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**RESEARCH ARTICLE**

# Role of Microvessel density assessment in prostate cancer: correlation with clinical stage and Gleason's grade

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## *Abstract*

Prostate cancer is a classic malignancy and second most leading cause of cancer deaths. This study was conducted to investigate the diagnostic performance characteristics of MVD, assessed by the analysis of CD34 immuno reactivity in cases of CaP and correlating it with Serum PSA levels in determination of clinical staging. 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 PSA and free PSA were determined using quantitative immunoassay (ELISA- enzyme linked immunosorbent assay). Microvessel density assessment was done in digitalized images using BIOVIS image analysis system. CD34 immunohistochemistry performed on FFPE with anti CD 34 by Streptavidin Biotin Method as per standardized protocol. Statistical analysis was carried out using SPSS 15.0 version. The mean difference between BPH (84.40) and CaP (175.99) patient is statistically significant for CD34 staining. The CaP patients were categorized in 2 groups on the bases of MVD score (MVD<170, MVD>170). In clinical stage, data shows that the difference was statistically significant except stage A. In Gleason's score group, the distribution of MVD score shows significance difference in CaP patients. The present study confirms the existence of significant correlation between MVD and pathological stage of prostate cancer and MVD may suitable marker for the diagnosis as well as prognosis of BPH and CaP.

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## INTRODUCTION

Prostate cancer (CaP) is a typical malignancy and second most leading cause of cancer deaths among men in USA (Jemal et al, 2008). Worldwide, the incidence of cancer varies and has been increased per year. Benign prostatic hyperplasia (BPH) is a progressive condition characterised by prostate enlargement accompanied by lower urinary tract symptoms (LUTS) (Roehrborn et al, 2009, Bechis et al, 2014). Although BPH is uncommon before age 40, roughly 50% of men develop BPH related symptoms at 50 years of age. Prostate specific antigen (PSA) produced by both normal as well as neoplastic prostate epithelial is the most widely used biomarker in prostate cancer (Bhatt et al, 2010).

It is well known that tumour growth does not start until tumour cells acquire the ability to induce neo vascularisation (Pastushenko et al, 2014), and any increase in tumour mass must be preceded by vascular supply which helps not only to support growth, but also dissemination, invasion and metastases of tumour cells (Weidner et al, 1993). Angiogenesis plays a critical role in CaP progression, and its significance in human CaP has been firmly established. Several independent studies have documented a significant correlation between microvessel density (MVD) with Gleason score, pathological stage, and patient survival (Borre et al, 1998, Bono et al, 2002). MVD is considered to be a marker of the neo-angiogenic process, which is responsible for local growth (Folkman et al, 1976) and metastases in tumours (Pastushenko et al, 2014). MVD can be assessed on archival pathological slides through the evaluation of immunoreactivity to some endothelial antigens (CD34, CD31, Factor VIII etc.). CD34 is a suitable endothelial marker for immunohistochemically visualising microvessels in BPH and CaP. It is more sensitive of the three investigated markers for angiogenesis in prostate cancer (Trojan et al, 2004).
The purpose of the present study was to see whether there is a correlation between MVD, assessed by the analysis of CD34 immuno reactivity in cases of CaP and correlating it with Serum PSA levels in determination of clinical staging.

