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Original Article

Histomorphological Features of Atypical Small Acinar Proliferations (ASAP) That Favor Malignancy

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ABSTRACT

Objective: Cases diagnosed as atypical small acinar proliferation (ASAP) in prostate transrectal ultrasound (TRUS) biopsies typically require rebiopsies, which are invasiveand associated with increased risk of complications. Therefore, reduction in the rates of ASAP diagnoses during initial biopsy interpretation will decrease the need forrebiopsy and limit the burden of new diagnostic procedures. The current study aimed to investigate patient demographics, serum PSA levels, and histopathological features of cases identified as "ASAP" during initial prostate biopsies and as "benign" or "malignant" in the rebiopsies.

Methods: This retrospective study included 187 cases and 257 core biopsies with a diagnosis of ASAP. Initial age and serum PSA levels were recorded, and the cores were analyzed histopathologically. The presence of nuclear enlargement, prominence of nucleoli, cytoplasmic amphophilia, luminal acellular secretions, cristalloids, infiltrative growth pattern, atrophy, inflammation, and number of the suspicious acini were recorded. Adenocarcinomas were identified using the Gleason score.

Results: The mean age and serum PSA levels were significantly higher in the intermediate-high grade malignant group compared to the other groups, while nuclear enlargement (>2 times) and prominent nucleoli were more frequently observed in the malignant group compared to the benign group. Amphophilic cytoplasm and luminal acellular amorphous eosinophilic secretions were more frequently observed in malignant groups, while the benign group exhibited higher rates of inflammation.

Conclusion: The findings of the current study showed enlargement of the nuclei (≥2 times), nucleolar prominence, amphophilic cytoplasm, luminal amorphous acellular secretion, and absence of inflammation were associated with malignancy. Moreover, higher mean age and serum PSA level were related with intermediate-high grade malignancy, and consideration of these factors during evaluation of initial TRUS biopsies may decrease the prevalence of ASAP diagnoses and prevent unnecessary interventions.

INTRODUCTION

Prostate transrectal ultrasound (TRUS) biopsy interpretation is often complicated by the presence of borderline cases, known as "atypical small acinar proliferations (ASAP)," which appear suspicious but do not meet the criteria for malignancy.[1–3] These lesions typically require repeat biopsies within 3–6 months,[2,3] approximately 40% of which eventually result in a diagnosis of prostate adenocarcinoma.[2–4] Prostate biopsies are invasive procedures that are a burden on both patients as well as the healthcare system,[3,5] and repeat biopsies are usually not preferred by doctors in about a third of the cases due to various factors such as age or clinical status.[6] Therefore, a reduction in the rates of ASAP diagnoses during initial biopsy interpretation will decrease the need for rebiopsies, and also, limit the burden of new diagnostic procedures.The current study aimed to investigate patient demographics, serum PSA levels, and histopathological features of cases identified as "ASAP" during initial prostate biopsies and as "benign" or "malignant" in the rebiopsies.

MATERIALS AND METHODS

This retrospective study included 477 cases that were initially identified as ASAP using 3946 TRUS biopsies per-

formed between 2009 and 2015 at our hospital. Cases with no archival and laboratory data, those with a diagnosis of ASAP after rebiopsy, and those with no rebiopsy were excluded from this study. The final cohort consisted of 187 ASAP cases with 257 primary biopsies. Laboratory and biopsy data were extracted from the medical records, serum PSA levels measured prior to initial diagnosis were noted, and demographic data such as age were recorded for all cases.

Histological analysis of all cores with a diagnosis of ASAP was carried out using archived 4–6 µm thick, hematoxylin-eosin (HE) stained sections prepared from formalin fixed, paraffin embedded tissues. All slides were evaluated by two pathologists, one of whom specialized in uropathology. Moreover, each core was evaluated separately in case of multiple cores diagnosed with ASAP, and the presence of nuclear enlargement, prominence of nucleoli, presence of hyperchromasia, cytoplasmic amphophilia, luminal acellular secretions, cristalloids, infiltrative growth pattern, atrophy, inflammation, and number of suspicious acini seen was recorded. Where adenocarcinomas were detected in the rebiopsies or in different cores from the same case, re-analysis was carried out using current approaches based on the Gleason score. The highest Gleason score detected in TRUS biopsy, TUR, or radical prostatectomy during follow-up was recorded, and the rebiopsies were classified into benign, low grade malignancy (Gleason score 3+3), or intermediate-high grade malignancy (higher Gleason score).

