 doi: 10.5146/tjpath.2010.01025

Differences in the Cytological Features of Atypical Adenomatous Hyperplasia and Low-Grade Prostatic Adenocarcinoma

Atipik Adenomatöz Hiperplazi ve Düşük Dereceli Prostatik Adenokarsinomların Sitolojik Özelliklerindeki Farklılıklar

Ahmet MİDİ[1], Tülay TECİMER[2], Süheyla BOZKURT[2], Naziye ÖZKAN[2]

Department of Pathology, [1]Maltepe University, Faculty of Medicine, [2]Marmara University, Faculty of Medicine, İSTANBUL, TURKEY

ABSTRACT

Objective: The aim of this study was to review the histological features and to define parameters distinguishing atypical adenomatous hyperplasia from prostatic adenocarcinoma grade 1 and 2. We evaluated 14 parameters related with cytological properties.

Material and Method: We found 11 atypical adenomatous hyperplasia (22 foci) and 15 prostatic adenocarcinoma grade 1 and 2 (22 foci) lesions in 105 radical prostatectomy specimens. Basal cell-specific keratin (34βE12) was applied and based on the fact that prostatic adenocarcinoma grade 1 and 2 lesions do not have basal cells we grouped the lesions as atypical adenomatous hyperplasia and prostatic adenocarcinoma grade 1 and 2.

Results: Statistically significant differences were found between atypical adenomatous hyperplasia and prostatic adenocarcinoma grade 1 and 2 lesions for some parameters including the largest nuclear diameter, nuclear location, 1-2 μm nucleolus, > 2 μm nucleolus, and nuclei containing multiple nucleoli. We found similar properties between the two lesions for the following parameters: irregularity of nuclear membrane, median diameter of the nucleolus, chromatin pattern, pynotic nucleus, nuclear pleomorphism, < 1 μm nucleolus, nucleolar margination, and the ratio of nucleus to cytoplasm and the appearance of cytoplasm in the secretory cells.

Conclusion: Evaluation of the overall histomorphological criteria is important in the differentiation of atypical adenomatous hyperplasia and prostatic adenocarcinoma grade 1 and 2 lesions.

Key Words: Prostate, Hyperplasia, Adenocarcinoma

ÖZ

Amaç: Bu çalışmada, atipik adenomatöz hiperplazi'nin histolojik özelliklerinin gözden geçirilmesi ve prostatik adenokarsinom Gleason grade 1 ve 2'den ayırt edebilecek parametrelerin ortaya konması amaçlanmıştır. Sitolojik özellikleri ile ilişkili 14 parametre incelenmiştir.

Gereç ve Yöntem: Yüz beş radikal prostatektomi materyalinde 11 atipik adenomatöz hiperplazi (22 odak) ve 15 prostatik adenokarsinom Gleason grade 1 ve 2 (22 odak) tespit edilmiştir. Bazal hücrelere özgü keratin (34βE12) uygulanmış ve prostatik adenokarsinom Gleason grade 1 ve 2 lezyonların bazal hücre içermemesi esas alınarak lezyonlar atipik adenomatöz hiperplazi ve prostatik adenokarsinom Gleason grade 1 ve 2 olarak gruplandırılmıştır.

Bulgular: İki lezyon arasında, en büyük nükleus çap ortalaması, nükleus yerleşimi, 1-2 μm nükleol, >2 μm nükleol, multipl nükleollu nükleus özellikleri bakımından istatistiksel olarak anlamlı fark bulunmuştur. İncelenen diğer parametreler olan nükleer membran düzensizliği, nükleus çap ortalaması, kromatin paterni, piknotik nükleus, nükleer pleomorfizm, <1 μm nükleol, nükleoler marginasyon (nükleer membrana değen nükleol varlığı), sekretuar hücre nükleus/sitoplazma oranı, sekretuar hücre sitoplazmik görünümü her iki lezyonda benzer özellik göstermiştir.

Sonuç: Çalışmamızda, histomorfolojik özelliklerin birlikte değerlendirilmesinin, atipik adenomatöz hiperplazi ve prostatik adenokarsinom Gleason grade 1 ve 2 lezyonların ayırıcı tanısında önemli olduğu sonucuna varılmıştır.

Anahtar Sözcükler: Prostat, Hiperplazi, Adenokarsinom

Received : 15.06.2009
Accepted : 05.10.2009

Correspondence: Ahmet MİDİ
Department of Pathology, Maltepe University, Faculty of Medicine, İSTANBUL, TURKEY
E-mail: ahmetmidi@yahoo.com Phone: +90 216 399 97 50

INTRODUCTION

Atypical adenomatous hyperplasia (AAH) is a small glandular proliferation usually with an orderly margin. Its frequency is 1.6-36.9% and it is more often seen in the transition zone (1-13). It has been called adenosis, AAH, atypical adenosis, atypical small acinar proliferation or small gland hyperplasia (1-7, 14-22) in other reports. AAH is focused around or in a hyperplastic nodule (17). It may display a lobular pattern and has uniform small glands under low magnification (21,23). Its border is usually distinct and orderly but may have focal irregularities (10,24). AAH consists of cells that have a clear or faded cytoplasm. The nucleus is round and is located at the basal portion of the cell. Its chromatin pattern is granular similar to a normal prostatic cell. It usually has a small nucleolus. Basal cells are inconspicuous (17,23).