## Materials and methods:

In this case control study the patients with CaP were considered as cases and BPH served as controls. Total of 100 subjects were included on the basis of confirmed histopathological report, 50 of CaP patients and 50 of BPH patients with PSA level >2ng/ml and abnormal digital rectal examination (DRE) investigation in time interval September 2008 to August 2010. The study was conducted in the Departments of Urology and Pathology, K.G Medical University, Lucknow, U.P. India (Former C.S.M. Medical University).
Blood sample were collected in plain vial for the total PSA analysis and Clinical staging of the disease was done under Whittmore-jewett. Grading was done by Gleason's scoring system. Prostate Biopsy samples were collected for the confirmation of histopathological diagnosis, Gleason's scoring, CD-34 Staining. The study was ethically approved by the Ethical Committee of the K.G Medical University, Lucknow, U.P. India (Former C.S.M. Medical University). The informed consent was obtained from all the participants prior to sample collection.
Patients with cases of BPH/Carcinoma complicated with any other primary debilitating disease like TB/AIDS, advanced liver or renal disease etc. or any systemic vascular disorder, cases associated with any other 2$^{nd}$ malignancy in past or present, cases of Benign Prostatic Hyperplasia on dutasteride therapy and patients on steroid therapy were excluded from the study.
Blood level of total PSA and free PSA test of cases and controls, was done by enzyme-linked immunosorbent assay **(**ELISA**)** with commercially available kits. Microvessel density assessment was done in digitalized images (Leica DFC 320 camera mounted on ZiessAxiolab microscope) using BIOVIS image analysis system (Expert Vision, Mumbai, India). CD34 immunohistochemistry performed on FFPE with anti CD 34 (Formalin Fixed Paraffin Embeded Tissue) by Streptavidin Biotin Method as per standardized protocol. For IHC staining antibodies were obtained from Vision biosystems Novocastra
For this purpose, ten areas of densely concentrated microvessels (hot spots) were located using 40 x magnification (objective lens x 4 and ocular lens x 10). In each case, these hot spots areas were used for microvessel counting at 400 x magnifications (40 x objective lens, 10 x ocular lens). A vascular unit, as a cell or group of endothelial cells of a brownish colour, clearly separated from adjacent microvessels, tumor cells and other connective tissue. A vessel lumen, although usually present, was not a criterion for defining a microvessel. Partially identified vessels that were not totally contained in the fields analysed were not considered in the count. It was established that the total number of vessels obtained in each case would be a result of the total sum of vessels counted in each of the 10 microscopic fields evaluated at 400 x magnifications. Microvessel density was defined as the average number of microvessels per field, calculated from the total number of microvessel in 10 fields. The CD-34 staining was determined by, the golden brown colour obtained by the endothelial cells. Large round of irregular cells were excluded from determining the MVD. Only the round small and newly formed blood vessels were considered MVD.

**Statistical analysis**

Data were summarized as Mean ± SE. Groups were compared by one factor repeated measures analysis of variance (ANOVA) using general linear models (GLM) and the significance of mean difference within and between the groups was done by Bonferroni post hoc multiple contrasts test. A two-sided ($\alpha$=2) $p<0.05$ was considered statistically significant.

## Results:

The Table 1 shows the Demographic and clinical characteristics of the studied patients. Significant difference was found between BPH (63.56) and cancer patients (67.56) for the mean age. Statistically modest significant difference

was found in the mean values of BMI (kg/m$^2$) in BPH and CaP patients. The no association was found between BPH and CaP patients with clinical characteristics and life style habits like diabetes, hypertension, diet, smoking, tobacco and alcohol. The CD 34 staining (Mean ± SE) in BPH and CaP patients present in Table 2. The mean difference between BPH (84.40) and CaP (175.99) patient is statistically significant at p<0.01 for CD34 staining. The CaP patients were categorized in 2 groups on the bases of MVD score (MVD<170, MVD>170). In stage A, no significant difference was found, whereas highly significant difference was observed in stage B patients. In clinical stage C1-D1, data shows that the difference was statistically significant (p=<0.05). Due to the less number to cases in D2 clinical stage the statistically analysis was not done (Table3). In each Gleason's score group, the distribution of MVD score according to gleason score shows statistical significance difference in CaP patients (Table 4).

**Table 1 Demographic and clinical characteristics of BPH and CaP Patients**

| Characteristics | BPH(n=50) | CaP (n=50) | p value |
|---|---|---|---|
| Age (yrs) | 63.56 ± 7.92 (40-87) | 67.56 ± 5.72 (46-90) | 0.005$^{\$}$ |
| BMI (kg/m$^2$) | 23.55 ± 2.53 (17.97-28.89) | 25.48 ± 3.82 (14.15-33.31) | 0.003$^{\$}$ |
| Non-diabetic | 47 (94.0%) | 46 (92.0%) | 0.700* |
| Diabetic | 3 (6.0%) | 4 (8.0%) | |
| Non-hypertensive | 46 (94.0%) | 43 (86.0%) | 0.335* |
| Hypertensive | 4 (8.0%) | 7 (14.0%) | |
| Non-vegetarian | 17 (34.0%) | 27 (54.0%) | 0.036* |
| Vegetarian | 33 (66.0%) | 23 (46.0%) | |
| Non-smoker | 37 (74.0%) | 43 (86.0%) | 0.126* |
| Current Smoker | 13 (26.0%) | 7 (14.0%) | |
| Non- Tobacco user | 35 (70.0%) | 36 (72.0%) | 0.828* |
| Tobacco user | 15 (30.0%) | 14 (28.0%) | |
| Non-alcoholic | 49 (98.0%) | 48 (96.0%) | 0.567* |
| Alcoholic | 1 (2.0%) | 2 (4.0%) | |

