 17.78pg/ml for those with Gleason score 7 and 19.0 pg/ml for those with Gleason score between 8-10 pg/ml.

Discussion

As is demonstrated by plenty of studies, angiogenesis play a crucial role in cancer pathogenesis, progression and metastasis, while tumor can't grow rapidly or metastasize to distant organs without vessels (Sitohy et al., 2012). The core processes was involved in the interaction of vessel oxygenation-perfusion and tumor stimulating (Carmeliet et al., 2011). Yamamoto et al. (1996) initially measured the circulating level of VEGF in serum samples. The level of VEGF was higher in cancer patients as compared to normal individuals. Subsequently, angiogenesis is the object of intense research. In our study, we correlated plasma levels of VEGF with various parameters associated to prostate cancer, such as age, dietary habit, total PSA, Free PSA, histological grade and Clinical stage. Sudip Shah et al. (2011) and Nath et al. (2012) found that levels of free PSA were higher in age categories of 60-75 and >74 and the mean f-PSA with in different groups was statistically significant (p=0.031).

We found higher circulating level of VEGF in prostate cancer subjects as compared to BPH controls and it was statistically significant (P=0.04, Table 1). This was in agreement with various studies in which higher level of VEGF in prostate cancer patients were found when compare with BPH or healthy controls (Caine et al., 2004; Shariat et al., 2004; Trapeznikova et al., 2004; Trapeznikova et al., 2005). On the other hand (Walsh et al., 1999; Peyromaure et al., 2005; Francisco et al., 2010) did not found statistically significant association between BPH and cancer patients in the circulating level of VEGF. We measured circulating levels of VEGF in plasma since it offers a more reliable measure of circulating VEGF levels as compared to serum because VEGF is present in platelet and is released during the clotting process. Circulating VEGF in serum from cancer patients may reflect an aggregate of tumor-cel-l and platelet-stored VEGF. One study also suggests that measurement of VEGF concentration in platelet-poor plasma may be most dependable method to measure circulating VEGF levels (Wynendaele et al., 1999).

These 7 studies (West et al., 2001; Fuduka et al., 2007; Green et al., 2007; Peyromaure et al., 2007; Mori et al., 2010; Wang et al., 2011; Weber et al., 2012) used cancer tissue specimen, while 3 studies (George et al., 2001; Shariat et al., 2004; Svatek et al., 2009) used plasma specimen and specially Bok's study using urine, VEGF level was detected as the major research target and also found high VEGF expression as an indicator of poor prognosis. The first prognostic significance of plasma VEGF levels in patients with hormone-refractory prostate cancer was first proved by George et al. (2001).

Such comparisons however, are not clinically relevant since elevated tPSA is the most common biomarker for the screening of prostate cancer and to recommend that who may go for prostatic biopsy, and reflect limited-challenge-bias (Rutjes et al., 2006). However, still there is a need for search of new diagnostic marker with high sensitivity and specificity to screen the prostate cancer because clinician can't always depend on PSA, since Thompson et al. (2004) detected prostatic cancer in 10.1 percent among those with PSA values of 0.6-1.0 ng/mL, 17.0 percent among those with values of 1.1-2.0 ng/mL, 23.9 percent among those with values of 2.1-3.0 ng/mL, and 26.9 percent among those with values of 3.1-4.0 ng/mL. These values can lead to a differential information bias that would cause an underestimation of the true association measure.

At present, many studies have demonstrated that the expression of plasma VEGF has no association with Gleason score in prostate cancer (Peyromaure et al., 2005; Trapeznikova et al., 2005). In our study, we also found that baseline levels of circulating VEGF were not significantly different across Gleason score groups, but the plasma level of VEGF was insignificantly higher in Gleason score ≥7 and in cancer stage C than D, A or B (P=0.13) (Figure 2). These findings suggest that VEGF have a role in risk of prostate cancer and tumor development and progression. Haojie et al. (2005) found no correlation in pre-diagnostic values of VEGF and Gleason score but opposite to our result they found drop off value of VEGF with advance stage of prostate cancer (stage C or D) (P=0.16).

Francisco et al. (2010) found no independent association between VEGF and prostate cancer when compared to BPH. Whereas Shariat et al. (2004) found a statistically significant preoperative elevated plasma level of VEGF in patients with Gleason score ≥7 (P=0.02) and in patients with localized cancer than healthy controls (P<0.001). Jie et al. (2006) had performed a study to investigate the expression of VEGF and VEGF-C between BPH and PCa tissues and found significantly elevated value of VEGF and VEGF-C in PCa tissue as compared to Benign tissue (P<0.01). VEGF could also be important from the clinical point of view if it's levels were higher in patients with worst prognosis prostatic cancer (those with higher Gleason score or in higher clinical stage) (Duque et al., 1999).

We have not separated our data in localized prostate cancer and metastatic prostatic cancer due to the limited number of cases. However, differences between localized and metastatic prostate cancer have been reported Haojie et al. (2005) and Kohli et al. (2003).

We do not find any correlation between circulating levels of VEGF-C and prostate cancer (P=0.62) (Table 1). Zeng et al. (2004) also didn't not find any significant relationship between VEGF-C and prostate cancer with advance stage. On the contrary, one study had found that the expression of VEGF-C in tissues with stage D had a significantly higher level of VEGF-C as compared to stage A,B or C (P<0.01) (Jie et al., 2006). Therefore circulating levels of VEGF-C may be less effective than tissue VEGF-C levels; ongoing researches on this matter will help to explain this difference. The ROC analysis revealed

that the cut-off value, for which a 20% of patients were correctly classified, was 25.96 pg/mL. However, Francisco et al. (2010) has reported this as 57% in his studies.

Another study conducted by Voss et al. (2008) VEGF-C was also not found to be co-related with tumor stage. The latest view as described by Jain et al. (2009) indicates that VEGF single nucleotide polymorphisms (SNPs) predict the cancer susceptibility and relate to interindividual variation in anti-VEGF therapeutic response of prostate cancers. However, all these exciting reports on this topic provide conflicting evidence and so far none of these reports have brought great change in clinical practice.

In conclusion, total PSA and free PSA are good biomarkers to differentiate between BPH and Cancer Prostate patients. Although VEGF-A is increased in prostate cancer patients but statistically significant correlation could not be established in this study. VEGF-C was not found to be a useful biomarker. Prostatic biopsy is the current gold standard for diagnosis and staging of cancer prostate; but is a painful procedure with several complications. Till date the useful biomarker to differentiate between BPH and cancer prostate is total and free PSA, however the need of the hour is to search for new biomarker for diagnosing and staging of cancer prostate.

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