Prostatic adenocarcinoma grade 1 (PACG 1) are round lesions composed of medium-size monotonous glands displaying minimal stromal invasion with cytoplasmic features similar to benign glands that expand by pushing surrounding tissues (25,26). Prostatic adenocarcinoma grade 2 (PACG 2) lesions display early infiltrative findings and mild size differences in glands in addition to PACG 1 features (25,26). There are many studies on the diagnostic criteria of AAH and differentiating AAH from PACG 1 and 2 (17,27-30). These studies have reported that nucleolus size, basal cell-specific keratin immunohistochemistry characteristics (34βE12, eIF 3/p66), aspect of the glands' luminal side, and existence of acidic mucin are the principal criteria. However, additional data are required when these are equivocal. Our previous main studies evaluated the distinguishing features of the AAH and PACG grade 1 and 2. Structural and secretory features were published as two separate articles (31,32). In this article we evaluated 14 cytological criteria (cytoplasmic, nuclear, nucleolar) in addition to the histological criteria of AAH and low-grade prostatic adenocarcinoma. The aim of this study was to determine the parameters that would enable differentiating these two lesions.

MATERIAL and METHOD

Case Selection

A total of 105 consecutive radical prostatectomy specimens evaluated at the Marmara University Medical Faculty between October 1999 and September 2004 were included in the study. The slides was re-evaluated and those containing AAH and PACG 1 and 2 lesions were selected. Three cases where the lesion consisted of small glands with inadequate areas representing the lesion in the tissue following serial sections were excluded. Eleven (22 foci) cases of AAH, and 15 (22 foci) cases of PACG 1 and 2 were evaluated for the study.

34βE12 immunohistochemistry was performed to show the basal layer on the 4 μm sections obtained from the paraffin blocks of the PACG 1 and 2 where the diagnosis was suspected and in all AAH lesions.

Evaluated Parameters: We studied AAH, PACG 1 and 2 lesions by dividing them using 14 parameters regarding nuclear, nucleolar and cytoplasmic features into 3 groups. These groups and the parameters are presented at Table I.

Evaluation of the Parameters: Table II presents the evaluation of the nuclear, nucleolar and cytoplasmic parameters.

Largest Mean Nuclear Diameter: All the lesions were scanned under x400 magnification and the diameter of 10 large nuclei was measured with the ocular micrometer. The largest nucleus found was measured again at x1000 magnification with the ocular micrometer and the result was specified as the largest nuclear diameter for that lesion.

Mean Nuclear Diameter: 10 fields at random were analyzed under x1000 magnification; 10 cells were measured in each field with the ocular micrometer and the mean value was determined.

The chromatin pattern was evaluated comparatively under x1000 magnification and all nuclei in the lesion were examined. A value of 1 to 4 was assigned depending on the homogeneity and presence of a chromocenter.

Nuclear membrane irregularity was determined comparatively under x1000 magnification and all cells in a randomly chosen area were examined. A value of 1 to 3 was assigned according to the number of nuclei with these features.

<1 μm nucleolus, 1-2 μm nucleolus, an >2 μm nucleolus parameters were evaluated in an independent and quantitative manner. The number of glandular nuclei in three randomly chosen areas under x1000 magnification were counted and a value of 0 to 3 was assigned after the ratio of nuclei containing <1 μm nucleolus, 1-2 μm nucleolus or >2 μm nucleolus was determined. Oval-round, eosinophilic or amphophilic intranuclear structures were defined as nucleolus and measured with an ocular micrometer. (The grid interval is equal to 25, 10, 5, 2.5 and 1 μm on x40, x100, x200, x400 and x1000 magnification respectively on ocular micrometer measurements).

Nucleolar margination (the number of nucleoli touching the nuclear membrane) and nuclei with multiple nucleoli parameters were evaluated quantitatively and all cells in a randomly chosen area were counted. The nuclei with these characteristics were assigned a numerical value from 0 to 3.

Immunohistochemical staining method: (34βE12); keratin, HMW Ab-3 (1/50; clone 34 beta E12; MS-1447-S1; Neomarkers, CA, USA). The streptavidin biotin/horseradish peroxidase (Str.AB/HRP) method was used to show keratin expression. A drop of Ultra V Block (Ultra Vision Kit; TP-125-HL; Lab Vision, CA, USA) was used on the slides to prevent nonspecific staining. The tissues were incubated

Table I: Grouping of evaluated parameters

Cytological features	Nuclear	Irregularity of nuclear membrane, largest median diameter of nucleus, median diameter of nucleus, chromatin pattern, pycnosis, nuclear location, pleomorphism
	Nucleolar	<1μm nucleolus, 1-2 μm nucleolus, >2 μm nucleolus, nucleolar margination, nucleus including multiple nucleoli
	Cytoplasmic	Ratio of nucleus to cytoplasm in the secretory cells, appearance of cytoplasm of glandular cells

Table II: Evaluation of the parameters of the cytological features

Histological parameter	**Evaluation**			
Nuclear				
Pleomorphism (relative)	Absent	Mild	Distinct	
Pycnosis	Absent	Present		
Location (relative)	Basal	Basal and medium	Basal, medium and apical	
Membrane irregularity (% of cells)	None	<%5	%5-50	>%50
Largest median diameter of nucleus, median diameter of nucleus	Quantitative with ocular micrometer (μm)			
Chromatin pattern	Homogeneous	Finely dotted	Coarsely dotted	Including chromocenter
Nucleolar				
< 1μm nucleolus, 1-2 μm nucleolus, or >2 μm nucleolus (% of cells)	Absent	<%5	%5-50	>%50
Nucleolar margination, nucleus including multiple nucleoli (% of cells)	Absent	<%5	%5-50	>%50
Cytoplasmic				
Appearance of cytoplasm	Clear	Mildly eosinophilic	Eosinophilic	
Ratio of nucleus to cytoplasm in secretory cells	In favour of nucleus	Equivalent	In favour of cytoplasm (low columnar cells)	In favour of cytoplasm (high columnar cells)

for 10 minutes in biotinylated secondary antibody (Ultra Vision Kit; TP-125-HL; Lab Vision, CA, USA). Streptavidine peroxidase (Ultra Vision Kit; TP-125-HL; Lab Vision, CA, USA) was applied. DAB (TA-125-HD, Lab Vision) was used as the chromogen. Cytoplasmic brown staining of the basal cells was accepted as positive.

