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Original Research Article

Evaluation of angiogenesis as a prognostic marker in prostatic neoplasm especially carcinoma of prostate

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ABSTRACT

Background: Prostatic carcinoma shows an unusually wide range of biological potential with well-known disparity between incidence and mortality for disease. Clinico-pathological studies suggests that angiogenesis and tumor neovascularity contributes to pathogenesis of prostate cancer. The aim of this study to present study was done to assess the validity of angiogenesis as a suitable prognostic marker in various prostatic disease specially the neoplasm's including the malignant ones. Settings and design are Retrospective study.
Methods: The present study of evaluation of angiogenesis as a prognostic marker in prostatic neoplasm especially carcinoma of prostate was done with 40 biopsy sample. The biopsy sample were obtained by suprapubic prostatectomy specimen and trans rectal needle biopsy specimen. Tissue sections were subjected to routine H and E staining. For demonstration of angiogenesis staining for reticulin fibers was applied.
Results: The microvessels density increases as the severity of lesion increases from benign to pre-neoplastic to frankly malignant. The micro vessel density in malignant lesions is approximately thrice that in benign lesions.
Conclusions: Very few studies have been done in prostatic lesions Hence an attempt is made to demonstrate and correlate angiogenesis as a tumour marker.

Keywords: Angiogenesis, Benign prostatic hyperplasia, Micro vessal density, Prostatic carcinoma

INTRODUCTION

Prostate disease now a day is fairly common. More so after the age of 45 years when the prostate gland is afflicted with hyperplasia in form of benign prostatic hyperplasia which is the major cause of morbidity and urinary problems in male patients.

Further cancer of prostate is most common cancer in men only next to lung cancer, as a cause of cancer related deaths which has been confirmed by autopsy studies. Prostatic carcinoma manifests an unusually wide range of biological potential with well-known disparity between incidence and mortality for disease. This has made it difficult for assessing best treatment for prostatic carcinoma.[1]

Premalignant lesions of the prostate present difficulties in their diagnosis. These include prostatic intraepithelial Neoplasia (PIN) and atypical adenomatous hyperplasia (AAH). The frequency of PIN in cancerous prostate is significantly greater than is frequency in non-cancerous prostate. The development of accurate prognostic markers that correlate with outcome would help to determine which patients may require aggressive adjuvant therapy because of being at high risk for carcinoma recurrence and death.[2] Stage, grade and serum PSA are the strongest predictive tools available at

present.[1] Newer-measurements of cell proliferation in prostatic carcinoma include bromodeoxyuridine labeling, ki-67 nucleolar organizing regions. There are also conflicting data as to whether the following markers are independent predictors of biological behaviour, extent of angiogenesis, extent of neuroendocrine expression, c-erb B-2, E-cadherin and p53.[3] Much experimental evidence exists showing that tumor growth and metastasis are dependent on tumor angiogenesis. Tumor angiogenesis is the growth of new vessels towards and within a tumor.[4] First evidence that intensity of angiogenesis in a human tumor could predict the probability of metastasis was reported for cutaneous melanoma.[5] In many studies, intratumor microvessel density was found to have independent prognostic significance when compared with traditional prognostic markers by multivariate analysis. This has been shown in studies of patients with carcinomas of breast, Testes and ovarian carcinoma.[6,7]

Bigler et al, 1992 studied micro vessel density (MVD) in invasive carcinoma prostate and noted a significantly increase in density of micro vessels within invasive carcinoma prostate relative to normal prostatic stroma. These studies define MVD as potential prognostic indicator for carcinoma prostate.[8] Hyaluronidase and other matrix degrading enzymes also have been detected at high levels in carcinoma prostate and may also promote angiogenesis.

METHODS

The Institute ethical committee approved the study. All sample were coded and labelled properly. The design is a retrospective study was done in the Department of pathology, M.L.B. Medical college Bundelkhand University, Jhansi, Uttar Pradesh, India.

Study material

Prostatic biopsy Specimens were submitted either as suprapubic prostatectomy or transrectal needle biopsy specimen for a period of 15 months. Biopsy were thus collected and preserved in 10% formal saline and were subjected to routine hematoxylin and eosin staining. For demonstration of angiogenesis staining for reticulin fibers was applied. The method used was GARDON and SWEET'S METHOD.

This method was simple reliable and can be used both for paraffin wax and frozen sections. Two great advantages of this method for reticulin fibers were the rapidity of impregnation of fibers and reversibility of reaction. Reticulin fibers are staned brownish black in untoned and dark purple in toned preparation. Microvessels can also be highlishted by staining endothelial cell for factor VIII-related antigen (F8-RA) also called as vWF (von Will brand Factor) using a standard immunoperoxidase technique. CD 31 as well as other endothelial markers such as CD 34 could be used in the same way as F8-RA.

