ORIGINAL ARTICLE

Quantitative evaluation by interactive image analysis system and its correlation with clinico-histopathological parameters in benign versus malignant lesions of prostate

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ABSTRACT
BACKGROUND: Morphometry is a potentially useful adjunct in surgical pathology for identifying malignant cells in lesions largely composed of benign appearing cells, distinguishing benign and malignant lesions with similar appearances or in distinguishing between similar appearing types of malignant neoplasm. The use of quantitative information on cellular details has been demonstrated to improve the accuracy and reproducibility of histopathological diagnosis. **MATERIALS AND METHODS:** The present study was performed on fifty cases, twenty five each of BPH and carcinoma prostate to evaluate the morphometric parameters – mean nuclear area (MNA), mean cytoplasmic area , N:C ratio , mean nuclear diameter , mean nuclear perimeter and mean nucleolar size in BPH and carcinoma prostate and its correlation with presenting symptoms and investigatory findings including- digital rectal examination , serum PSA levels , ultrasonography , histopathologic diagnosis. **RESULTS:** MNA ranged from 17.25-43.69 um^2 (mean 26.42 ± SD 7.22) and 11.71-80.00 um^2 (mean 37.15 ± SD15.17) respectively in BPH and carcinoma prostate. Mean cytoplasmic area ranged from 40.95-126.19um^2 (78.34 ± SD 21.48) and 11.65-84.59um^2(mean 54.60± SD 16.81) respectively in BPH and carcinoma prostate .N:C ratio in BPH and carcinoma prostate ranged from 0.18-0.69(mean 0.34 ± SD 0.11) and 0.26-0.81um (mean 0.62± SD 0.12) respectively. Mean diameter in BPH and carcinoma prostate ranged from 1.00-2.00um (1.54 ± SD 0.29) and 1.00-2.20um (mean1.74 ±SD 0.44) respectively. Mean perimeter ranged from 4.20-8.90um (mean 6.80± SD 0.99) and 5.20-12.2um (mean8.00 ± SD 1.80) in BPH and carcinoma prostate respectively. Nucleolar size in BPH and carcinoma prostate ranged from 0.10-0.30um (mean 0.03 ± SD 0.08) and 0.04-0.40um (mean 0.13 ± SD 0.16) respectively. **CONCLUSION:** The variation in MNA, mean cytoplasmic area, N:C ratio mean diameter , mean perimeter and mean nucleolar size between BPH and carcinoma prostate were statistically significant with a p value of <0.05.

Keywords: BPH, Carcinoma prostate, Morphometry, Mean nuclear area , Mean cytoplasmic area, N:C ratio, Mean diameter, mean perimeter.

INTRODUCTION

Prostate is an important sexual organ in males. Development of prostatic hyperplasia continues to be a leading cause of morbidity in elderly males, causing obstruction to urinary outflow. The clinical incidence of this disease is about 8% during the fourth decade, but it reaches upto 50% in the fifth decade and 75% in the eighth decade.[1] Prostate cancer is an important growing health problem presenting a challenge to urologists, radiologists, oncologists and pathologists as well. Prostate cancer is the most common nondermatologic cancer, yet despite its frequent occurrence, the clinical course is often unpredictable. Most prostate cancers are slow growing and may not manifest during the man's lifetime; in fact, many men are found to have incidental microscopic foci of prostate cancer at postmortem examination. Thus, many men die with prostate cancer rather than from prostate cancer; however, some prostate cancers are aggressive, with a rapidly worsening course .[2]

The word morphometry means "measurement of form". Morphometry is the quantitative description of geometric features of structures such as cell, nuclei or nucleoli. It is being increasingly established in medical centers as an important research tool and also accepted as a method of diagnostic and prognostic statements. Computerized image analysis has become an important tool in the pathology laboratory for

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quantitative morphologic analysis. It utilizes a high resolution video camera, a digital frame grabber and image analysis software in a computer in a very efficient and precise method for estimating stereologic parameters of the cells.[3-5]

The sharp increase of interest in the application of quantitative pathology in cancer diagnosis and prognosis is mainly due to the following reasons:

(a) Increased social demands of quantitation and objectivity;
(b) Improvement in, and widespread availability of adequate technology;
(c) Awareness that changes can be detected with quantitative analysis which would otherwise escape observation;
(d) The improvement of therapeutic possibilities for cancer patients.