Slides were used to detect and count suspicious acini in benign glands, and the nucleus sizes were scored (0: no nuclear enlargement; 1: mild enlargement; 2: ≥2 times enlargement) following comparison with the epithelium of the gland. Nucleolar prominence was considered positive if seen at 20x objective, and morphometry was not used. Hyperchromasia was considered to be positive if the chromasia did not allow analysis of nuclear details. Infiltrative pattern (presence of a suspicious focus in between benign glands), atrophy (intermediate and advanced atrophy seen in the surrounding prostate), and inflammation (moderate or more stromal/glandular inflammation detected) were recorded. Presence of a cellular amorphous secretions, cristalloids in glandular lumens, and cytoplasmic amphophilia were also analyzed.

SPSS 23.0 (SPSS Inc.) and StatXact-10 (Cytel Software) were used for all statistical analyses. Pearson's chi-square and Fisher's exact chi-square tests were used to evaluate differences, while the Shapiro-Wilk test was used to assess normality of data distribution. The Kruskal-Wallis H test was used to evaluate differences if the data was not normally distributed, and $p<0.05$ was considered statistically significant.

This study was approved by the Health Sciences University Kartal Dr. Lutfi Kirdar City Hospital Ethics Committee on 31.01.2017 (decision no: 2017/514/100/3).

RESULTS

Of the 3946 TRUS biopsies (12%) carried out at our pathology clinic between 2009 and 2015, 477 were reported as ASAP and 187 cases were included in the current study. Of the 98 (52.5%) cases in the cohort classified as malignant, 44 (23.5%) were low grade and 45 (24%) were intermediate-high grade. The mean age of the study cohort was 64.22±8.11 years (range: 43–84 yrs mean), with the corresponding values in the benign, low grade malignant, and intermediate-high grade malignant groups being 62.65 (±7.58), 65.14 (±8.57), and 66.76 (±8.16) years, respectively (Table 1). The difference in age between the benign and intermediate-high grade malignant groups was statistically significant ($p<0.05$).

When the groups were evaluated by age (40–59, 60–79, and >80 yrs), 60.4% (32/50 patients) of the 40–59 year age group were benign, 22.6% (12/50 patients) were low grade malignant, and 17% (9/50 patients) were intermediate-high grade malignant. Patients were equally distributed between the malignancy categories in the 60–79 age group, while 83.3% (5 /6 patients) of the >80 year age group were malignant. The differences in diagnoses between age groups was statistically significant, particularly with regard to the benign and intermediate-high grade malignant diagnoses ($p<0.05$) (Table 1).

The serum PSA levels were 8.44 ng/ml in the benign group and 12.62 ng/dl in the malignant group. Upon evaluation by risk, the corresponding values were 8.73 ng/dl and 16.42 ng/dl in the low grade and intermediate-high grade malignancy groups, respectively, and this difference was statistically significant (Table 1). However, this significance was lost if a cutoff value of 4 g/dl was used for the PSA screening test.

Histopathological evaluation

Nuclear enlargement in suspicious foci were assessed through comparison with the surrounding benign epithelium, and ≥2 times enlargement was seen in 22% of benign rebiopsy cases and 31.4% of all malignant cases ($p<0.05$). Moreover, the corresponding values in the low and intermediate-high grade malignant groups were 30% and 33%,

Table 1. Age and initial serum PSA levels in ASAP cases with benign and malignant diagnosis at rebiopsies (n=187)

	Benign (n=98)	Malignant		P value
		Low grade (n=44)	Intermediate-high grade (n=45)	
Age (mean, years)	62.65	65.14	66.76	0.013
Serum PSA level (mean, ng/ml)	8.44	8.73	16.42	0.00

Table 2. Histopathological characteristics according to rebiopsies (n=257)

	Benign (n=136)	Malignant		P value
		Low grade (n=60)	Intermediate-high grade (n=61)	
≥2 times nuclear enlargement	22%	30%	33%	p=0.001, between malignant and benign groups p=not significant, between malignant groups
Nucleolar prominence	27.2%	71.6%	72.1%	p=0.000, between malignant and benign groups p=not significant, between malignant groups
Amphophilic cytoplasm	21.3%	26.6%	42.6%	p=0.008, intermediate-high grade vs benign group p=0.017, malignant group vs benign group
Luminal amorphous acellular secretion	8.8%	51.6%	49.1%	p<0.05, between malignant and benign groups p=not significant, between malignant groups
Inflammation	47.8%	33.3%	11.5%	p=0.000

respectively (p-value not statistically significant) (Figs. 1 and 2, Table 2).

