The increase in capillary vessel density observed in neoplastic processes is an occurrence that has been argued and speculated upon recently. The question is whether it is a result of neoplasia or is an important critical factor in the pathogenesis of the neoplasia.

EFFECT OF CHRONIC PROSTATITIS ON ANGIOGENIC ACTIVITY AND SERUM PROSTATE SPECIFIC ANTIGEN LEVEL IN BENIGN PROSTATIC HYPERPLASIA

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The aim of this study was to evaluate the effect of chronic inflammatory pathology on the angiogenic activity in benign prostatic hyperplasia (BPH). Besides the presence of a relationship between serum prostate specific antigen (PSA) values and microvessel density (mvd), the intensity and extent (widespread or focal) of tissue PSA expression was also examined. The distribution of 30 cases according to the diagnosis groups was as follows: group 1, nine cases with prostatic adenocarcinoma; group 2, 10 cases with BPH and chronic prostatitis; group 3, 11 cases with BPH. The biopsy materials obtained by tru-cut biopsy (five cases) and transurethral resection (25 cases) were evaluated retrospectively. The evaluation of angiogenesis was made by CD34 immune marker, while the analysis of immunohistochemical tissue PSA expression was verified by PSA immune marker. Serum PSA levels and other clinical parameters were obtained from the clinical files of the patients. The mean age of the patients was 68±3 years (range, 48–83 years). The difference between the mean mvd values of the groups was statistically significant ($\chi^2 = 10.492, p = 0.005$). Group 1 showed higher mean mvd value than the other two groups. Although group 2 showed higher mean mvd value than group 3, the difference was not statistically significant ($p = 0.863$). There was no correlation between the mean mvd and serum PSA levels in any group. The intensity of PSA expression in prostate specimens was different in all groups. Maximum cases in group 3 showed high tissue PSA expression ($\chi^2 = 12.442, p = 0.014$). In group 1, there was a significant relationship between the intensity of PSA expression and the mean mvd ($U = 1, p = 0.032$). In group 2, a statistically significant correlation was noted between the mean serum PSA levels and the widespread occurrence of PSA expression ($U = 0, p = 0.017$). In the present study, we determined that chronic prostatitis had no effect on mvd in BPH cases. The correlation between tissue PSA expression and mvd was contradictory to the reports in the literature. Analyses in larger series are needed to prove the presence of a probable effect of chronic prostatitis on angiogenesis.

**Key Words:** angiogenesis, benign prostatic hyperplasia, chronic prostatitis, microvessel density, prostate specific antigen

The values of microvessel density (mvd) determined in malignant neoplasms are considerably higher than those in benign neoplasms and normal tissues [1]. Many chemical mediators suggested to have a role in the formation of new capillary vessels exist. Based on the results of clinical and experimental studies carried out earlier, it was suggested that some of these mediators were released by inflammatory cells associated with neoplasia, while the others were produced by transformed neoplastic cells [2–4].

The effect of chronic inflammation on the pathogenesis of neoplasia has been argued for a long time and is suggested to be an important factor in the pathogenesis of some neoplasms. Inflammatory pathology, which accompanies some epithelial malignant tumors, is now accepted to play an important role in the development and progression of malignancy via autocrine and paracrine factors released by the inflammatory cells. A part of this role was suggested to be created via the stimulation of angiogenesis [4].

Although an organ-specific hormone prostate specific antigen (PSA) is determined in high serum levels in patients with prostate carcinoma, serum PSA levels also reach higher values in benign prostatic hyperplasia (BPH), chronic prostatitis (CP), and after the biopsy procedure [5–8]. The effect of CP on the serum PSA level causes problems of differential diagnosis in prediagnosis of prostate carcinoma and the follow-up postoperative care of patients with prostate carcinoma. The studies which investigated the probable relationship between serum PSA level and angiogenic activity are present in the literature [9,10].

In this study, we investigated the correlation between inflammatory process and mvd, serum PSA level, and tissue PSA expression in cases of BPH with and without CP and prostate carcinoma.

