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Neovascularization in prostatic adenocarcinoma as determined by CD34: A retrospective study

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Abstract

Background Microvessel density is one of the variables that are thought to affect the natural history of prostatic carcinoma in as much as its degree influences tumor progression including the ability of invasion and metastasis.

Objectives To assess microvessel density of prostatic adenocarcinoma, and determine its relationship to serum levels of prostatic specific antigen, and carcinoma grade, as determined by Gleason's score.

Methods Thirty patients with prostatic adenocarcinoma were studied. Parameters assessed are patients' age, serum levels of prostatic specific antigen, and the grade of the carcinomas according to (Gleason's scoring system). The values of the serum levels of prostatic specific antigen and the Gleason score were divided into 3 subgroups for statistical purposes. The degree of angiogenesis was evaluated by assessing microvessel density in sections stained immunohistochemically with CD34.

Results The microvessel density ranged from 10 to 35 (average 20.24 ± 5.95). Statistically significant correlation was found between the mean of microvessel density and serum prostatic specific antigen when the cutoff point of was 11 ng/ml, and with Gleason score when the cutoff point was 7.

Conclusion Microvessel density determination can predict the potential biologic behavior of prostatic adenocarcinoma in individual cases. The incorporation of the serum levels of prostatic specific antigen levels and Gleason scores with the former makes such predictions more practical.

Key words Prostatic adenocarcinoma, microvessel density, serum prostate specific antigen, Gleason score.

Introduction

Prostatic adenocarcinoma (PAC) is a significant cause of morbidity and mortality in elderly males. The occult, clinically undetectable forms are even more common. Thus, it is important to understand the changes seen with the early stages of the disease as well as those that are associated with progression. One of these changes is the cancer cell-mediated neovascularization [(1)]. Any increase in the tumor mass must be preceded by a sufficient vascular supply that helps support the growth, as well as augments its ability to invade and metastasize [(2)]. Cancer cells have been found to produce several angiogenic factors, including vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). Angiogenesis is initiated as proliferation of endothelial cells with subsequent formation of new vessels from the already existing vascular bed [(3)]. This process is normally under a tight regulatory control through a balance between angiogenic stimulators and inhibitors [(4)].

Neovascularization is assessed by microvessel density (MVD). The most common method utilized to quantify MVD is through calculating the number of vessels in specified areas

microscopically [3]. The latter task can be performed more accurately by staining histological sections with antibodies that specifically identify, and thus highlight endothelial cells. CD34 is one of the most commonly used antibodies for this purpose.

The aims of this study are to assess MVD in PAC, to determine whether there is a correlation between serum levels of prostatic specific antigen (PSA) and microvessel density and to correlate the above findings with the Gleasons score of the tumors.

Methods

A retrospective study from August 2004 through August 2008, thirty patients with PAC was studied. Samples were collected from laboratory of the hospital of specialized surgeries. These were represented by thirty specimens of formalin-fixed, paraffin-embedded blocks along with their histo-pathological reports. Parameters assessed included: the patients' age, serum levels of prostatic specific antigen (S-PSA), and the Gleason's score of the tumor. The nature of the surgical specimens submitted for evaluations included 26 samples were transurethral resection (TURP); prostectomies; and 4 samples were needle core biopsies.

The S-PSA levels were divided into 3 subgroups for statistical purposes; levels less than 10 ng/ml, levels between10-29 ng/ml and levels of 30 ng/ml and more.The range of Gleason score was similarly divided into three categories; 4 or less (well differentiated PAC), 5-6 (moderated differentiated PAC), and 7 or higher (poorly differentiated PAC). Two sections, 3 micron each were cut from paraffin blocks. One was stained with H&E stain, and the other with CD34 immunostain (mouse antiendothelial cell marker CD34 class II monoclonal antibody. Clone QBEnd/10. (Dako EnVisionTM code N1632). Both positive (a hemangioma) and negative (replacing the primary antibody with BPS buffer) controls were run together with the every batch of sections. A positive reaction is indicated by a red-brown colored cytoplasmic precipitate. The degree of angiogenesis was evaluated by assessing microvessel density (MVD) [5].