Nucleolar prominence was seen in 48.2% of all ASAP groups, while the nucleoli were prominent in 27.2% of benign and 71.9% of all malignant cases (p<0.05; Figs. 1 and 2). No statistically significant difference in nucleolar prominence was observed between the low and intermediate-high grade malignant groups (Table 2).

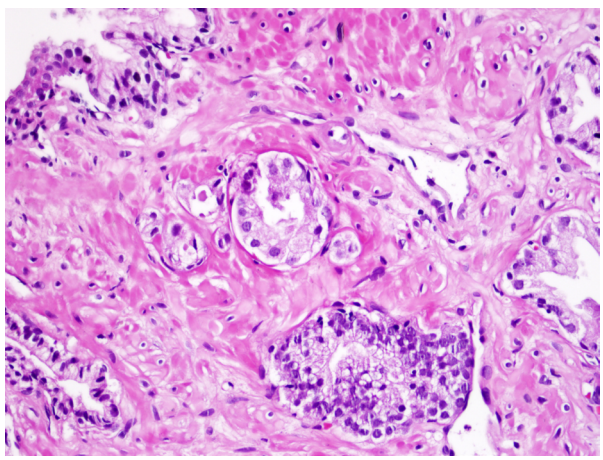

Figure 1. Few number of small glands with amphophilic cytoplasm scattered between benign prostate glands (HEX400).

Nuclear hyperchromasia was seen in 14.7% of the benign group, 15% of the low grade malignant group, and 18% of the intermediate-high grade malignant group, although these differences were not statistically significant (p>0.05; Not shown in Table 2).

Amphophilic cytoplasm was seen in 21.3% of the benign-group, 34.7% of the malignant group (p=0.017), and 42.6% of the intermediate-high grade malignancy group (statistically significant difference with benign group; p=0.008; (Figs. 1 and 2a and b, Table 2).

Only 8 patients (3.2%) in the study cohort exhibited intraluminal cristalloids, of which 4 (50%) were benign and 4 (50%) were malignant (p>0.05; not shown in Table 2).

Luminal acellular amorphous eosinophilic secretions were seen in 8.8% (12/136) of benign and 50.4% (61/121) of all malignant cases (statistically significant difference with benign group, p<0.05 and not statistically significant between the malignant risk groups) (Fig. 2, Table 2).