Finally, the opinion of pathologists have not always proved consistent or reproducible while quantitative pathological analyses are more reproducible and capable of preventing under- and overtreatment.[6]

Specific areas in which morphometry could be helpful

1. To identify malignant cells in lesions that are largely composed of benign appearing cells (e.g. follicular thyroid neoplasms).
2. To define reference points in apparent continua (e.g. progression from normal colon to adenoma to adenocarcinoma).
3. To distinguish between benign and malignant lesions with similar appearances (e.g. fibromatosis and fibrosarcoma of soft tissue).
4. To distinguish between similar – appearing types of malignant neoplasms (e.g. between small cell carcinoma of lung and small cell lymphoma).[7]

There seems to be number of facets of morphometric studies which hinder their wider application by the diagnostic pathologist. These include the following:

1. Lack of large conclusive studies.
2. Requirement of expensive computer equipment.
3. Lack of clear and standardized protocols for potential applications.
4. Poor understanding or fear of technology and numerical methods.
5. Time consuming.[4]

As there is overlapping appearances in the ultrasonographic and the prostate specific antigen values, histopathological examination followed by quantitative evaluation by interactive image analysis is required to characterize the lesions . The present study has been undertaken to evaluate the morphometric parameters – nuclear area , cytoplasmic area , N:C ratio , nuclear diameter , nuclear perimeter and nucleolar size in BPH and carcinoma prostate and its correlation with presenting symptoms and investigatory findings including- digital rectal examination , serum PSA levels , ultrasonography , histopathologic diagnosis.

MATERIALS AND METHODS

This prospective study was carried out in the Department of Pathology, Pandit Bhagwat Dayal Sharma Post Graduate Institute of Medical Sciences, Rohtak . A total of fifty cases, including 25 cases each of carcinoma prostate and BPH constituted the material for the present study.

Morphometric Analysis: Hematoxylin and Eosin stained slides, processed by conventional technique were studied for routine histopathological examination using light microscope and diagnosis were made as far as possible. The quantitative morphometric studies will be done by image analysis. Images provided by a charge device video camera coupled with Olympus BX51 microscope at a magnification of 400X (40X x 10X) were stored on a host computer based on Pentium 4 processor with operating system Microsoft Windows XP through a digital frame grabber and processing was done by image analysis software Image Pro Plus Version 6.3. One hundred epithelial cells were randomly selected and measured in each case. The cells of interest were identified on the screen and the contours of their nuclear and cytoplasmic profiles were traced manually. Inside each tracing, a semiautomatic procedure consisting of threshold based boundary detection was implemented to determine the nuclear and cytoplasmic areas.

Following parameters were studied:

1. Nuclear area
2. Cytoplasmic area
3. N:C Ratio

4. Nuclear diameter
5. Nuclear perimeter
6. Nucleolar size

The results thus obtained were tabulated and analyzed using statistical method – unpaired t test (p value < 0.05 was taken as significant) to find out differences between benign and malignant lesions of prostate and correlate them with clinical findings, ultrasonographic findings, PSA expression and histopathological appearance .

Statistical analysis: The results thus obtained were tabulated. The statistical difference of the average values for mean nuclear area, cytoplasmic area, N:C ratio, nuclear diameter , nuclear perimeter and nucleoli between BPH and carcinoma prostate was analyzed using the Student unpaired t test .The correlation between Gleason's grade and mean nuclear area was analyzed using the Pearson correlation coefficient. Statistical significance was considered when $p < 0.05$.