**Materials and Methods**

Transurethral resection (TUR) and tru-cut biopsy material of 30 cases were evaluated in the study. Five of these cases were tru-cut needle biopsy, while 25 cases’ tissue specimens consisted of TUR material. The distribution of cases according to the histopathologic diagnoses was as follows: group 1, nine cases with prostatic adenocarcinoma; group 2, 10 cases with BPH and CP; group 3, 11 cases with BPH. The presence of lymphoplasmocytic cell infiltration in stromal areas was accepted as CP. However, lymphoplasmocytic cell infiltrations spreading to prostatic acini and ductuli and abscess formation were also accepted as CP. Five cases in group 1 were diagnosed by tru-cut needle biopsies, while the diagnoses of four cases were reached via prostate TUR materials. All prostate carcinoma cases had moderately differentiated prostate adenocarcinoma. Gleason score of the cases was between 4 and 7. All the other cases were diagnosed with prostate TUR materials.

CD34 antibody was used for analysis of angiogenic activity in the prostate tissues. The most demonstrative biopsy tissue examples of cases diagnosed as prostate adenocarcinoma were separately blocked and immunohistochemical analyses were performed on these blocks. In BPH cases, the paraffin blocks that contained the most demonstrative tissue fragments were used for immunohistochemical analysis. The sections were prepared in 4 µm thickness and standard dewaxing process was performed after incubation at 37°C for one night. Subsequently, antigen retrieval processing was done in citrate tampon solution using a microwave oven. CD34 (monoclonal, QBEnd/10, 1/100, NeoMarkers) and PSA (polyclonal, 1/200, NeoMarkers) antibodies were applied by using the streptavidin-peroxidase method. The vessels away from the tumor and hyperplasia focus served as an internal positive control for CD34 antibody. Benign prostate tissues were used as positive control for PSA antibody. To evaluate angiogenic activity in prostate tissue stained with CD34, all capillary vessels, endothelial cells, and buds were counted (Figure 1). Middle and large vascular structures were disregarded. The capillary vessels and endothelial buds in all tumor tissues of prostatic carcinoma cases, which were diagnosed by tru-cut needle biopsy materials were counted. In prostate TUR materials, the most demonstrative 10 high-power fields in prostate tissues with tumor were designated for the capillary vessel counting. If tumor focus was limited to a smaller area than in the 10 high-power fields, all tumor areas were included for the analysis. The area of tumor was calculated in mm² with ocular grid in tru-cut needle biopsy materials. In TUR materials, the sum of microvessels counted in 40× power (0.196 mm², Nikon E600) and the number of counted tissue areas was taken into account and then mvd was calculated. For the evaluation of PSA expression in the prostate tissues, the extent of PSA expression was classified into widespread and focal;
the intensity of staining into low (1+), intermediate (2+), and high (3+) were independently evaluated (Figure 2). Positive staining values \( \geq 30\% \) in the most demonstrative areas of the prostate parenchyma tissue examined were accepted as widespread PSA staining and the lower values were regarded as focal PSA staining.

Serum PSA levels were obtained from the clinical files of the patients. Only those serum PSA levels that were measured before the prostate biopsy procedure were considered for the analyses and the normal accepted range of serum PSA level was 0–4 ng/mL.

Kruskal-Wallis variants analysis, Mann–Whitney U test with Bonferonni correction, and \( \chi^2 \) tests (likelihood ratio) were used for the statistical analysis. The \( p \) value < 0.05 was accepted as significant.

**RESULTS**

The patients’ ages ranged from 48 to 83 years. The mean age was 68 ± 3 years. The mean ages and age ranges according to diagnosis groups were as follows: group 1, 70.7 years and 64–79 years; group 2, 67.2 years and 50–83 years; group 3, 66.5 years and 48–80 years.

The comparison between the groups was made by Mann–Whitney U test with Bonferonni correction as the difference between the groups was significant for mean mvd (\( \chi^2 = 10.492, p = 0.005 \)). The mean mvd values and serum PSA levels for all groups are given in Table 1. The differences between group 1 and group 2, and group 1 and group 3 were significant (\( U = 11, p = 0.004 \) and \( U = 12, p = 0.003 \)). The difference between group 2 and group 3 was statistically insignificant.
The difference between mean PSA values of group 1 and other groups was not statistically significant ($\chi^2 = 5.262, p = 0.863$) because the distribution of serum PSA levels of patients in group 1 was not homogeneous. In group 1, the lowest value for serum PSA was 2.17 ng/mL, while the highest value was 813 ng/mL. A correlation between the mean mvd and serum PSA levels was not determined in each group and between groups. The positive correlation in group 2 was not significant ($p = 0.216$).

