Mast cells numbers and peritumoral microvessel density of the prostatic adenocarcinomas and correlation with prognostic parameters

Havva Erdem¹, M. Ali Kayikci², Murat Oktay¹, Ali Kemal Uzunlar¹, Ali Tekin², Ebru Sener³, Handan Ankarali⁴, Nesrin Gursan⁵, Cem Şahiner⁴, Nilüfer Kadioğlu¹

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ABSTRACT

Aim To determine the utility of mast cell numbers and microvascular density (MVD) in evaluating acinar type of prostatic adenocarcinoma (PCa), and to ascertain a relationship between the number of mast cells with prognostic parameters (larger tumor volume, high Gleason score, lymphovascular, perineural, seminal vesicles invasion, metastatic lymph node).

Methods The study comprised 97 radical prostatectomy specimens. The paraffin sections were stained with anti-CD31, anti-CD34 and Toluidine Blue. The numbers of positive staining of cells and microvessels in 10 high-power fields were counted systematically.

Results A statistically significant relationship was found between MVDn and number of MC (r=0.218 and p=0.032). There was no correlation between age and MC and MVD (p=0.406 and p=0.671, respectively).

Conclusion A correlation between mast cell number and microvascular density cannot depend on tumor angiogenesis or this relationship can be an independent parameter. More comprehensive studies could reveal relationship with prognostic parameters.

Key words: CD31, CD34, Toluidine Blue, Gleason score, tumor volume

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INTRODUCTION

Prostate adenocarcinoma (PC) takes second place following lung cancer as a cause of male cancer-related deaths in developed countries (1). The variability in the clinical and biological behavior of PC, the inadequacy of markers used for assessing prognosis cause difficulties in the management of these patients (2). Searching for therapeutic modalities and new prognostic parameters has been the aim of many researches (2).

Solid tumors recruit blood vessels from neighboring tissue by angiogenesis with the sprouting of capillaries from pre-existing vessels that migrate into the tumor and form a new vascular network (3). Prevascular tumors may remain dormant in situ for months to years, then the “switching” of a subgroup of prevascular tumor cells to an angiogenic phenotype enables rapid growth and progression, metastasis (3). A significant increase in MCs number has been observed in malignant tumors, in both experimental models and human specimens and conflicting results have been published about the prognostic role of mast cells in a large variety of human tumors (4). Recently, some studies reported the presence of mast cells (MCs) in various malignancies and their role in tumor growth (3-5).

The present study was designed to determine the utility of mast cell number (MCn) and microvascular density (MVD) in evaluating malignant prostate lesions, and to ascertain a relationship between a number of MC with the prognostic parameters.

PATIENTS AND METHODS

This retrospective study used formalin-fixed tumor samples taken from patients with acinar type of prostatic adenocarcinomas (PCa), who were diagnosed between 2005 and 2011 at the Department of Pathology, Duzce University Medical Faculty. This study comprised 97 radical prostatectomy (RP) specimens and did not comprise tru-cut biopsy.

The study protocol was reviewed and approved by the Ethics Committee of Duzce University, Duzce, Turkey.

All of the hematoxylin and eosin sections of the cases were examined retrospectively (including tumor areas). One or two blocks in compliance with Gleason score were elected. Prognostic data of all cases were reviewed. All the diagnoses were made by two pathologists, following the Pathology of World Health Organization Classification of Tumors (6).

All biopsies and RP specimens were graded according to the Gleason grading system, which included the Gleason major and Gleason minor score, and the combined score. The ISUP decided in 2005 that the Gleason score should include the primary and secondary patterns, with a separate comment on the presence of a tertiary pattern of higher grade in the RP specimen (7).

Other variables such as larger tumor volume, high Gleason score, lymphovascular, perineural, seminal vesicles invasion, metastatic lymph node etc. were also determined.

Selected paraffin block was performed by toluidine blue, CD34, CD31 stain. Slides were examined by light microscopy (Olympus BX50). The numbers of positively stained cells in ten high power fields were counted systematically. In PCa cases, peritumoral regions were evaluated separately. Intratumoral areas were not calculated for a few MCn.