(The values are given in Mean ± SD)
Numbers in parenthesis indicates the range (min-max) and the percentage (%) as applicable, *p value by Chi sq-test

**Table 2 CD 34 staining (Mean ± SE) of two groups**

| CD 34 staining | BPH (n=50) | CaP (n=50) | p value |
|---|---|---|---|
| CD34 staining (MVD) | 84.40 ± 11.38 (65.13-99.45) | 175.99 ± 42.40 (103.70-248.44) | p<0.01 |

**Table 3 Correlation between MVD and clinical stage**

| Clinical stage | n | MVD<170 (Mean±SE) | n | MVD>170 (Mean±SE) | p-value |
|---|---|---|---|---|---|
| A | 2 | 144.18 ± 25.90 | 2 | 214.10 ± 28.17 | NS |

| | | | | | |
|---|---|---|---|---|---|
| B | 4 | 137.73± 8.04 | 4 | 213.10±10.99 | <0.001 |
| C1 | 9 | 127.36± 6.73 | 3 | 197.49 ± 26.04 | <0.05 |
| C2 | 5 | 159.16± 3.87 | 11 | 210.33 ± 6.39 | <0.05 |
| D1 | 3 | 133.97 ± 22.45 | 3 | 212.57 ± 21.77 | <0.05 |
| D2 | 1 | 135.85 | 3 | 218.78 ± 18.32 | NC |

NC= Not Calculated, NS= Non Significant

**Table 4 Distribution MVD according to gleason score in CaP patients**

| Gleason score | MVD<170 | MVD>170 | *P value |
|---|---|---|---|
| 2-4 | 4 | 3 | <0.01 |
| 5-6 | 4 | 4 | <0.05 |
| 7 | 5 | 7 | <0.05 |
| >7 | 11 | 12 | <0.001 |
| Total | 24 | 26 | |