Couting procedure

Micro vessel density was passed by light microscopy in areas of invasive tumor containing the highest number of capillaries and small venules per area (neovascular hot spots). After the area of highest neovascularisation was identified individual micro vessel count was made on an x400 field. Any brown staining endothelial cell or endothelial cell cluster clearly separate from adjacent micro vessels, tumor cells and other connective tissue elements was considered a single countable vessel. Vessel lumen was not necessary for a structure to be defined as a micro vessel and red cells were not used to define a vessel lumen. Results were expressed as highest number of micro vessels defined within any single x400 field.

RESULTS

The present study of evaluation of angiogenesis as a prognostic marker in prostatic neoplasm especially carcinoma of prostate has been done in Department of Pathology, MLB Medical College, Jhansi Uttar Pradesh, India. The clinical findings along with observations were recorded on a preset proforma for clinico-pathological correlation and analysis. Over a period of 15 months total 40 cases were studied Majority of patients were from rural areas of Bundelkhand region Most of the patients were above 50 years of age, both benign and malignant ones. Table 1 shows distribution of cases according to histo-pathological diagnosis. Preneoplastic lesion was present in 4 cases (10%) while frank neoplasia was present in 6 cases (15%). The most common histological type of carcinoma in prostate was Adenocarcinoma. As the age advances the incidence of preneoplastic and neoplastic conditions increases.

Table 1: Histo-pathological diagnosis of prostatic lesions (40 cases).

Histopathological diagnosis	No. of cases	Percentage (%)
Benign prostatic hyperplasia with chronic prostatitis	28	70
Benign prostatic hyperplasia with tuberculosis	01	2.5
Benign tumor- Leomyoma	01	2.5
Preneoplastic lesion	04	10
Neoplstic lesion	06	15

The micro vessel density increases as the severity of lesion increases from benign to pre-neoplastic to frankly malignant. The micro vessel density in malignant lesions is approximately thrice that in benign lesions. Table 2 shows comparative study of MVD in different lesions of prostate increase in MVD as the severity of lesion increases i.e. from benign to malignant. As is seen the range of MVD in benign lesions is 2-8/hpf and mean 5/hpf on H and E while it is 3-8/hpf and mean is 6/hpf on reticulin staining. In Pre-neoplastic lesions the range of

MVD is 8-14/hpf and mean 11/hpf on H and E while it is 9-18/hpf and mean is 14/hpf on reticulin staining. In adenocarcinoma the range is high i.e. 10-24/hpf with a mean of 17 on H and E while it is 12-26 with mean 19 pm reticulin staining.

Table 2: comparative study of MVD in different lesions of prostate (40 cases).

Histopathological diagnosis	MVD (H and E)/HPF		MVD (Reticulin)/HPF	
	Range	Mean	Range	Mean
Benign lesions	2-8	5	3-8	6
Pre-neoplastic lesion	8-14	1	9-18	14
Adenocarcinoma	10-24	17	12-26	9

DISCUSSION

The incidence of prostatic lesions has risen sharply in the past decade owing to early detection methods and treatment. Benign Prostatic hyperplasia, being the most common prostatic pathology with an incidence of 50% in fifth decade rising to 75% in eight decade. Prostatic cancer is the most common carcinoma in men and only second to lung cancer as the leading cause of mortality, autopsy study showing about 80% of the persons harboring as the disease of eighth decade. The present study of evaluation of angiogenesis as a prognostic marker in prostatic neoplasm especially carcinoma of prostate comprises of 40 cases total. Majority of cases were from rural background 62.5% when compared to only 37.5% cases from urban areas. The high ratio of rural to urban areas i.e. 1.7: I. It may be attributed to lack of newer modalities of treatment available to rural people other than total prostatectomy.

More than 90% of cases of Benign Prostatic Hyperplasia were of greater than 50 years of age with maximum (40%) in 61-70yrs age Group. This is in accordance to the clinical incidence of disease by Berry et al that its incidence is 8% in fourth decade and rises to 50% in fifth decade and to 75% in eighth decade.[9] All the pre-neoplastic and neoplastic cases were of >50yrs of age with an increase in the incidence of disease as the age increases thereafter. As regards evaluation of micro vessel density in benign cases employing reticulin stained sections, varied from 3-8/hpf with a mean of 6/hpf, mostly present along the basement membrane and at the periphery of adenomatous nodule (Figure 1 and 2).

Bigler(1993) studied micro vessel density in benign and malignant prostatic tissue in 15 cases.[10] Micro vessels in tissue sections were quantified by marking, the vascular endothelial cells by antibodies to factor- VIII related antigen and found that in benign prostatic tissue the capillaries were restricted for most part to the peri-glandular stroma immediately adjacent to epithelium. In pre-neoplastic lesions in our series micro vessel density ranged from 9-18/hpf with a mean of 14/hpf. So, when these findings were compared to that of benign lesions a useful ratio of 2.3:1 was recorded. (Figure 3 and 4).