Immunohistochemical staining was performed on the 3-μm sections of formalin fixed, paraffin-embedded material. The sections were deparaffinized with xylene and rehydrated with ethanol. Non-enzymatic antigen retrieval was performed on each slide and they were washed with 10 Mm phosphate-buffered saline (PBS), pH 7.5. Immunohistochemical staining was performed manually using the Standard avidin–biotin peroxidase complex technique (Neomarkers, Thermo Fisher Scientific, Fremont, CA, USA). Briefly, the slides were incubated at 37 °C for 60 min with the following primary antibodies (Neomarkers, Thermo Fisher Scientific): anti-CD31 (Ab-1, MS-353-S1, 1:40 dilution) and anti-CD34 (GTX61737, dilution of 1:100).

Histochemically, NovaUltra Toluidine Blue Stain kit was used. Toluidine blue should stain mast cells red-purple (metachromatic staining) and the background blue (orthochromatic staining).

The MVD and MC counting procedure was performed on Olympus BX50 microscope. Ten peritumoral areas were counted systematically (Figure 1. Microvessels stained positively with anti-CD31 and anti-CD34 were counted on 10 high-power fields (Figure 1).
Microvessels were identified as individual or clusters of cells with or without lumens that were positively stained by anti-CD31 and anti-CD34 primary antibody using the immunohistochemistry procedure described above. The lumen diameter had to be smaller than approximately eight red blood cells. Areas of fibrosis, necrosis and inflammation, and vessels with muscle wall were excluded from counting. Counting was preferred in manual method and fields were counted systematically.

Descriptive statistics were computed as mean±SD or number/percentage of frequency. The correlation between mast cells numbers, peritumoural microvessel density and prognostic parameters were used by using Pearson or Spearman rank correlation analysis. P values which are less than 0.05 are accepted as statistically significant.

RESULTS

The age range of the cases was between 44 and 79 years (63.7 ± 7.2 years). Tumor volume ranged between 0.5 and 90% (14.4 ± 14.1). In 32 (33%) cases, the tumors were Gleason score 6, in 53 (54.6%) cases Gleason score was 7, in five (5.2%) cases Gleason score was 8, and in seven (7.2%) cases the Gleason score was 9. There were 85 cases with PT2 (tumor confined within prostate) and 12 cases with PT3 (tumor extends through the prostate capsule).

A statistically significant relationship was found between MVDn and number of MC (r=0.218 and p=0.032) (Table 1). There was no correlation between MC and MVD according to age (p=0.406, p=0.671, respectively).

Table 1. Correlations between numerical variables

<table>
<thead>
<tr>
<th>Tumor volume</th>
<th>Gleason score</th>
<th>MC</th>
<th>MVD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Correlation coeff. (r)</td>
<td>0.024</td>
<td>0.172</td>
</tr>
<tr>
<td>p</td>
<td>0.185</td>
<td>0.092</td>
<td>0.406</td>
</tr>
<tr>
<td>Tumor volume</td>
<td>Correlation coeff. (r)</td>
<td>0.399</td>
<td>-0.171</td>
</tr>
<tr>
<td>p</td>
<td>0.000</td>
<td>0.094</td>
<td>0.931</td>
</tr>
<tr>
<td>Gleason score</td>
<td>Correlation coeff. (r)</td>
<td>-0.087</td>
<td>-0.237</td>
</tr>
<tr>
<td>p</td>
<td>0.398</td>
<td>0.019</td>
<td></td>
</tr>
<tr>
<td>MC</td>
<td>Correlation coeff. (r)</td>
<td>0.218</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.032</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MC, mast cells; MVD, microvascular density

The number of MC was 32.2±13.2 in PT2 tumors, and 25.4±8.0 in PT3 tumors. The number of MVD were 27.8±9.7 in PT2 tumors and 28.0±4.8 in PT3 tumors.