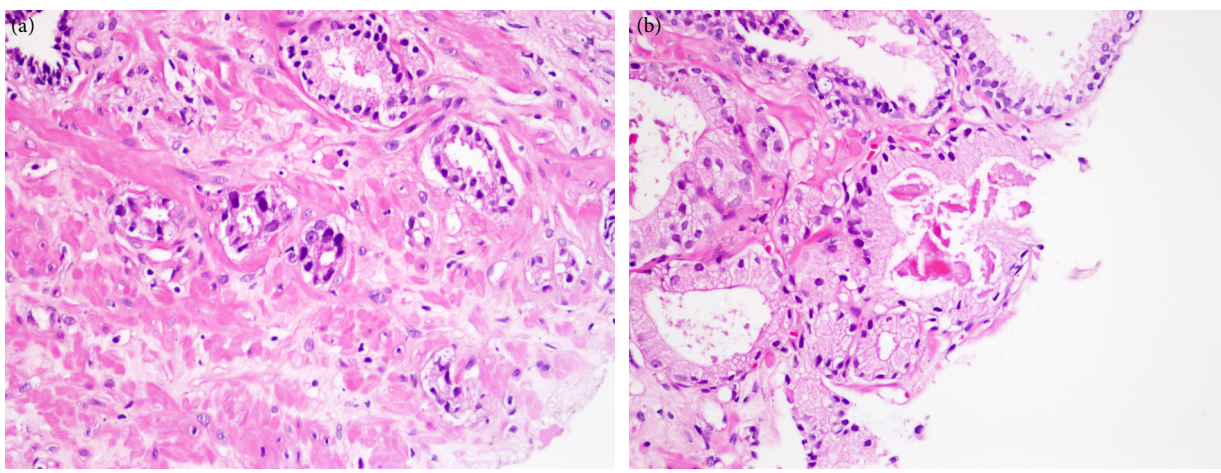

Figure 2. (a, b) Suspicious glands with nucleomegaly, nucleolar prominence, cytoplasmic amphophilia and intraluminal amorphous eosinophilic secretion (HEX400).

With regard to the infiltrative patterns of suspicious foci within large benign glands, 61 of the 126 cases (48.4%)with this pattern were benign and 65 (51.6%) were malignant. Infiltrative pattern was seen in almost half of the cases, and no statistically significant differences were seen (not shown in Table 2). Atrophy of the surrounding tissues was present in 36/257 cases (14%), of which 24 (66.7%) were benign and 12 (33.3%) were malignant, while atrophic changes were observed in 17.6% (24/136) of benign and 9.9% (12/121) of malignant cases. These differences, although not statistically significant, did exhibit a trend toward the same (p=0.06; p>0.05; not shown in Table 2).

Inflammation of the surrounding tissues was observed in 47.7% of benign and 22.3% of malignant cases (p=0.000), of which 33.3% were in the low grade and 11.5% were in the intermediate-high grade malignancy groups (p=0.000) (Table 2). Only 7 (7.6%) of the 92 core biopsies with inflammation were in the intermediate-high grade malignancy group.

DISCUSSION

Appoximately 1%–5% of prostate biopsies typically have a diagnosis of "atypia" or ASAP due to the absence of adequate histomorphological signs allowing differentiation of the atypical focus from a carcinoma, high grade prostatic intraepithelial neoplasia, or certain lesions mimicing cancer.[7] Limited number of glands in the biopsy, cytological (nucleomegaly and nucleolomegaly) and architectural (irregular distribution of small acini with no nuclear atypia) atypia in some glands, intraluminal blue mucin, and presence of cristalloids or pink proteinous secretions are associated with a diagnosis of ASAP,[2] while fixation, paraffin embedding, and problems with sectioning and staining may hinder correct evaluation of prostate biopsies.[6] Atypia may also be more frequently reported by pathologists that fail to examine a significant number of TRUS specimens. [5] In the current study, the ASAP rate was higher than that observed in the literature (12%), and this could be attributed to variations in the experience of pathologists evaluating the initial biopsies.

Age and PSA: The current study first assessed age and PSA values in patient groups. Although advanced age is a well known risk factor for prostate cancer,[6,8] current evidence on the predictive value of age for malignancy in ASAP patients is conflicting.[9] Several studies reporta positive correlation between PSA levels and malignancy in ASAP patients.[10,11] Leone et al.[10] reported that ASAP patients (n=89) diagnosed with cancer by means of their rebiopsies exhibited higher mean serum PSA levels (6.7 ng/dl) compared to patients (n=175) who did not have a diagnosis of cancer (5.8 ng/dl, p=0.01), while Brimo et al.[11] suggested that ASAP patients with serum PSA levels <5 ng/dl exhibited a low correlation with malignancy. However, Warlick et al.[12] reported that PSA density and velocity exhibited higher predictive values than PSA.[4] In the current study, the age of patients diagnosed with ASAP exhibited an association with the grade of malignancy, with the mean age of the high grade malignancy group (67 yrs) being significantly higher than that of the benign group (63 yrs; p<0.05). Moreover, in concordance with previous literature, the serum PSA levels of the intermediate-high grade malignancy group was significantly higher than that of the other groups (p<0.05). These results suggest that advanced age and high PSA levels may indicate increased risk of of high grade malignancy in patients diagnosed with ASAP. However, the current PSA cutoff values used for screening (4.0 ng/dl) may hinder prediction of malignancy, highlighting the need for a higher cutoff value.