Statistical evaluation: The results of the study were analyzed by SPSS (Statistical Package for Social Sciences for Windows, version 11.0) package software.

Mann-Whitney U test was used to compare the numeric variables between types (largest mean nuclear diameter, mean nuclear diameter, largest mean glandular diameter, mean glandular diameter, lesion width, and lesion height parameters).

Chi-square test was used to compare the non-numerical data between the types. Accordingly, the nuclear pleomorphism, nuclear membrane irregularity, chromatin pattern, stratification, <1 μm nucleolus, 1-2 μm nucleolus, >2 μm nucleolus, nucleolar margination, multinucleolated and multinucleated nucleus, secretory cell cytoplasmic appearance, and secretory cell nucleus/cytoplasm ratio parameters were statistically evaluated.

Fisher exact probability test, a subgroup of the Chi-square relation test, was used when evaluating four-cell tables where the expected values were less than 5 (pynotic nucleus, nucleus localization).

The results were evaluated at the $p<0.05$ significance level.

RESULTS

The cytoplasmic parameter results and statistical evaluation for AAH (Figure 1A) and PACG 1 and 2 (Figure 1B) are presented in Table III.

The nuclei tended to localize at the basal cytoplasm in PACG 1 and 2 and mixed in both the basal and middle sections of the cytoplasm in AAH (Figure 2A,B). There was a significant difference between AAH and PACG 1 and 2 lesions for nuclear localization ($p<0.05$). The largest mean nucleus diameter was smaller in AAH than PACG 1 and 2. The difference was statistically significant ($p<0.05$).

There was no statistically significant difference between the groups for nuclear pleomorphism (Figure 3A,B), nuclear membrane irregularity (Figure 4), pynotic nucleus, chromatin pattern and mean nucleus diameter parameters ($p>0.05$).

We found <1 μm nucleolus in all AAH and PACG 1 and 2 lesions in our study and there was no significant difference between the groups for <1 μm nucleolus ($p>0.05$).

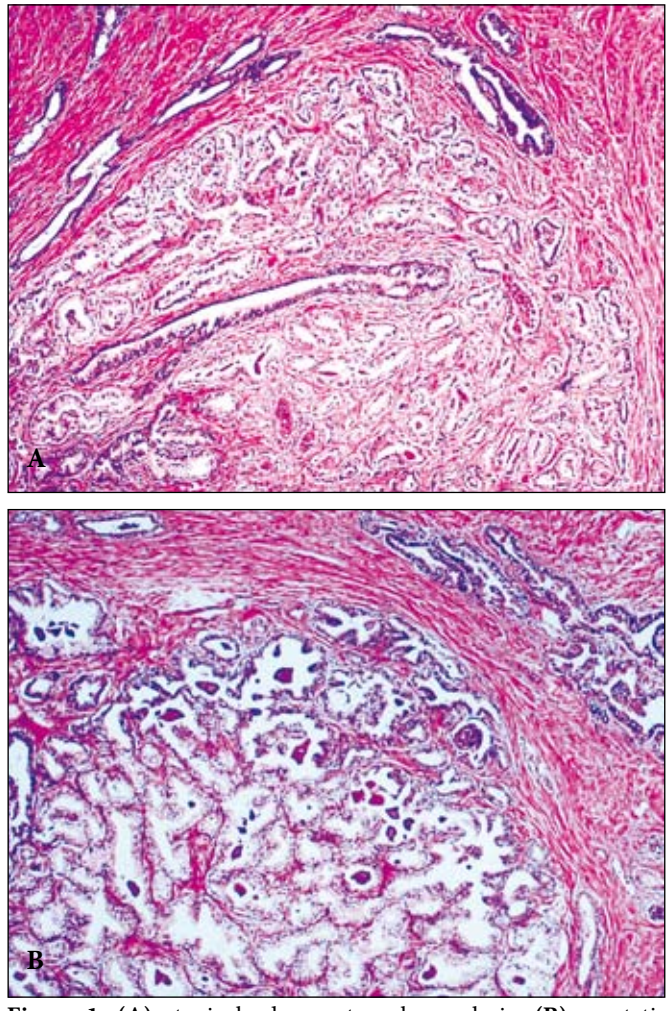

Figure 1: (A) atypical adenomatous hyperplasia, **(B)** prostatic adenocarcinoma grade 1 and 2 (H&E, x100).

The percentage of 1-2 μm nucleolus was 63.6% for AAH (Figure 3A), and 95.5 for PACG 1 and 2 lesions. PACG 1 and 2 lesions had >2 μm nucleolus at a rate of 50% (Figure 4) while no AAH lesions had such nucleolus. There was a significant difference between AAH and PACG 1 and 2 lesions for 1-2 μm nucleolus and >2 μm nucleolus ($p<0.05$).

The statistical significance of the 1-2 μm nucleolus parameter in PACG 1 and 2 was due to the higher percentage of nuclei with 1-2 μm nucleolus than in the AAH group and the statistical significance of the >2 μm nucleolus parameter in PACG 1 and 2 was due to none of the AAH lesions containing >2 μm nucleolus.

