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Original Article

Estimation of Nestin and AMACR markers expression in Iraqi patients with prostate tumor: histopathological and immunohistochemical study

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ABSTRACT

Background: Prostate tumors constitute a major cause of urological morbidity among Iraqi men. Prostate cancer was one of the top six male cancers, with an age-standardized incidence rate (ASIR) of 8.64 per 100,000 in Iraq, according to GLOBOCAN 2020. By contrast, BPH continues to be the most common resected prostatic lesion globally, impacting up to 50% of men by the age of 50 and more than 80% by the age of 80, Prostate tumor evaluation using Nestin, an intermediate filament protein linked to stem/progenitor cells, and α-methylacyl-CoA racemase (AMACR), a lipid metabolism enzyme, is a promising marker. Objective: To compare Nestin and AMACR expression in Iraqi patients' and correlate with clinicopathological characteristics. Methods: In this retrospective study, a total of 85 prostate tissue specimens (including 40 carcinomas, 30 benign hyperplasias and 15 control) were retrieved from the archives of Iraqi hospitals in medical city. Immunohistochemical staining for AMACR and Nectin was independently evaluated by two pathologists using the H-score method (range 0–300), calculated as the product of staining intensity (0–3) and percentage of positive cells (0–100%). Statistical analyses were performed in SPSS program. Results: Nestin H-scores were substantially higher in malignant tissues compared to benign and controls. A moderate positive connection was found between Nestin H-score and Gleason score, AMACR expression was significantly higher in malignant cases than in benign and control tissues, independent of Gleason grade. Patient age did not affect either marker. Conclusion: Nestin and AMACR can consistently differentiate malignant from benign prostate tissues in this Iraqi population. Nestin correlates marginally with tumor grade, suggesting prognostic potential, while AMACR has excellent sensitivity and specificity regardless of grade adding diagnostic panels may improve accuracy in difficult instances.

Keywords: AMACR, cancer, Nestin, prostate cancer.

1 Introduction

Prostate conditions represent a significant health burden among elderly men, with benign prostatic hyperplasia (BPH) and prostate cancer (PCa) being the most prevalent forms of primary organ disease [1-3]. BPH is a non-malignant proliferation of prostate tissue, commonly causing lower urinary tract symptoms (LUTS) in older males. Its histological prevalence is notably high, affecting 50-60% of men in their 60s and increasing to 80-90% in those over 70 years of age. Histopathologically, BPH results from an imbalance favoring cellular proliferation of periurethral epithelial and stromal cells, leading to increased cell numbers [4-6]. It predominantly originates and develops within the transition zone (TZ) of the prostate. In contrast, prostate cancer is a malignant neoplasm, representing the most common non-skin cancer and the second leading cause of cancer-related mortality among men in the United States [1,7-9]. In Iraq, PCa is a significant health concern, ranking as the sixth most common cancer among males in Karbala province, where it accounts for 7.1% of all cancer cases. Unlike BPH, PCa typically forms in the prostatic periphery. Both conditions can present with similar LUTS, making clinical differentiation challenging. However, PCa may be suspected with a recent worsening of LUTS, the presence of blood in urine or semen, or systemic symptoms such as low back or bone pain, or pathological fractures. Pathologically, PCa exhibits distinct features suggestive of malignancy within the cells, which are systematically grouped using the Gleason grading system [10,11].

Importance of Accurate Differentiation

The accurate differentiation between BPH and PCa is paramount for effective patient management, primarily due to the considerable overlap in their clinical presentations. Misdiagnosis can lead to significant consequences, including over-diagnosis

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and subsequent over-treatment of benign conditions, or, conversely, under-diagnosis and delayed intervention for malignancies [12]. Prostate-specific antigen (PSA) testing, while widely used for prostate cancer screening, presents a notable limitation in its specificity. Elevated PSA levels can be observed not only in PCa but also in benign conditions such as BPH, infections, or chronic inflammation. This lack of specificity often leads to false positives, prompting unnecessary biopsies and patient anxiety. Despite this inherent limitation, mass screening programs incorporating PSA have contributed to a significant increase in the diagnosis of prostatic cancer. This rise in diagnoses, while seemingly beneficial for early detection, also highlights a public health challenge: balancing the benefits of identifying potentially curable cancers with the risks of over-detecting indolent cancers that may never cause harm, leading to over-treatment. Consequently, the widespread use of PSA, despite its poor specificity, underscores the absolute necessity of definitive tissue-based diagnosis to confirm the presence of malignancy and accurately distinguish it from benign mimics [4,13].