Nuclear and nucleolar prominence: In the current study, nuclear and nucleolar prominence were the primary significant criteria in histological evaluation of cases diagnosed with ASAP. In their study, Varma et al.[13] observed a prominent nucleolus in 94% of malignant cases and 25% of benign cases; in contrast, another study reported no prominent nucleolus in 24% of prostate malignancies using prostate fine needle biopsies.[14] Previous studies have reported mild enlargement of the nucleus in patients diagnosed with ASAP and mild to moderate enlargement in patients with carcinomas.[6] As the evaluation of these criteria are affected by factors such as fixation, section thickness, and routine staining procedures, comparison with the benign epithelium may be beneficial. Although nuclear enlargement is often associated with adenocarcinoma,[15] its low sensitivity and limited diagnostic value must be kept in mind.[13]

Histological evaluation of the current study cohort showed higher prevalence of nucleolar enlargement in the malignant group compared to the benign group, suggesting that prominent nucleolus in a suspicious focus may serve as a warning for undetected malignancy. Moreover, the malignant group also exhibited nuclei that were twice the size of those exhibited by the benign group, and the absence of nuclear enlargement in this group was also significantly lower (p<0.05). However, no significant differences were observed between the malignancy subgroups.

Inflammation: Inflammation may lead to alterations mimicking carcinomas or might complicate differential diagnoses further,[6] with previous studies suggesting that chronic inflammation exhibited a negative correlation with prostate cancer and possibly even prevented its development.[16] Moreover, histological inflammation has been reported to increase PSA levels and is frequently observed in benign prostate pathologies, often leading to unnecessary rebiopsies.[17] In accordance with the literature, the prevalence of inflammation in ASAP cases with intermediate-high grade malignancies was significantly lower than that of the other two groups (p<0.05).

Infiltrative growth pattern: Previous studies have suggested that infiltrative growth patterns are frequently observed in patients diagnosed with ASAP (68–75%),[18] and this was in accordance with the findings of the current study (prevalence of infiltrative growth pattern: 52%).

However, the difference between the benign and malignant groups was not statistically significant.

Intraluminal cristalloids, amorphous eosinophilic material, and cytoplasmic amphophilia: Some studies have suggested that intraluminal cristalloids do not increase the risk of cancer,[6] while others have reported that they are frequently observed in cases with malignancies (40.6% in malignant cases vs 1% in benign cases).[13] In the current study, intraluminal cristalloids were evenly distributed between the groups. Intraluminal amorphous eosinophilic materials are typically are in benign tissues (2%), but are more commonly found in ASAP cases (66%–74%) and those with malignancies (86.7%).[6,13,16] In the current study, the prevalence of amorphous eosinophilic material was significantly higher in the malignant group, suggesting a possible role in the prediction of malignancy in ASAP cases. Previous studies have also reported a higher prevalence of cytoplasmic amphophilia in malignant glands compared to benign glands,[13] and this was in accordance with the findings of the current study. Moreover, the difference between the groups was statistically significant ($p<0.05$).

Immunohistochemical (IHC) evaluation: IHCs have limited potential for differentiating ASAPs from carcinomas in TRUS biopsies, although its sensitivity may be increased if basal cell markers such as P63 (nuclear staining) and 34bE12 (cytoplasmic staining) are used together.[2,6] Immunohistochemical racemase (a-Methyl acyl-CoA racemase) staining is typically strong and appears diffused positive in 97%–100% of prostate cancers and 8–12% of benign cases,[2] while ERG gene rearrangements are seen in 40%–50% of prostate carcinomas, although its high specificity and low sensitivity limits its use.[6] Some studies suggest considering several markers such as ERG, and using AMACR+P63 or AMACR+P63+34bE12 with single and double chromogene reactions, respectively, may allow differentiation of malignant (P504S+/basal cell marker-) and benign (P504S-/basal cell marker+) lesions.[6] Therefore, maximum diagnostic potential may be achieved through the combined use of HE and IHC analyses in suspicious foci that may be malignant.[2] However, IHC analysis was not carried out in the current study as the design aimed to implement diagnosis using morphological characteristics via HE only.