RESULTS

Table 1: Clinicopathological features in BPH and carcinoma prostate

Serum PSA levels	BPH (no. of cases/percentage)	Carcinoma prostate (no. of cases/percentage)
<4ng/ml	5/20%	1/4%
4-10ng/ml	14/56%	0/0%
11-20ng/ml	2/8%	5/20%
21-30ng/ml	3/12%	2/8%
31-50ng/ml	0/0%	7/28%
>50ng/ml	1/4%	10/40%
USG findings		
Hyperechcogenicity	32%	28%
Hypoechogenicity	32%	20%
Isoechogenicity	16%	12%
Mixed echogenicity	20%	40%
Homogenous texture	80%	24%
Heterogenous texture	20%	76%

Table 1 shows correlation of serum PSA with BPH and carcinoma prostate. Maximum number of cases (56%) had a serum PSA level 4-10 ng/ml in cases of BPH while in carcinoma prostate most of the cases (40%) had serum PSA level >50 ng/ml. In ultasonographic findings most of the cases were of mixed echogenicity in carcinoma prostate followed by hyperechoic, hypoechoic and isoechogenicity. In BPH, both hyperechoic and hypoechoic lesions were present in equal proportion followed by cases of mixed and isoechogenicity. Maximum number of cases (76%) in carcinoma prostate and 80% in BPH were of heterogenous and homogenous texture respectively.

Table 2: Morphometric features in BPH and carcinoma prostate

Morphometric values	BPH Range(mean±SD)	Carcinoma prostate Range(mean±SD)
Mean nuclear area (um^2)	17.25-43.69 (26.42±7.22)	11.71-80.00 (37.15±15.17)
Mean cytoplasmic area (um^2)	40.95-126.19 (78.34±21.48)	11.65-84.59 (54.60±16.81)
N:C ratio	0.18-0.69 (0.34±0.11)	0.26-0.81 (0.62±0.12)
Mean diameter (um)	1.00-2.00 (1.54±0.29)	1.00-2.20 (1.74±0.44)
Mean perimeter (um)	4.20-8.90 (6.80±0.99)	5.20-12.2 (8.00±1.80)
Nucleolar size (um)	0.10-0.30 (0.03±0.08)	0.04-0.40 (0.13±0.16)

Nuclear area in 25 cases of BPH were in the range of 17.25-43.69μm^2 with a mean of 26.42±7.22 μm^2 whereas in 25 cases of carcinoma prostate nuclear area were in the range of 11.71-80.00 μm^2 with a mean of 37.15±15.17 μm^2.The variation in nuclear area between BPH and carcinoma prostate was statistically significant.

Cytoplasmic area in BPH was in the range of 40.95-126.19μm^2 with a mean of 78.34±21.48μm^2, whereas in carcinoma prostate cytoplasmic area was in the range of 11.65-84.59μm^2 with a mean of 54.60±16.81μm^2. The variation in cytoplasmic area between BPH and carcinoma prostate was statistically significant with a p value of <0.05. N:C ratio in BPH was in the range of 0.18-0.69 with a mean of 0.34±0.11, whereas in cases of carcinoma prostate N:C ratio was in the range of 0.26-0.81 with a mean of 0.62±0.12. The variation in N:C ratio between BPH and carcinoma prostate was significant(<0.05). Nuclear diameter in cases of BPH was in the range of 1.00-2.00um with a mean of 1.54±0.29, whereas in carcinoma prostate it was in the range of 1.00-2.20um with a mean of 1.74±0.44. The variation in nuclear diameter between BPH and carcinoma prostate was significant. Nuclear perimeter in BPH was in the range of 4.20-8.90um with a mean of 6.80±0.99um, whereas in carcinoma prostate nuclear perimeter was in the range of 5.20-12.2um with a mean of 8.00±1.80um. The variation in nuclear perimeter between BPH and carcinoma prostate was also significant.