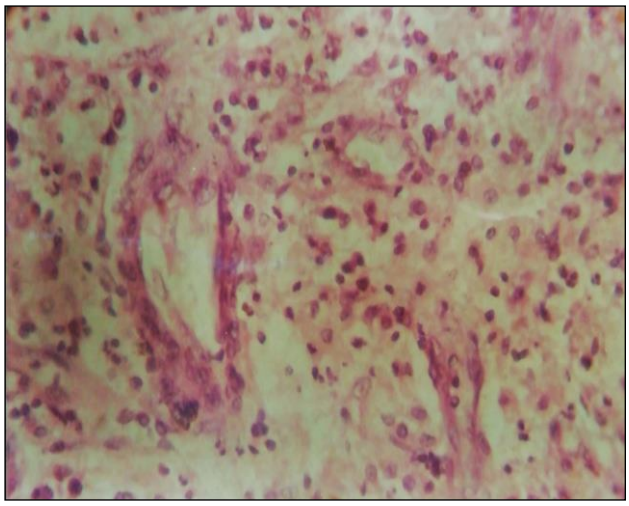

Figure 1: Photomicrograph of benign prostatic hypeplasia showing 3-4 vessels of medium to large size in the stroma some with thick walls (H and E, 400X).

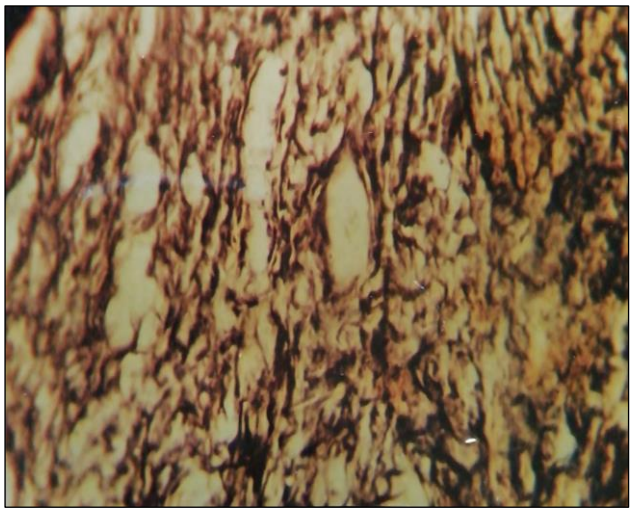

Figure 2: Photomicrograph of benign prostatic hypeplasia showing 3-4 vessels of medium to large size in the stroma some with thick walls (reticulin ,400X).

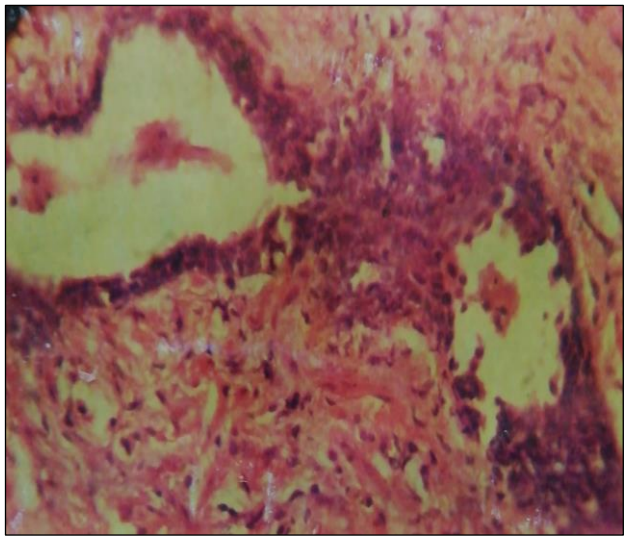

Figure 3: Photomicrograph of preneoplastic lesion of prostate showing 8-9 small to medium sized micro vessels around acini and along basement membrane. (H and E 400X).