In conclusion, the enlargement of nuclei (≥2 times), nucleolar prominence, amphophilic cytoplasm, luminal amorphous acellular secretion, and absence of inflammation were found to be associated with high risk of malignancy, with nuclear enlargement, amphophilic cytoplasm, absence of inflammation, and higher mean age and serum PSA levels being correlated with intermediate-high grade malignancy. Therefore, taking age, PSA levels, and significant histopathological characteristics into consideration during interpretation of the initial biopsy may help decrease the rate of ASAP diagnoses, allow prediction of malignancies and high grade cancers, and prevent unnecessary interventions and related complications.

Ethics Committee Approval

This study was approved by the Health Sciences University Kartal Dr. Lutfi Kirdar City Hospital Ethics Committee (date: 31.01.2017, no: 2017/514/100/3).

Peer-review

Internally peer-reviewed.

Authorship Contributions

Concept: A.G.S., Ş.S., N.Ö.B.; Design: A.G.S., Ş.S., N.Ö.B., S.Ş.; Data: A.G.S., C.C.B., S.Ş., Ş.S.; Analysis: S.Ş., C.C.B., A.G.S., Ş.S.; Literature search: N.Ö.B., C.C.B., S.Ş.; Writing: A.G.S., S.Ş., N.Ö.B.; Critical revision: S.Ş., C.C.B.

Conflict of Interest

None declared.

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Atipik Küçük Asiner Proliferasyon Tanılı Olguların Histomorfolojik Özelliklerinin Malignite Tanısındaki Önemi

Amaç: Transrektal ultrasonografi (TRUS) eşliğinde alınan prostat biyopsilerinde atipik küçük asiner proliferasyon (ASAP) tanısı alan olgular invaziv bir işlem olan biyopsi tekrarı ile takip edilmektedir. ASAP sıklığının azaltılması rebiyopsi gereksinimi ve yeni tanısal girişimlere bağlı yükleri azaltabilir. Bu çalışmada, ilk prostat biyopsisi örneklerinde ASAP tanısı verilen ve sonraki biyopsilerinde benign ya da malign olduğu saptanan olguların başlangıçtaki hasta demografik özellikleri ve serum PSA değerleri yanı sıra çeşitli histopatolojik özellikleri yönünden tartışılması amaçlanmıştır.

Gereç ve Yöntem: Bu geriye dönük çalışmaya, ASAP tanısı almış 187 olgu ve 257 kor biyopsi dahil edildi. Olguların, başlangıçtaki yaş ve PSA düzeyleri kaydedildi. Histopatolojik incelemede, ayrı ayrı değerlendirilen her kor için şüpheli asinüslerin sayısı, nükleer büyüme, nükleol belirginliği, sitoplazmik amfofili, luminal amorf aselüler sekresyon, kristaloid varlığı, infiltratif görünüm, atrofi ve enflamasyon bulguları kaydedildi. Adenokarsinom varlığında Gleason skoru saptandı.

Bulgular: Orta-yüksek dereceli malign grubun yaş ortalaması benign gruba göre anlamlı düzeyde yüksektir. Serum PSA düzeyi de orta-yüksek malignite grubunda diğer gruplardan anlamlı yüksek bulundu. Nükleusta ≥2 kat büyüme oranı, malign tanı alan grupta benign gruptan anlamlı düzeyde fazlaydı. Nükleol belirginliği de malign grupta anlamlı olarak daha sık görüldü. Amfofilik sitoplazma, lümende aselüler amorf eozinofilik sekresyon varlığı malign olgularda anlamlı düzeyde daha sık iken enflamasyon, benign grupta daha fazlaydı.

Sonuç: Bu çalışmada, nükleuslarda ≥2 kat büyüme, nükleol belirginliği, amfofilik sitoplazma, lüminal amorf aselüler sekresyon ve enflamasyonun yokluğu malignite ile ilişkili bulunmuştur. Ortalama yaş ve serum PSA düzeylerinin yüksek olması orta/yüksek dereceli malignite lehine kriterlerdir. İlk TRUS biyopsinin bu kriterler eşliğinde değerlendirilmesi ASAP tanısını ve sonraki gereksiz invaziv girişimleri önleyebilir.

Anahtar Sözcükler: Atipik küçük asiner proliferasyon; prognoz; prostat; TRUS biyopsi.