In cases of BPH, number of nuclei with nucleoli was 7.6±21.26% in cases of BPH, whereas in

cases of carcinoma prostate number of nuclei with nucleoli was 32.8±34.46%. Nucleolar size was in the range of 0.10-0.30um with a mean of 0.03±0.08um in BPH, whereas in carcinoma prostate nucleolar size were in the range of 0.04-0.40um with a mean of 0.13±0.16um. The variation in nucleolar size and number of nuclei with nucleoli between BPH and carcinoma prostate was significant.

DISCUSSION

Prostate cancer is an important growing health problem with often unpredictable course and presenting as diagnostic challenge to urologists, radiologists and pathologists. Prostatic lesions can be diagnosed by digital rectal examination, ultrasonography and prostate specific antigen value, but few indicators currently distinguish progressive prostatic tumors from those that are more indolent. As there is overlapping appearances in the ultrasonographic and the prostate specific antigen values , histopathological examination followed by quantitative evaluation by interactive image analysis is required to characterize the lesions .This study was planned to differentiate benign from malignant prostatic lesions by quantitative evaluation on morphometry and to correlate various parameters like clinical evaluation , ultrasonographic findings , PSA expression and histopathological appearance of the disease .

Table 3: Comparison of mean nuclear area in cases of carcinoma prostate

Study group	Mean nuclear area (um^2)
Aragona et al[8]	34.6
Tardiff et al[9]	55.8±12.7
Mohler et al[10]	29.9±6.6
Zang et al[11]	36.2
Hironori et al[12]	20.94±5.82
Bektas et al[13]	22.43±7.43
Present study	37.15±15.17

Aragona et al measured the mean nuclear area of cases of carcinoma prostate and adenosis. They found that mean nuclear area of carcinoma and adenosis was 34.6um^2 and 22.9um^2 respectively.[8]

The findings of present study revealed that mean nuclear area of carcinoma prostate and BPH were 37.15±15.17um^2 and 26.42±7.22um^2 respectively with a statistically significant difference between the mean nuclear areas of both the groups.

There were no other studies found in the literature comparing the mean nuclear areas of BPH and carcinoma prostate.

Tardif et al used nuclear morphometry to compare histologic and cytologic specimens obtained from patients who underwent radical prostatectomy for clinically localized prostatic carcinoma. Nuclei of Diff-Quik preparation were nearly twice as large as in Papanicolaou and histologic preparations. Nuclear area in histologic preparation was 55.8±12.7um^2 .[9]

Mohler JL et al compared nuclear morphometry on automatic biopsy and radical prostatectomy specimens in patient with prostatic carcinoma. They concluded that nuclear size was smaller in biopsy specimens (area 29.9 ±6.6 um^2) than in prostatectomy specimens (area 48.2± 8.7 um^2).[10]

Zhang et al compared nuclear morphometric values and Gleason scores between biopsy and radical prostatectomy specimens in patients with clinically localized prostate cancer. The average value for the mean nuclear area of the biopsy specimens was 36.2 um^2 which was smaller than those of prostatectomy specimen (51.4 um^2). Gleason score were identical in 32% cases.[11]

Hironori et al who evaluated the efficacy of morphometry as a prognostic factor in 35 localized prostate cancers (15 stage B cases, 20 stage C cases). All cases were diagnosed as moderately or poorly differentiated adenocarcinoma by needle biopsy. Mean nuclear area was 20.94±5.82.[12]

Bektas et al evaluated nuclear parameters such as roundness factor, form ellipse , area , length and perimeter on 130 prostatic adenocarcinoma cases (77% needle biopsies and 23% prostatectomy specimens). They concluded that nuclear size and shape factors especially mean nuclear area were concordant with the Gleason score and the mean nuclear area & mean nuclear perimeter were smaller in cases of needle biopsy specimens than in prostatectomy specimen.[13]

The findings of present study was consistent with Aragona et al , Tardif et al ,Mohler JL et al, Zhang et al , Hironori et al and Bektas et al .