Microvessels were counted without prior knowledge of the grade of the diseases [6]. For the MVD quantitation, the stained slides were screened to identify areas of highest density of neovascularization ("hot spots"). In each section, five hot spots were chosen. The microvessel count (MVC) is the number of microvessels present within a 200X field (equivalent to 0.740 mm^{2}). The average of the MVCs was obtained by dividing the sum of the five values by 5 [7-9]. All the statistical analyses were performed through the SPSS program (version-12) and Excel application. Inferential statistics used included Binomial ANOVA test, and t-test.

Results

This study included 30 cases of PAC. The range of the patients' age was 52 to 85 year (mean 69.18 ± 7.59 years). The Gleason score was calculated for only 26 out of the 30 cases studied; in four cases the material present were insufficient for such determination being represented by needle biopsy samples. The diagnosis of carcinoma in such cases was made primarily on the presence of perineural invasion, vascular and lymphatic invasions. The mean of MVD was from 10 to 35 (average 20.24 ± 5.95). The mean of MVD in specimens collected from patients with a PSA level of less than 10 ng/ml (3 cases) was 13.2 ± 3.6, whereas those with PSA level of 30 ng/ml or more (11 cases) showed a mean MVD of 22.4 ± 5.2. Put it in another way, all of those with a PSA level of less than 10 ng/ml showed a MVD less than 10. Those cases with a PSA levels lying between 10 to 29 ng/dl (16 cases) showed a mean MVD of 19.5+5.43. Statistical analysis showed that there was significant difference in the mean of MVD in those patients with PSA level of 11 ng/ml or more compared with those expressing PSA level of less than 11 ng/ml. (P = 0.005) as seen in figure 1.

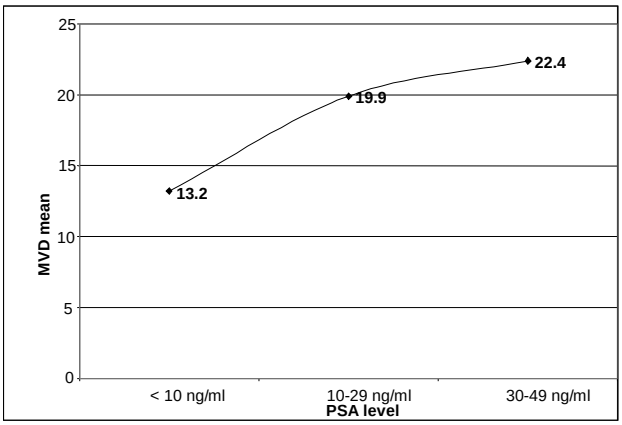

Figure 1. Distribution of the mean MVD in relation to serum PSA level

Regarding the relation between MVD & the grade of PAC, the mean MVD in cases of well differentiated PAC (Gleason score 4 or less) was 16.2 ± 5.7 versus 23.02 ± 4.9 for poorly differentiated PAC cases (Gleason score 7 +) as seen in figure 2.

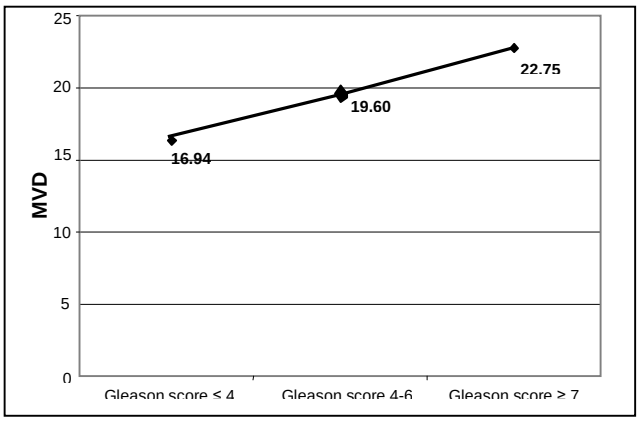

Figure 2. Distribution of the mean MVD in relation to the grade of prostatic cancer (Gleason score)

The majority of the latter group (Ten out of 12 cases; 83.3%) had a MVD count of 20 or more. A statistically significant difference was noted among the means of MVD of those with Gleason score below and those above 7 (p = 0.0036) as seen in (figures 3 and 4).