Multinucleolated nuclei were present in more cells in PACG 1 and 2 lesions than AAH lesions and this result was statistically significant ($p<0.05$). The statistical significance of the multinucleolated nuclei was due to a higher chance

Table III: Comparison of the atypical adenomatous hyperplasia (AAH) and prostatic adenocarcinoma grade (PACG) 1, 2 lesions in terms of cytological parameters and statistical results

Histological parameters	Evaluation	AAH n (%)	PACG 1, 2 n (%)	p value
Largest median diameter of nucleus		9.86 ±1.8/ 6-13 µm	11.63 ± 2.3/ 8-16 µm	Mann-Whitney U test; p<0.05
Median diameter of nucleus		6.68 ± 0.99/ 4-8 µm	7.13 ± 0.88/ 5-9 µm	Mann-Whitney U test; p>0.05
Nuclear pleomorphism	Absent	14 (63.6)	8 (36.4)	Chi square test; p<0.05
	Mild	6 (27.3)	8 (36.4)	
	Distinct	2 (9.1)	6 (27.3)	
Nuclear membrane irregularity	Absent	17 (77.3)	13 (59.1)	Chi square test; p<0.05
	<%5	3 (13.6)	4 (18.2)	
	%5-50	2 (9.1)	5 (22.7)	
	>%50	0 (0)	0 (0)	
Chromatin pattern	Homogeneous	0 (0)	0 (0)	Chi square test; p<0.05
	Finely dotted	11 (50)	9 (40.9)	
	Coarsely dotted	10 (45.5)	7 (31.8)	
	Chromocenter	1 (4.5)	6 (27.3)	
Pycnotic nucleus	Absent	21 (95.5)	21 (95.5)	Fisher definite possibility test; p<0.05
	<%5	1 (4.5)	1 (4.5)	
	%5-50	0 (0)	0 (0)	
	>%50	0 (0)	0 (0)	
Nuclear location	Basal	6 (27.3)	19 (86.4)	Fisher definite possibility test; p=0.0001
	Basal and medium	16 (72.7)	3 (13.6)	
	Basal, medium and apical	0 (0)	0 (0)	
<1 µm nucleolus	Absent	0 (0)	0 (0)	Chi square test; p>0.05
	<%5	16 (72,7)	11 (50)	
	%5-50	6 (27.3)	9 (40.9)	
	>%50	0 (0)	2 (9.1)	
1-2 µm nucleolus	Absent	8 (36.4)	1 (4.5)	Chi square test; p<0.05
	<%5	8 (36.4)	6 (27.3)	
	%5-50	6 (27.3)	9 (40.9)	
	>%50	0 (0)	6 (27.3)	
>2 µm nucleolus	Absent	22 (100)	11 (50)	Chi square test; p<0.05
	<%5	0 (0)	4 (18.2)	
	%5-50	0 (0)	5 (22.7)	
	>%50	0 (0)	2 (9.1)	
Nucleolar margination	Absent	2 (9.1)	4 (18.2)	Chi square test; p=0.057
	<%5	14 (63.6)	5 (22.7)	
	%5-50	5 (22.7)	11 (50)	
	> %50	1 (4.5)	2 (9.1)	
Nucleus including multiple nucleoli	Absent	9 (40.9)	6 (27.3)	Chi square test; p<0.05
	<%5	10 (45.5)	5 (22.7)	
	%5-50	3 (13.6)	7 (31.8)	
	>%50	0 (0)	4 (18.2)	
Appearance of cytoplasm	Eosinophilic	1 (4.5)	3 (13.6)	Chi square test; p>0.05
	Mildly eosinophilic	19 (86.3)	18 (81.8)	
	Clear	2 (9.0)	1 (4.5)	
The ratio of nucleus to cytoplasm in secretory cells	In favour of cytoplasm (high columnar cells)	3 (13.6)	8 (36.3)	Chi square test; p>0.05
	In favour of cytoplasm (low columnar cells)	14 (63.6)	10 (45.4)	
	Equivalent	5 (22.7)	4 (18.1)	
	In favour of nucleus	0 (0)	0 (0)	

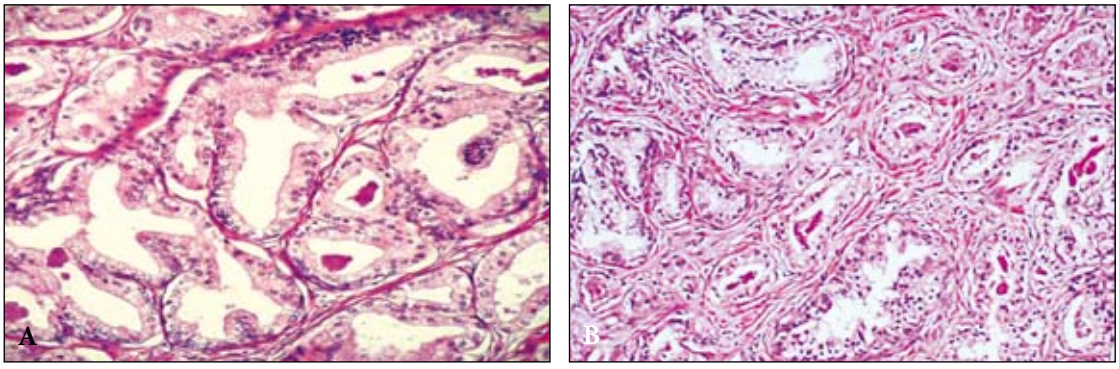

Figure 2: (A) Basal localization of the nuclei in prostatic adenocarcinoma grade 1 and 2, **(B)** Localization of the nuclei in the basal part and center of the cell in atypical adenomatous hyperplasia (H&E, x200).