When the intensity of PSA expression was evaluated, a statistically significant difference between the groups was found ($\chi^2 = 12.442, p = 0.014$) (Table 2). The eight cases (72.7%) in group 3 showed high PSA expression. The other three cases (27.3%) of this group had intermediate PSA expression. There was no significant difference between the groups for the extent of PSA expression ($\chi^2 = 0.479, p = 0.789$) (Table 3).

In all groups, the presence of a probable relation between mvd, intensity, and extent of PSA expression and serum PSA levels was investigated with Mann–Whitney U test.

In group 1, the mean mvd according to the intensity of PSA expression showed significant difference ($U = 1, p = 0.032$). The mean mvd of cases, which showed low PSA expression was lower than that of cases which showed high PSA expression. When the means of serum PSA levels according to the distribution of PSA expression were evaluated, the difference was not statistically significant, although the cases which showed low PSA expression had higher mean serum PSA levels than those cases which showed high PSA expression ($U = 8, p = 0.73$).

In group 2, although higher mean mvd value was determined in cases which showed high PSA expression, the difference was statistically insignificant ($U = 7, p = 0.714$). In this group also, no significant relation between serum PSA levels and the intensity of PSA expression was determined ($U = 6, p = 0.548$). The same correlation between mean mvd and the intensity of PSA expression was noted in group 3. The cases which showed intermediate PSA expression had higher mean mvd values than those with low PSA expression. The difference was statistically significant ($U = 0, p = 0.012$).
In group 3, no significant correlation between the intensity of PSA expression and serum PSA levels was found ($U=9$, $p=0.630$).

A correlation between the extent of PSA expression and the other two parameters (mean mvd and serum PSA value) was not determined in group 1. The $U$ and $p$ values for the extent of PSA expression and mvd were 4 and 0.5, respectively, while these values for the extent of PSA expression and serum PSA levels were 6 and 0.889, respectively.

A significant relationship was observed between the mean serum PSA level and the extent of PSA expression in group 2. The mean serum PSA level of cases which showed widespread PSA expression was higher than that of cases which showed focal expression. The difference was statistically significant ($U=0$, $p=0.017$). In this group, a significant relation between the extent of PSA expression and the mean mvd were not determined ($U=8$, $p=0.667$).

In group 3 too, a significant relationship between the extent of PSA expression, the mean mvd, and serum PSA levels were not determined ($U=10$, $p=0.527$; $U=11.5$, $p=0.648$).

**DISCUSSION**

The effect of angiogenesis on development of neoplasia is a popular research subject, which has been extensively investigated in malignant and benign neoplasms. In the literature, it is frequently reported that especially malignant neoplasms have high capillary vessel density. Whether angiogenesis is an
outcome of neoplasia or is a factor which triggers neoplasia development is still debated. It is suggested that various factors stimulate angiogenesis and these angiogenic factors are released by malignant neoplastic cells [11–15]. In some studies, it is suggested that angiogenic factors with tumor necrosis factor and basic fibroblastic growing factor are released together by macrophages [12,13,16]. It has been speculated that the effect of chronic inflammation on tumorigenesis could be via stimulating angiogenesis or both factors could be effective via synergic interaction.

Serum PSA values that are used as an important parameter in the early diagnosis and clinical follow-up of prostatic carcinomas could also be increased to higher levels than normal in BPH and CP [5–8]. The deterioration of cellular mechanisms is suggested to be the cause of elevation in serum PSA levels in BPH and prostatic carcinomas [9]. PSA, which is released to the extracellular area as a result of this deterioration, enters into the systemic circulation via local microcirculation [9]. Although the release of PSA in systemic circulation has not been understood well, the increase in the density of capillary vessel network near the basal layer of prostate ductuli and acini could be accepted as a facilitative factor for the release of PSA into the systemic circulation [9]. The effect of capillary vessel density on serum PSA level has not been precisely explained [17,18]. However, the results of an in vitro investigation revealed that PSA converts plasminogen to biologically active angiostatin-like fragments. Authors suggested that these fragments similar to angiostatin would be able to suppress angiogenesis and, therefore, also tumor growth and tumor metastasis [19]. In the study by Papadopoulos et al [10], it was found that high PSA expression by prostate cancer cells was accompanied by low intratumoral angiogenesis. The relation between high PSA expression and low intratumoral angiogenesis seems to be correlated with the finding that poorly differentiated prostate cancer expresses significantly less PSA than benign prostate and low-grade prostate cancer [20,21].