*Chi square p value

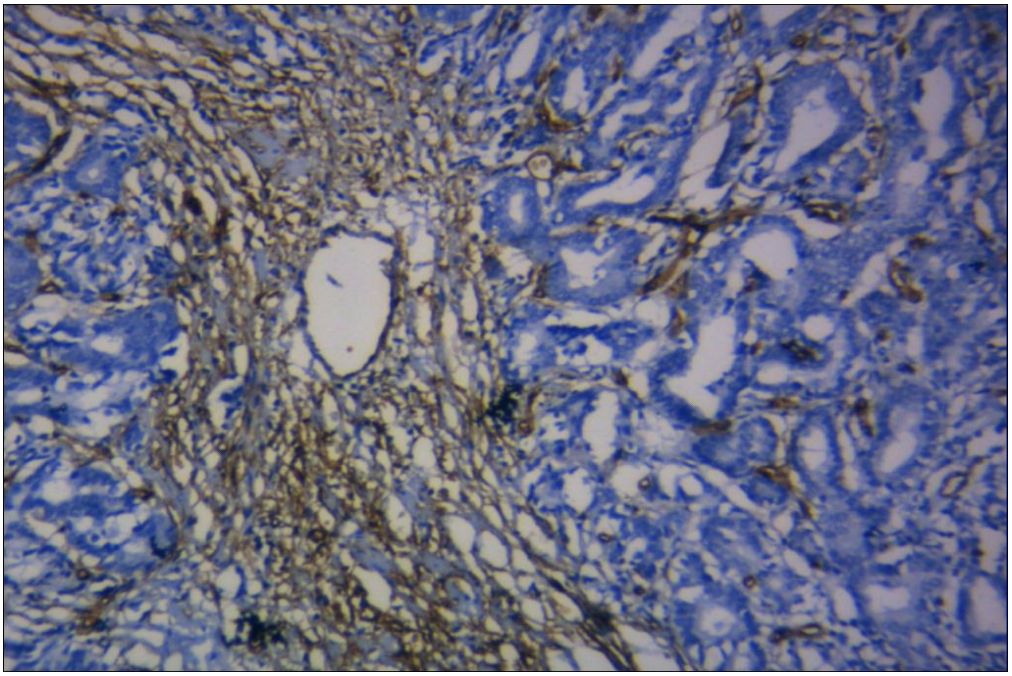

**Fig 1(a) Low power view of a case of PCa showing a hotspot area for MVD counting**

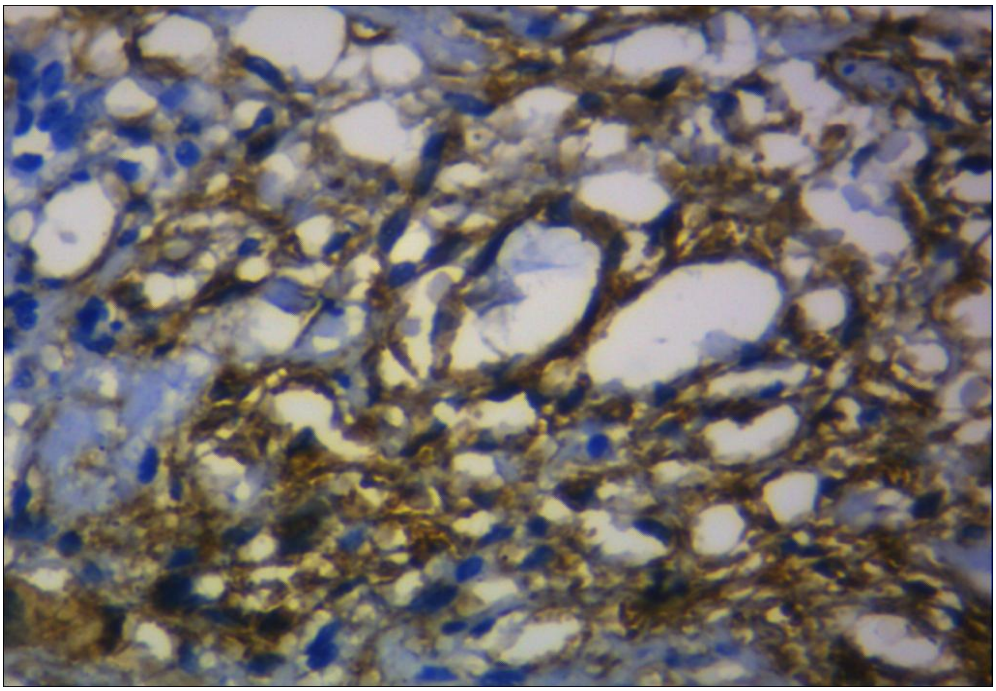

**Fig 1(b) High power view (×400) of PCa immune stained with CD34 demonstrating high MVD**

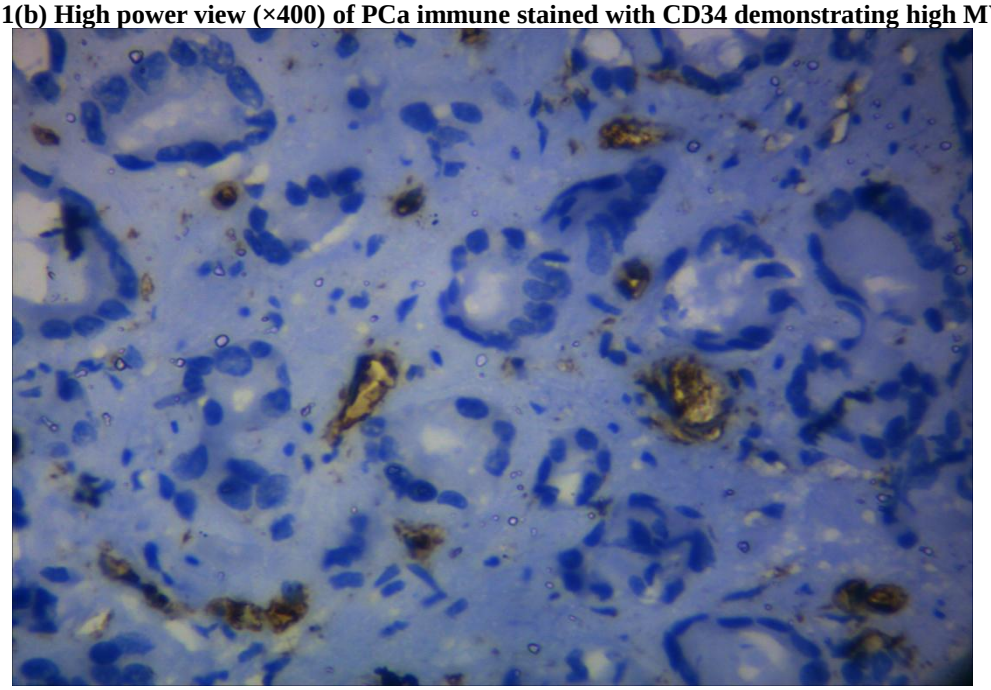

**Fig 2(a) Low power view of a case of BPH showing low MVD**

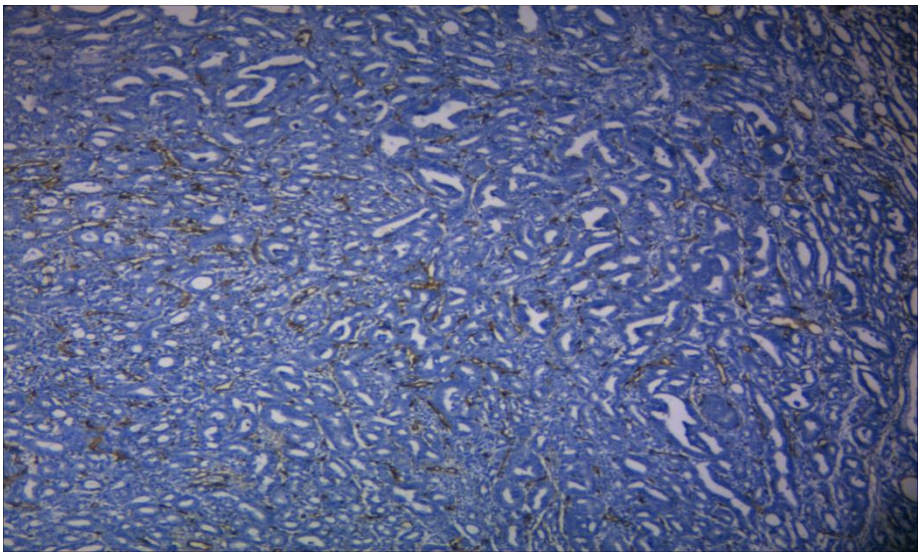

**Fig 2(b) High power view of a case of BPH showing low MVD.**

## Discussion:

Our observations confirm the existence of significant correlation between MVD and pathological stage of prostate cancer (Fig 1a, b and Fig 2a, b). This is in agreement with some reports **(L**uczynska et al, 2013), although in contrast with other study (Silbermann et al, 1997). The microenvironment, in which prostate cancer (PC) cells live, is crucial for the survival, progression and metastasis.The PC cells can either be stimulated or inhibited by specific stromal environments (Condon et al, 2005). Tumour associated angiogenesis, measured as vascular density by the use of immunohistochemistry (IHC) markers like CD34, to visualise the vasculature, has been shown to give prognostic information in variety of solid tumours including prostate carcinoma (Weidner et al, 1993). A correlation between angiogenesis and tumour grading has previously been reported in breast, gastric and cervix cancer (Warnberg et al, 2001; Tenderenda et al, 2001; Hollingsworth et al, 1995). Trojan et al., (2004) reported the, lack of significant correlation between MVD and clinical staging but observed significant correlation between MVD and Gleason's scoring. Our results have an agreement with this study. Our data showed that MVD assessed by using CD34 marker can be used as prognostic marker for CaP.

The assessment of angiogenesis is still experimental and not used in routine clinical diagnostics. Available research reports on angiogenesis in prostate cancer may well underline the importance of MVD assessment as a prognostic factor. We conclude that MVD assessment by CD34, is suitable marker for the diagnosis as well as prognosis of BPH and CaP and also useful for designing treatment strategy based on angiogenesis.