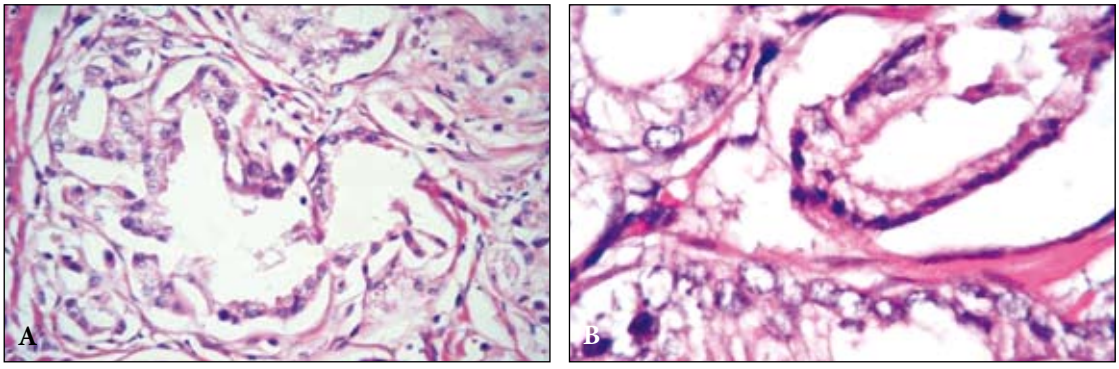

Figure 3: (A) Large nuclei containing 1-2 μm nucleoli and nucleolar margination in atypical adenomatous hyperplasia (H&E, x400), **(B)** Large nuclei in prostatic adenocarcinoma grade 1 and 2 (H&E, x1000 imm. oil).

of multiple nucleoli in 5-50% of nuclei in PACG 1 and 2 and no AAH lesion containing multiple nucleoli in >50% nuclei.

Nucleolar margination was present in more cells in PACG 1 and 2 lesions (Figure 5) and this was very close to being statistically significant (p=0.057). This result was due to a low rate of nucleolar margination in 5-50% of nuclei in AAH lesions.

AAH and PACG 1 and 2 lesions have a slightly eosinophilic cytoplasm. The rate of lesions consisting of high columnar cells was higher in PACG 1 and 2 (Figure 6) but there was no significant difference between the groups for secretory cell cytoplasmic appearance and secretory cell nucleus/cytoplasm parameters (p>0.05).

DISCUSSION

Evaluating lesions consisting of small acini in the prostate gland with a suspicion of malignancy is an often-encountered problem in pathology. The inadequacy of the tissue representing the lesion and the insufficient histological criteria in needle biopsies have led to some cancers being reported as benign lesions and vice versa (33).

We did not find any studies on nuclear membrane irregularity in AAH and PACG 1 and 2 lesions. We found nuclear membrane irregularity at a rate of 23% in AAH and 41% in PACG 1 and 2 lesions with no statistically significant difference. We believe that nuclear membrane irregularity is not a criterion that can be used to differentiate between these lesions. However, more studies are needed to make a definite comment.

Some studies have reported that nuclear anaplasia is important in PACG 1 and 2 lesions (17,24,34-36). The study by Bostwick DG et al. reported a significant difference between AAH and PACG 1 and 2 lesions for nuclear size while there was no difference between the groups for nuclear pleomorphism (17). We used nuclear pleomorphism, largest nucleus diameter and mean nucleus diameter to evaluate nuclear atypia in our study. AAH and PACG 1 and 2 lesions were similar for nuclear pleomorphism and average nucleus diameter. The largest nucleus diameter was higher in cancer (11.64 μm) than in AAH (9.76 μm) and the difference was statistically significant.

AAH has a uniform granular chromatin structure similar to normal prostatic cells (17,37). Bostwick DG et al. have reported the chromatin pattern in all AAH and PACG 1 and 2 lesions as generally uniform and finely dotted (17). Other studies have also not found the chromatin pattern useful to differentiate AAH from adenocarcinoma (27,28,30,38). We found the chromatin finely and coarsely dotted in AAH

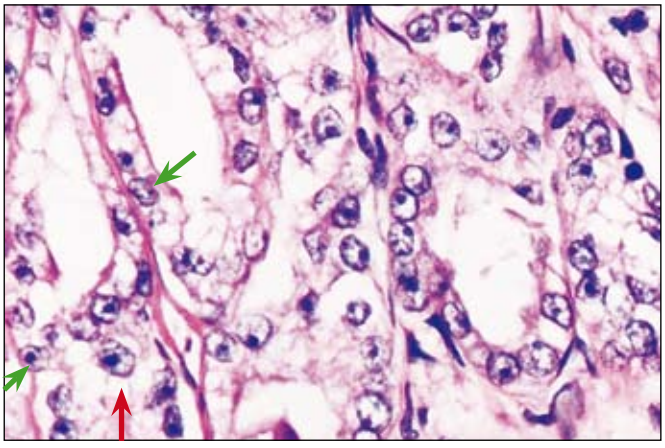

Figure 4: >2 µm nucleoli (red arrow), nuclear membrane irregularity in (green arrow) prostatic adenocarcinoma grade 1 and 2 (H&E, x1000, imm. oil).

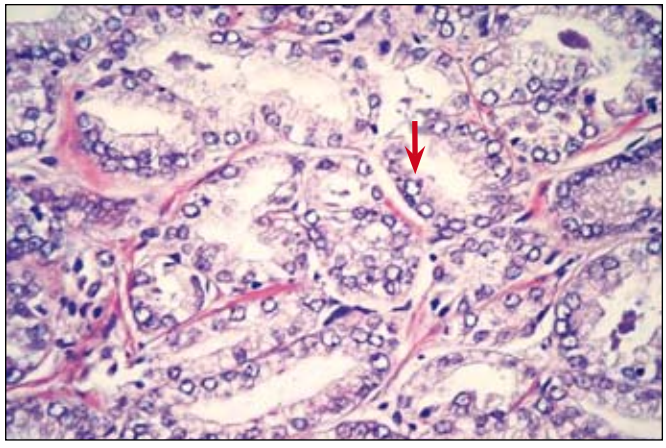

Figure 5: Nucleolar margination in prostatic adenocarcinoma grade 1 and 2 (H&E, x400).