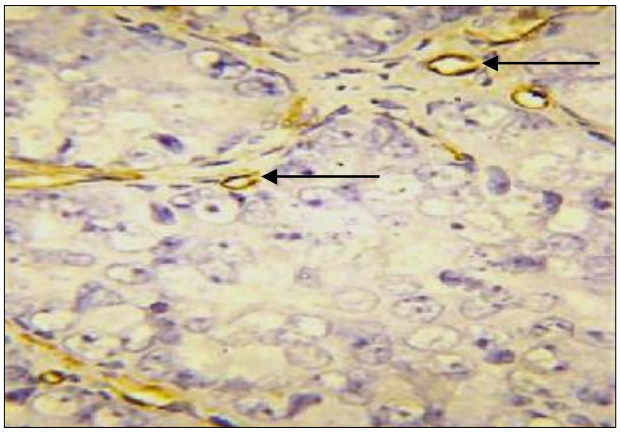

Figure 3. Poorly differentiated adenocarcinoma (Gleason score 9) showing prominent MVD showed the CD34 immunostain as brownish cytoplasmic discoloration highlighting the vascular endothelium. (40x).

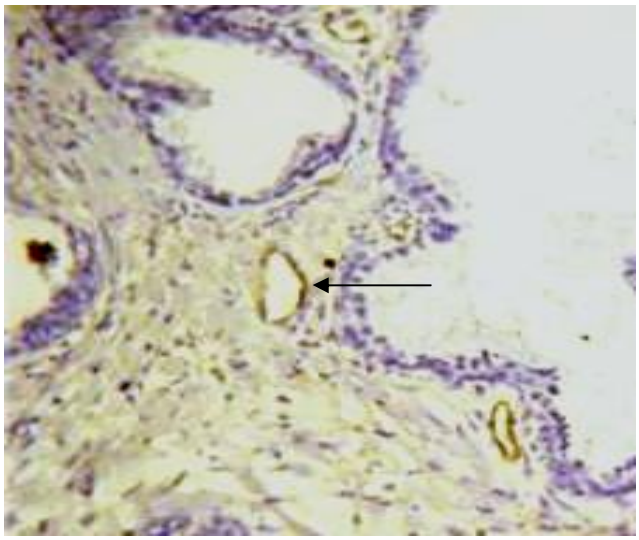

Figure 4. Well differentiated adenocarcinoma (Gleason's score 3) showing low microvessel density. The vessels are highlighted by CD34 immunostaining as brownish cytoplasmic discoloration. (10x).

Discussion

Angiogenesis of malignant tumors is essential for cancer growth, invasion and metastases [10]; PAC is no exception. Several studies have shown that the degree of angiogenesis does indeed influence the natural history of PAC through its correlation with the tumor aggressiveness and metastatic potential [10, 11] A

close correlation has been observed between MVD and patient survival in several malignant tumors including PAC.[7] The correlation between MVD using CD34 marker and such variables as tumor grade (Gleason score), and S-PSA levels were investigated.

This study has substantiated, indirectly, the significance of angiogenesis in relation to the biological behavior of PAC. In this study there was a definite correlation between the MVD of PAC and S-PSA levels; this has reached a statistical significance when the patients were segregated into two groups with a cutoff point of 11 ng/ml. Our results is supported by a similar study carried out by Strohmeyer et al [(12)], despite the fact they used a different marker; a polyclonal antibody against factor VIII. On the other hand, these results were contradicted by Kaygusuz et al [(13)] using CD34, who claimed that no such correlation existed. However, more independent studies on this particular point have to be carried out to clarify the relation between these two variables.

In this study there was a positive correlation between MVD and the grade of PAC as determined by the well-known Gleason score. This correlation also assumed a statistical significance when the cutoff point of the score was 7. These findings were augmented by two other studies; the findings of Bettencourt et al [(14)] and those of Kaygusuz et al [(13)]. In these two studies, like ours, CD34 monoclonal antibody was implemented. As it nearly always the case, the aforementioned conclusion was opposed by Rubin et al [(15)] and Silberman et al[16] through their application of CD31 monclomal antibody (instead of CD34). Although their samples size was larger than ours; but studies utilizing CD34 marker (including ours) are admittedly more reliable since CD34 is acknowledged of being more reliable than CD31 as endothelial cells markers.

Finally, we concluded that the MVD determination can predict the potential biologic behavior of PAC in individual cases. The incorporation of the S-PSA levels and Gleason scores with the former makes such predictions more accurate.

We recommend further studies of similar nature to be carried out with larger samples size, and to investigate in addition other prognostic variables of PAC in relation to MVD. Moreover, to establish a research program to assess the efficacy of angiogenesis therapy alone and in conjugation with radiotherapy and chemotherapy.

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