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## References

1. Jemal, A., Thun, M.J., Ries, L.A., Howe, H.L., Weir, H.K., Center, M.M., Ward, E., Wu, X.C., Eheman, C., Anderson, R., Ajani, U.A., Kohler, B., Edwards, B.K. (2008): Annual report to the nation on the status of cancer, 1975-2005, featuring trends in lung cancer, tobacco use, and tobacco control. J. Natl. Cancer. Inst., 3:1672-1694.
2. Roehrborn, C.G. (2009): Efficacy of alpha-Adrenergic Receptor Blockers in the Treatment of Male Lower Urinary Tract Symptoms. Rev, Urol., 11(Suppl 1),S1-8.
3. Bechis, S.K., Otsetov, A.G., Ge, R. and Olumi, A.F. (2014): Personalized Medicine for Management of Benign Prostatic Hyperplasia. J Urol. doi: 10.1016/j.juro.2014.01.114.
4. Bhatt, A.N., Mathur, R., Farooque, A., Verma, A. and Dwarakanath, B.S. (2010): Cancer biomarkers - current perspectives. Indian. J. Med. Res.,132:129-149.

5. Pastushenko, I., Vermeulen, P.B., Carapeto, F.J., Van, den, Eynden, G., Rutten, A., Ara, M., Dirix, L.Y. and Van, Laere, S. (2014): Blood microvessel density, lymphatic microvessel density and lymphatic invasion in predicting melanoma metastases: systematic review and meta-analysis. Br. J. Dermatol., 170:66-77.
6. Weidner, N. (1993): Tumor angiogenesis: review of current applications in tumor prognostication. Semin. Diagn. Pathol., 10:302-313.
7. Borre, M., Offersen, B.V., Nerstrøm, B., and Overgaard, J. (1998): Microvessel density predicts survival in prostate cancer patients subjected to watchful waiting. Br. J. Cancer.,78:940-944.
8. Bono, A.V., Celato, N., Cova, V., Salvadore, M., Chinetti, S. and Novario, R.(2002): Microvessel density in prostate carcinoma. Prostate. Cancer. Prostatic. Dis., 5:123-127.
9. Folkman, J., Cotran, R. (1976): Relation of vascular proliferation to tumor growth. Int. Rev. Exp. Pathol., 6:207-248.
10. Trojan, L., Thomas, D., Friedrich, D., Grobholz, R., Knoll, T., Alken, P. and Michel, M.S.(2004): Expression of different vascular endothelial markers in prostate cancer and BPH tissue: an immunohistochemical and clinical evaluation. Anticancer. Res., 24:1651-1656.
11. Luczynska, E., Gasinska, A., Wilk, W. (2013): Microvessel density and expression of vascular endothelial growth factor in clinically localized prostate cancer. Pol. J. Pathol., 64: 33-38.
12. Silbermann, M.H., Gyssens, I.C., Endtz, H.P., Kuijper, E.J. and van, der, Meer, J.T.(1997): Two patients with recurrent melioidosis after prolonged antibiotic therapy. Scand. J. Infect. Dis., 29:199-201.
13. Condon, M.S. (2005): The role of the stromal microenvironment in prostate cancer. Semin. Cancer. Biol., 15:132-137.
14. Weidner, N., Carroll, P.R., Flax, J., Blumenfeld, W. and Folkman, J. (1993): Tumor angiogenesis correlates with metastasis in invasive prostate carcinoma. Am. J. Pathol., 143: 401-409.
15. Warnberg, F., Nordgren, H., Bergkvist, L., and Holmberg, L. (2001): Tumour markers in breast carcinoma correlate with grade rather than with invasiveness. Br. J. Cancer., 85: 869-874.
16. Tenderenda, M., Rutkowski, P., Jesionek-Kupnicka, D., Kubiak, R. (2001): Expression of CD34 in gastric cancer and its correlation with histology, stage, proliferation activity, p53 expression and apoptotic index. Pathol. Oncol. Res., 7:129-134.
17. Hollingsworth, H.C., Kohn, E.C., Steinberg, S.M., Rothenberg, M.L. and Merino, M.J. (1995): Tumor angiogenesis in advanced stage ovarian carcinoma. Am. J. Pathol., 147: 33-41.
18. Trojan, L., Thomas, D., Friedrich, D., Grobholz, R., Knoll, T., Alken, P. and Michel, M.S. (2004): Expression of different vascular endothelial markers in prostate cancer and BPH tissue: an immunohistochemical and clinical evaluation. Anticancer. Res., 24:1651-1656.