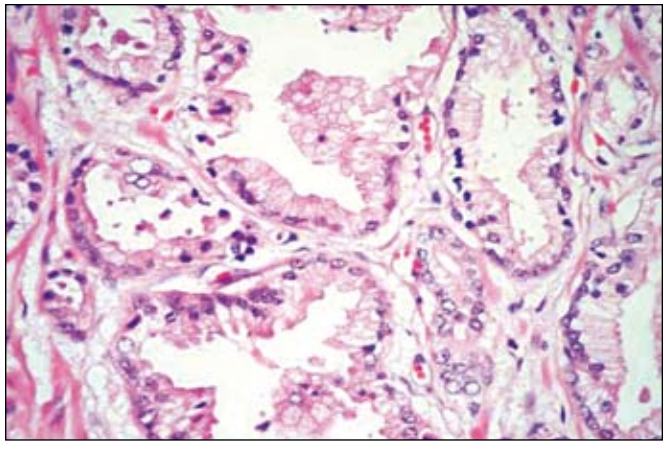

Figure 6: High columnar cells with eosinophilic cytoplasm in prostatic adenocarcinoma grade 1 and 2 (H&E, x200).

and found a chromocenter in 4.5% of the lesions. PACG 1 and 2 lesions were similar to AAH but a higher percentage of lesions had a chromocenter (27.3%). However, this difference was not statistically significant.

Kramer CE et al. and Haussler O have reported a higher rate of apoptosis in PIN and cancer than AAH (24,25). McNeal JE et al found 8 PACG 1 lesions in 77 radical prostatectomy specimens and compared these with PACG 2 and surrounding areas of benign nodular hyperplasia. A pynotic nucleus was seen in almost all large magnification fields in 47% of the PACG 1 cases (22). We only observed pynotic nuclei from an apoptotic nucleus characteristic in AAH and PACG 1 and 2 lesions. We found pynotic nuclei in a single lesion of AAH and PACG 1 and 2, a lower rate than the results in the references. There are few studies on the subject and the use of different fixatives on the tissues may have led to the different results.

PACG 1 lesions have nuclei placed linearly basally while this arrangement disappears in PACG 2. We did not find an English study comparing AAH with PACG 1 and 2 for nucleus localization. In our study, there were 6 AAH lesions (27.3%), and 19 PACG 1 and 2 lesions (86.4%) with basal arrangement of nuclei. The nuclei of non-malignant epithelia were generally arranged in a mixed manner at the basal, center and apical parts of the cytoplasm. We felt that basal localization of nuclei was an important histological feature for PACG 1 and 2 while central and apical localization was an important histological feature for AAH. This difference was very significant statistically.

One of the most important criteria reported for the differential diagnosis of AAH and PACG 1 and 2 lesions is the prominence of nucleoli (36,37,39-45). However, there are no common criteria for defining prominent nucleolus. Various studies have defined prominent nucleolus as measuring 1-3 µm (17,36,37,39-41,43-45). We defined three groups in our study for the presence of nucleoli as <1 µm, 1-2 µm, and >2 µm. There was no difference between the AAH and PACG 1 and 2 lesions for the presence of <1 µm nucleoli but 1-2 µm nucleoli and >2 µm nucleoli were more common in PACG 1 and 2 lesions. There was a statistically significant difference between the two lesions.

The only comparative study between AAH and PACG 1 and 2 lesions for the cytological criterion nucleolar margination has been by Montironi et al. They have reported a significant finding of nucleolar margination in more than 50% of the cells in 60% of cancer lesions (37). We found a difference very close to statistical significance (p=0.057). This difference was due to the presence of nucleolar margination

in 5-50% of the nuclei in 22.7% of AAH lesions and 50% of PACG 1 and 2 lesions.

Nuclei with multiple nucleoli have been studied in a single study comparing AAH and PACG 1 and 2. Montironi R et al. have found the rate of multiple nucleolated nuclei as 30% and 70% in AAH and cancer respectively (37). This rate was 59% in AAH and 72% in PACG 1 and 2 in our study and the difference was statistically significant ($p<0.05$). This significant difference was due to the presence of multiple nucleoli in more than 50% of nuclei in 4 PACG 1 and 2 lesions and no AAH lesions and the presence of multiple nucleolated nuclei in 5-50% of nuclei in 13.6% of AAH lesions and 31.8% of PACG 1 and 2 lesions. More studies are needed on the matter for a more comprehensive evaluation.

Cytoplasmic clarity is a characteristic feature of normal prostatic secretory cells. This clarity is lost in dysplastic and malignant prostatic epithelial cells and amphophilic (dark) cytoplasmic staining is encountered (35). AAH and PACG 1 and 2 consist of cells with pale or clear cytoplasm. Continuing cytoplasmic clarity in PACG 1 and 2 may be a sign of well differentiation. Many vesicles filling the cytoplasm are responsible for this cytoplasmic clarity (46). These vesicles are not observed if the tissue is fixed with fixatives such as 95% ethyl alcohol, 4% and 10% buffered formaldehyde, 1% buffered glutaraldehyde, B5 or Bouin's. However, if the tissue is fixed with 3% and 5% buffered gluteraldehyde, the cytoplasmic granules are stained dark eosinophilic in routine H&E staining as the secretory granules are preserved. Cytoplasmic clarity is therefore thought of as a fixation artifact. These granules immunohistochemically stain positively with prostate-specific antigen and prostatic acid phosphatase. Eosinophilic staining is not found in routine H&E staining in high-grade dysplasia and cancer as the granules decrease in number or disappear. (46). Montironi R et al. have observed the secretory cell cytoplasmic appearance in AAH in PACG 1 and 2 as clear in 70% and 30% of lesions and granular (eosinophilic) in 30% and 70% of lesions respectively (37). However, the cytoplasmic staining quality has not been found useful in differentiating AAH from adenocarcinoma in some studies. We found an eosinophilic appearance in 13.6% of PACG 1 and 2 lesions and 4.4% of AAH lesions. The mild eosinophilic appearance and cytoplasmic clarity was similar in both lesions and there was no statistically significant difference. The results are consistent with other reports (27,28,30,38).