Statistically significant relationship between Gleason score and MVD of the cases was found (p=0.019) but this correlation was negative. Incorrect result may have been due to the small number of cases (Gleason score 8 = 5 cases). Statistically significant relationship between Gleason score and MCn (p=0.398) was not found. Six cases were localized in left lobe, seven in right lobe and, 84 cases were localized in both right and left lobes. The relationship between location and number of MC and MVC was not statistically significant (p=0.222, p=0.800, respectively). There were tertiary patterns in nine cases, metastatic lymph nodes (MLN) in three, invasion of seminal vesicles (ISV) in five, and lymphovascular invasion (LVI) in two cases. There was perineural invasion (PI) in 54 cases. There was no correlation prognostic parameters such as larger tumor volume, high Gleason score, ISV, MLN, LVI, PI.

DISCUSSION

Sustained tumor growth requires a positive balance between tumor cell proliferation and cell death or apoptosis (8). Using an experimental animal model, it has been shown that the initiation of angiogenesis appears concomitantly with a decrease in tumor cell apoptosis, while the levels of tumor cell proliferation remain constant, thus leading to net tumor growth (8). Preinvasive malignant cells are known to remain dormant until they become angiogenic, and this is followed by a phase of rapid tumor growth (9-11). Angiogenesis is the outcome of an imbalance between positive and negative angiogenic factors produced by both tumor and host cells (10). Among the host...
cells, which produce and release pro-angiogenic and angiogenic factors are mast cells (8). Mast cells are an important source of several proangiogenic and angiogenic factors (8).

The evaluation of MC was first described in PCa by Gupta (11), and a few MCs were found around the prostate acini and in the stroma of PCa. However, only a few studies (12) have confirmed these results. As the connective tissue stroma increases with age, an increase in the MCn in human prostate has been described (13). Aydin et al. did not find relationship between mast cell number and age in PCa (14).

Many studies have reported better survival with higher numbers of MC (15). Lachter et al. have reported that MC is a beneficial prognostic factor (16). However, Elezoğlu et al. found a significant relationship between MC number and survival and grade in colorectal carcinoma. They found a statistical correlation between MC number and MVD and a statistically significant relationship between MC number and survival (17). Pittoni et al. have found that MCs infiltrating prostate tumors in transgenic adenocarcinoma of the prostate of mice and humans increase in number upon disease progression (5).

The present study has not demonstrated relationship between MC and the prognostic parameters. It has been demonstrated that Gleason 7 tumor is more aggressive than Gleason 5 to 6, and should not be lumped together as intermediate-grade tumor (18). In this study, no statistical difference between Gleason score groups was found and there was no interaction between score groups and peritumoral regions. Sari et al. did not find a connection between Gleason grade and number of MCs around the tumor in prostate (12).

Tomita M et al. presented two reasons for conflicting reports on the role of mast cells (19). The cytotoxic functions of mast cells that suppress tumor activities might be present initially when the mast cells infiltrate the tumor tissue. However, after infiltration, the tumor cells might promote the angiogenic properties of mast cells while suppressing their cytotoxic functions, thereby leading to tumor angiogenesis (19). The effect of mast cells against cancer cells might depend on the concentration of mast cell products in the microenvironment. Tomita M et al. found a correlation between MVD and MCD with a linear increase in MVD as the MCD, suggesting a positive correlation (19). In the present study, significant relationships were found between MVD and MC in prostate carcinomas.

Deng et al. found significant difference in the mean mast cell numbers between well-differentiated groups and poorly-differentiated groups (20). In the present study, significant relationships were not found between MC and Gleason score groups in prostate carcinomas.

In conclusion, a correlation between mast cell number and microvascular density can not depend on tumor angiogenesis or this relationship can be an independent parameter. More comprehensive studies could reveal relationship with prognostic parameters.

**FUNDING**

This study was supported by Duzce University of Medical Sciences.

**TRANSPARENCY DECLARATION**

Competing interests: none declare.

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