Mean cytoplasmic area of BPH and carcinoma prostate were 78.34±21.48μm^2 and 54.60±16.81μm^2 respectively. N:C ratio of BPH and carcinoma prostate were 0.34±0.11µm and 0.62±0.12µm respectively . Both show

significant difference between BPH and carcinoma prostate. We could not find any previous studies, which had included cytoplasmic variables for differentiating benign cells from malignant cells.

Table 4: Comparison of mean nuclear diameter in cases of carcinoma prostate

Study group	Mean nuclear diameter(um)
Argona et al[8]	5.59±0.91
Bektas et al[13]	6.54±1.07
Present study	1.74±0.29

In present study there was a significant difference between nuclear diameter of BPH (1.54±0.29µm) and carcinoma prostate (1.74±0.29µm). In contrast to the previous studies, low mean nuclear diameter was observed in our study as the maximum cases of carcinoma prostate were diagnosed on needle biopsy (22cases). Aragona et al conducted a morphometric study to distinguish prostatic dysplasia from well differentiated adenocarcinoma. He concluded that mean nuclear diameter greater than 5mu predict malignancy.[8]

Bektas et al evaluated mean nuclear diameter of prostatic adenocarcinoma cases diagnosed on needle biopsies and prostatectomy specimens. Mean nuclear diameter in needle biopsy and prostatectomy specimen were 6.54±1.07um and 6.75±1.06um respectively.[13]

In the present study mean nuclear perimeter in cases of BPH and carcinoma prostate were 6.80±0.99µm and 8.00±1.80µm respectively and there was a significant difference of nuclear perimeter between BPH and carcinoma prostate. But the values were lower than the previous studies.

Mohler JL et al compared nuclear morphometry on automatic biopsy and radical prostatectomy specimens in 20 patients with prostatic carcinoma. He concluded nuclear size was smaller in biopsy specimens with nuclear perimeter 17±4.9 um.[10]

Bektas et al evaluated nuclear perimeter of prostatic adenocarcinoma cases diagnosed on needle biopsies and prostatectomy specimens. Nuclear perimeter in needle biopsy and prostatectomy specimen were 18.43±3.07um and 19.77±3.25um respectively.[13]

Helpap et al discovered in his study that with increasing grade of malignancy in prostatic carcinoma, the proportion of nuclei containing one or more nucleolus also increases. They also concluded that in BPH, the nuclei containing nucleoli was upto 0.5% and in carcinoma prostate percentage increases as the grade of carcinoma increases. Careful analysis of the number and localization of nucleoli can be helpful in the analysis of the differential diagnosis between carcinoma and atypical hyperplasia of the prostate, which can be difficult in histopathological and cytopathological material.[14]

In the present study, numbers of nuclei with nucleoli were 7.6±21.26% in cases of BPH, whereas in cases of carcinoma prostate numbers of nuclei with nucleoli were 32.8±34.46%. The variation in nucleolar size and number of nuclei with nucleoli between BPH and carcinoma prostate was statistically significant and consistent with the findings of the study of Helpap et al.

Bostwick et al studied morphologic criteria for distinction between atypical adenomatous prostatic hyperplasia and well-differentiated carcinoma. He measured mean nucleolar diameter and found that it was 0.69 um and 1.78um in atypical adenomatous hyperplasia and carcinoma prostate respectively.[15]

In the present study we found that mean nucleolar diameter was lower than the above mentioned study. But the difference in the values between BPH and carcinoma was significant.

Kavantzas et al noticed that the higher the percentage of nucleolated nuclei, the bigger the nuclear and nucleolar areas.[16]

In the present study the percentage of nucleolated nuclei and nuclear area was not significantly correlated (paired t test $p > 0.05$).

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