AAH and PACG 1 and 2 lesions consist of cuboidal or columnar cells (17,27,28,30,38,47). The quality of cytoplasm has not been reported to help differentiate between AAH and adenocarcinoma (17,27,28,38). We also observed that AAH and PACG 1 and 2 lesions were made of low and high columnar cells and did not find a significant difference for the nucleus/cytoplasm ratio of secretory cells between the groups. We believe that cell size is not a criterion that can be used in the differential diagnosis of AAH and PACG 1 and 2 lesions.

In conclusion, we accepted the absence of basal cells in PACG 1 and 2 and their interrupted presence in AAH as the most important diagnostic criterion. The percentage of cells with nuclei containing 1-2 μm nucleolus or multiple nucleoli was higher in the PACG 1 and 2 lesions than AAH lesions while AAH lesions did not have the >2 μm nucleolus observed in PACG 1 and 2 lesions and these were important parameters for the differentiation of these lesions. We also concluded that the nuclear localization, reported for the first time, was important for differentiation between AAH and PACG 1 and 2.

The difference between the groups for nucleolar margination, observed more commonly in PACG 1 and 2 almost reached significance. Although prominent nucleolus, crystalloid, mucin, lobular pattern and the presence of basal cells are important parameters to differentiate between AAH and PACG 1 and 2, the differential diagnosis is still difficult in some cases where a limited number of histological parameters can be evaluated, especially following needle biopsies. It is important to evaluate the histological parameters in AAH lesions where basal cells are focal and limited in number.

REFERENCES

1. ***Gaudin PB, Epstein JI:*** Adenosis of the prostate. Histologic features in transurethral resection specimens. Am J Surg Pathol 1994,18:863-870
2. ***McNeal JE, Redwine EA, Freiha FS, Stamey TA:*** Zonal distribution of prostatic adenocarcinoma. Correlation of histologic pattern and direction of spread. Am J Surg Pathol 1988, 12:897-906
3. ***Yang XJ, Wu CL, Woda BA, Dresser K, Tretiakova M, Fanger GR ve Jiang Z:*** Expression of alpha-Methylacyl-CoA racemase (P504S) in atypical adenomatous hyperplasia of the prostate. Am J Surg Pathol 2002, 26:921-925
4. ***Mittal BV, Amin MB, Kinare SG:*** Spectrum of histologic lesions in 185 consecutive prostatic specimens. J Postgrad Med 1989, 35:157-161
5. ***Qian J, Bostwick DG:*** The extent and zonal location of prostatic intraepithelial neoplasia and atypical adenomatous hyperplasia: relationship with carcinoma in radical prostatectomy specimens. Pathol Res Pract 1995, 191:860-867
6. ***Epstein JI:*** Interpretation of Prostate Biopsies. New York, Raven Press, 1989
7. ***Skjørten FJ, Berner A, Hervei S, Robsahm T, Tretti S:*** Prostatic intraepithelial neoplasia in surgical resections: relationship to coexistent adenocarcinoma and atypical adenomatous hyperplasia of the prostate. Cancer 1997, 79:1172-1179