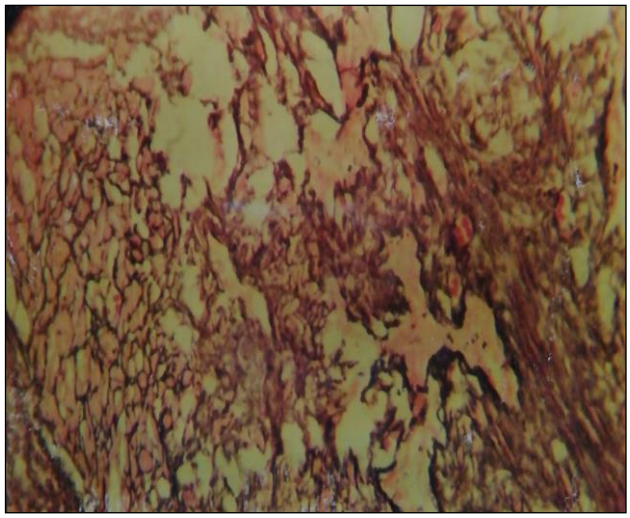

Figure 4: Photomicrograph of preneoplastic lesion of prostate showing small to medium sized micro vessels 10-12 in number, mostly thin walls, some with red blood cells around (reticulin 400X).

Brawer et al studied 25 cases of pre-neoplastic lesion for neovascularity relative to benign tissue.[11] They found that in Prostatic Intraepithelial Neoplasia the acini and ductules had increased neovascularity relative to benign epithelium in 18 of 25 cases. Linear vessel density ranged a from a low- of 2.5vV/mm (benign) to a high of 17.8vV/mm (Prostatic Intraepithelial Neoplasia). In frankly neoplastic cases, the range of micro vessel density was found to be 12-26/hpf with a mean of 19/hpf in reticulin stained sections. The micro vessels were haphazardly arranged and were seen between glands, in papillary projections and also along basement membrane. (Figure 5 and 6)

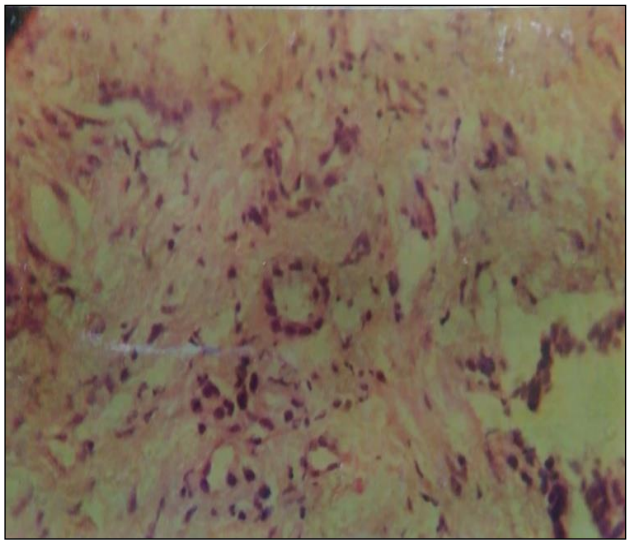

Figure 5: Photomicrograph of adenocarcinoma of prostate showing 12-13 micro vessels small to medium sized in the stroma and along basement membrane (H and E400X).

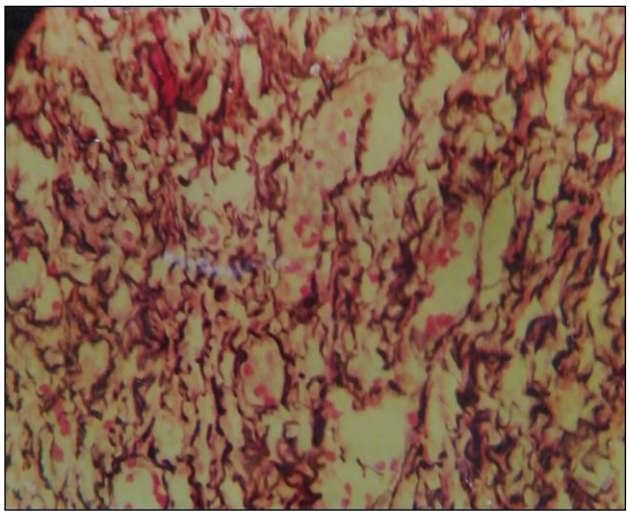

Figure 6: Photomicrograph of adenocarcinoma of prostate showing 12-13 microvessels mostly with red blood cells having thin walls (reticulin 400X).

To compare micro vessel density of neoplastic and non-neoplastic lesion a ratio of approx. 3:1 is obtained i.e. micro vessel density in neoplastic lesions is three times higher when compared with non- neoplastic lesions. Deering et al found that overall ratio of vessels/unit area in sections of carcinoma verses benign tissue was approximately double.[12] Bigler SA while studying morphology of blood vessels demonstrated that capillaries in benign tissue were restricted to petiglandular stroma immediately adjacent to epithelium whereas in carcinoma it appears to be more random. In present study, Reticulin staining was used to highlight the micro vessels. But it lacks sensitivity when compared with either marking, the vessels by antibodies to factor-

VIII related antigen or when a double labeling technique for Ki-67 and CD-34 was used.

So, to improve upon the result immunohistochemistry can be used for studying angiogenesis in different lesions in various organs.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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