In our study, a statistical difference for the mean mvd values was not observed between group 2 and group 3. The differences in the mean mvd between group 1 and the other two diagnosis groups (group 2 and group 3) were statistically significant (U = 11, p = 0.004; U = 12, p = 0.003). This was an expected finding but was not the aim of investigation in the present study because many studies have revealed that prostate carcinomas have higher values of mvd than BPH, prostatic intraepithelial neoplasia, and benign prostate tissues [22–25].

The effect of chronic inflammation on angiogenesis is suggested to occur via some autocrine and paracrine factors released by macrophages [16]. For this reason, it has been speculated that the capillary vessel density value in cases of BPH accompanied by chronic inflammatory pathology could be of a higher value than that of the cases with BPH without CP. In the present study, although the mean mvd value for the group of BPH with CP was higher than that of the BPH-without-CP group, the difference was not statistically significant. According to a view, the increase in serum PSA level in prostate inflammations could be due to structural and metabolic deterioration of acinic epithelial cells. In addition to this opinion, another hypothesis was also suggested that the increased capillary vessel density near the basal layer of the acini observed in BPH and especially prostate carcinoma could contribute to increased systemic circulation of escaped PSA [9]. Some other studies, which analyzed the effect of capillary vessel density on serum PSA level, did not prove such a relation [17,18]. In the present study, although serum PSA level and the mean mvd value in cases of BPH with CP were higher than those of BPH cases, the differences for both these parameters were not statistically significant.

The difference in the intensity of PSA expression between groups was clearly observed. The highest intensity of PSA expression was observed in two cases of group 3. When a probable relationship between the mean mvd and the intensity of PSA expression was analyzed in group 1, it was determined that the mean mvd value of the cases which showed low PSA expression was lower than the mean mvd value of prostate carcinoma cases which showed high PSA expression (U = 1, p = 0.032). This finding was not concordant with the results of Papadopoulos et al [10]. According to their study, high expression of PSA in prostate carcinoma was related to low intratumoral angiogenesis. In the present study, group 1 showed lower intensity of PSA expression than group 2 and group 3. This was an expected finding according to the literature [20,21]. But the correlation between mvd and PSA expression intensity in group 1 was contrary to the results of Papadopoulos et al [10]. A similar relationship between mvd and PSA expression intensity was also determined in group 2 and group 3.
This reserve correlation that was determined between mvd and PSA expression in the present study may result from a small number of cases in the groups.

There was no significant relation between the intensity of PSA expression and serum PSA levels in all groups. When the relationship between the extent of PSA expression and serum PSA levels was analyzed, it was found that a statistically significant correlation existed between both parameters in group 2. The mean serum PSA level of cases which showed widespread PSA expression was significantly higher than that of cases which showed focal PSA expression ($U = 0, p = 0.017$). We can consider that this finding was also contrary to the concerned literature data because widespread PSA expression may be related to lower mvd and, therefore, the release of PSA into the systemic circulation may occur at a lower rate [9,17,18,20,21].

Today, it is still not clear whether the increase in capillary vessel density is a consequence or a cause in the tumorigenesis process. A view widely accepted is that some angiogenic factors released by neoplastic cells are effective in angiogenesis. However, the angiogenic effects of secondary pathologies such as inflammation could not be clarified yet. Also, the effects of similar factors on the serum levels of some hormone or hormone-like markers used in clinical follow-up of neoplastic diseases could not be clearly explained.

In our study, we could not show that chronic inflammatory pathology has a positive effect on capillary vessel density in the cases of BPH. Additionally, a relationship between serum PSA level and mean mvd was also not observed according to the results of our study. Analyses of larger series of patients are needed to reveal a probable relationship between chronic inflammatory pathology and capillary vessel density.

**ACKNOWLEDGMENTS**

This study was funded by The Commission of Scientific Research Projects of Gaziosmanpasa University.

**REFERENCES**