8. ***Foster CS:*** Pathology of benign prostatic hyperplasia. Prostate Suppl 2000; 9:4-14
9. ***Young RH, Srigley JR, Amin MB, Ulbright TM, Cubilla AL:*** Tumours of the prostate gland, seminal vesicles, male urethra and penis. Atlas of tumor pathology, Third Series, Fascicle 28. Washigton D.C., Armed Forces Institute of Pathology, 2000, 1-25
10. ***Aumüller G, Holterhus PM, Konrad L, von Rahden B, Hiort O, Esquenet M, Verhoeven:*** Immunohistochemistry and in situ hybridization of the androgen receptor in the developing human prostate. Anat Embryol (Berl) 1998, 197:199-208
11. ***Parsons JK, Nelson CP, Gage WR, Nelson WG, Kensler TW, De Marzo AM:*** GSTA1 expression in normal, preneoplastic and neoplastic human prostate tissue. Prostate 2001, 49:30-37
12. ***McNeal JE:*** Prostate. In Sternberg SS (Ed): Histology for Pathologists. 2nd ed., Philadelphia, Lippincott- Raven,1997, 997-1017
13. ***Lowsley OS:*** The development of the human prostate gland with reference to the development of other structures at the neck of the urinary bladder. Am J Anat 1912, 13:299-349
14. ***Franks LM:*** Benign prostatic hyperplasia; gross and microscopic anatomy. In: Grayhack JT, Wilson JD, Scherbergke MJ, (eds). Benign prostatic hyperplasia. Bethesda, Maryland, National Institute of Health, 1973, 62-68
15. ***McNeal JE:*** The prostate and prostatic urethra: a morphologic synthesis. J Urol 1972, 107:1008-1016
16. ***Gaudin PB, Epstein JI:*** Adenosis of the prostate. Histologic features in needle biopsy specimens. Am J Surg Pathol 1995,19:737-747
17. ***Bostwick DG, Srigley J, Grignon D, Maskem J, Humphrey P, van der Kwast TH, Bose D, Harrison J, Young RH:*** Atypical adenomatous hyperplasia of the prostate: morphologic criteria for its distinction from well-differentiated carcinoma. Hum Pathol 1993, 24:819-832
18. ***Bostwick DG:*** Prospective origins of prostate carcinoma. Prostatic intraepithelial neoplasia and atypical adenomatous hyperplasia. Cancer 1996, 78:330-336
19. ***McNeal JE:*** Origin and development of carcinoma in the prostate. Cancer 1969, 23:24-34
20. ***Bostwick DG, Qian J:*** Atypical adenomatous hyperplasia of the prostate.Relationship with carcinoma in 217 whole-mount radical prostatectomies. Am J Surg Pathol 1995, 19:506-518
21. ***Qian J, Jenkins RB, Bostwick DG:*** Chromosomal anomalies in atypical adenomatous hyperplasia and carcinoma of the prostate using fluorescence in situ hybridization. Urology 1995, 46: 837-842
22. ***McNeal JE, Cohen RJ Brooks JD:*** Role of cytologic criteria in the histologic diagnosis of Gleason grade 1 prostatic adenocarcinoma. Hum Pathol 2001, 32:441-446
23. ***Epstein Jl, Fynheer J:*** Acidic mucin in the prostate: can it differentiate adenosis from adenocarcinoma? Hum Pathol 1992, 23:1321-1325
24. ***Kramer CE, Epstein JI:*** Nucleoli in low-grade prostate adenocarcinoma and adenosis. Hum Pathol 1993, 24:618-623
25. ***Häussler O, Epstein JI, Amin MB, Heitz PU, Hailemariam S:*** Cell proliferation, apoptosis, oncogene and tumor suppressor gene status in adenosis with comparison to benign prostatic intraepithelial neoplasia and cancer. Hum Pathol 1999, 30: 1077-1086
26. ***Lopez-Beltran A, Qian J, Montironi R, Luque RJ, Bostwick DG***: Atypical adenomatous hyperplasia (adenosis) of the prostate: DNA ploidy analysis and immunophenotype. Int J Surg Pathol 2005, 13:167-173
27. ***Brawer MK, Peehl DM, Stamey TA, Bostwick DG:*** Keratin immunoreactivity in the benign and neoplastic human prostate. Cancer Res 1985, 45:3663-3667
28. ***Grignon DJ, Ro JY, Ordoñez NG, Ayala AG, Cleary KR:*** Basal cell hyperplasia, adenoid basal cell tumor, and adenoid cystic carcinoma of the prostate gland: an immunohistochemical study. Hum Pathol 1988, 19:1425-1433
29. ***Jones EC, Clement PB, Young RH:*** Sclerosing adenosis of the prostate gland. A clinicopathologic and immunohistochemical study of 11 cases. Am J Surg Pathol 1991, 15:1171-1180
30. ***Amin MB, Schultz DS, Zarbo RJ:*** Analysis of cribriform morphology in prostatic neoplasia using antibody to high-molecular-weight cytokeratins. Arch Pathol Lab Med 1994 118:260-264
31. ***Midi A, Tecimer T, Bozkurt S, Ozkan N:*** Differences in the structural features of atypical adenomatous hyperplasia and low-grade prostatic adenocarcinoma. Indian J Urol 2008, 24:169-177
32. ***Midi A, Tecimer T, Bozkurt S, Özkan N:*** Differences in the secretory activity of the atypical adenomatous hyperplasia and low-grade prostatic adenocarcinoma. UroToday Int J 2008 1 (2).
33. ***Chan TY, Epstein JI:*** Follow-up of atypical prostate needle biopsies suspicious for cancer. Urology 1999, 53:351-355
34. ***Devaraj LT, Bostwick DG:*** Atypical basal cell hyperplasia of the prostate. Immunophenotypic profile and proposed classification of basal cell proliferations. Am J Surg Pathol 1993, 17:645-659
35. ***Epstein JI:*** Diagnostic criteria of limited adenocarcinoma of the prostate on needle biopsy. Hum Pathol 1995, 26:223-229
36. ***Mostofi FK, Sesterhenn IA, Davis CJ Jr:*** Prostatic carcinoma: Problems in the interpretation of prostatic biopsies. Hum Pathol 1992, 23:223-241
37. ***Montironi R, Bartels PH, Hamilton PW, Thompson D:*** Atypical adenomatous hyperplasia (adenosis) of the prostate: development of a Bayesian belief network for its distinction from well-differentiated adenocarcinoma. Hum Pathol 1996, 27:396-407
38. ***Bostwick DG, Brawer MK:*** Prostatic intraepithelial neoplasia and early invasion in prostate cancer. Cancer 1987, 59:788-794
39. ***Srigley JR:*** Small-acinar patterns in the prostate gland with emphasis on atypical adenomatous hyperplasia and small-acinar carcinoma. Semin Diagn 1988, 5:254-272
40. ***Kovi J, Mostofi FK:*** Atypical hyperplasia of prostate. Urology 1989, 34:23-27
41. ***Goldstein NS, Qian J, Bostwick DG:*** Mucin expression in atypical adenomatous hyperplasia of the prostate. Hum Pathol 1995, 26:887-891
42. ***Kelemen PR, Buschmann RJ, Weisz-Carrington P:*** Nucleolar prominence as a diagnostic variable in prostatic carcinoma. Cancer 1990, 65:1017-1020
43. ***Jones EC, Young RH:*** The differential diagnosis of prostatic carcinoma. Its distinction from premalignant and pseudocarcinomatous lesions of the prostate gland. Am J Clin Pathol 1994, 101:48-64
44. ***Totten RS, Heinemann MW, Hudson PB Sproul EE, Stout AP:*** Microscopic differential diagnosis of latent carcinoma of prostate. AMA Arch Pathol 1953, 55:131-141
45. ***Hoda SA, Reed RJ:*** Morphologic characteristics of nucleoli in the differential diagnosis of well-differentiated prostatic adenocarcinoma. Am J Clin Pathol 1991, 95: 271 [abstract].
46. ***Cohen RJ, McNeal JE, Edgar SG, Robertson T, Dawkins HJ:*** Characterization of cytoplasmic secretoty granules (PSG), in prostatic epithelium and their transformation-induced loss in dysplasia and adenocarcinoma. Hum Pathol 1998, 29:1488-1494
47. ***Gleason DF:*** The Veterans Administration Cooperative Urological Research Group. Histologic grading and clinical staging of prostatic carcinoma. In Tannenbaum M (ed). Urologic Pathology: Prostate Philadelphia, Lea & Febiger, 1977, 171–197