Microscopic Features Differentiating PCa from BPH

At the microscopic level, several key features allow pathologists to distinguish between PCa and BPH as (shown in figures 1 and 2):

- **Glandular Architecture:** BPH is characterized by a nodular proliferation of glands and intervening stroma, with glands often showing variable sizes and prominent papillary infoldings [12]. In contrast, PCa typically presents as infiltrative small glands that are haphazardly arranged, or as cribriform glands that are too large or irregular to be considered benign. Malignant glands are often described as single, separate, and well-formed, lacking the organized structure seen in BPH [14].
- **Basal Cell Layer Presence:** This represents a fundamental discriminator. Benign prostatic glands, including those in BPH, characteristically retain an intact or at least a fragmented basal cell layer[12]. This layer is crucial for maintaining glandular integrity. Conversely, prostate adenocarcinoma is defined by the absence of this basal cell layer, presenting as a single-cell layer of epithelial cells. This distinction is so foundational that the basal cell layer serves as a primary anatomical and pathological discriminator. Its consistent presence in benign conditions and typical absence in malignancy provides a robust morphological basis for diagnosis. The loss of this layer is a critical event in prostate carcinogenesis [14].
- **Cellular Atypia:** PCa exhibits significant nuclear atypia, including nuclear and nucleolar enlargement, reflecting the malignant transformation of cells [12]. In benign conditions like BPH, the cells generally lack these features of cancer[15]. High-grade prostatic intraepithelial neoplasia (HGPIN), considered a precursor to PCa, shows crowded, irregularly spaced epithelial cells with hyperchromatic, pleomorphic nuclei and small nucleoli, but critically, it still retains an intact or fragmented basal cell layer [12].

Other Features: The presence of corpora amylacea, which are round/ovoid eosinophilic bodies with laminations, is typically observed in benign glands. In contrast, eosinophilic proteinaceous debris can be associated with cancer. Mitotic figures are generally uncommon, even in high-grade PCa [16]. Minor criteria that may favor a diagnosis of PCa include intraluminal wispy blue mucin, pink amorphous secretions, and intraluminal crystalloids [14].

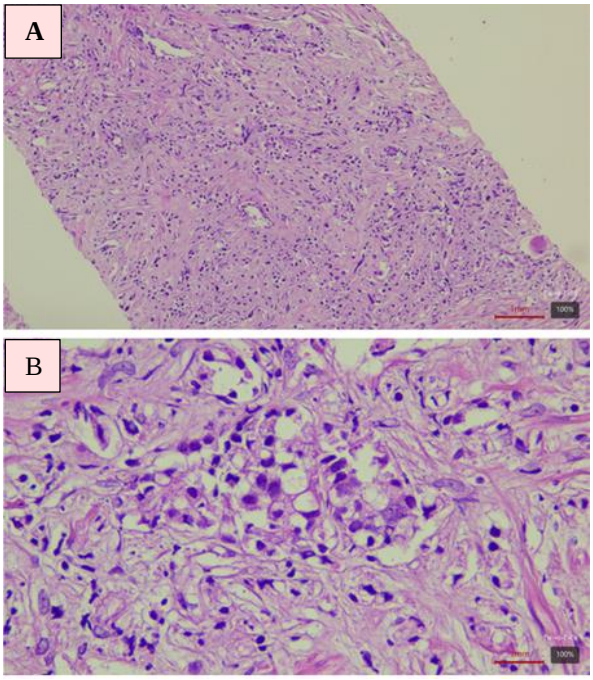


Figure 1: Prostatic acinar adenocarcinoma by H&E staining, power 10x (A), power 40x (B).

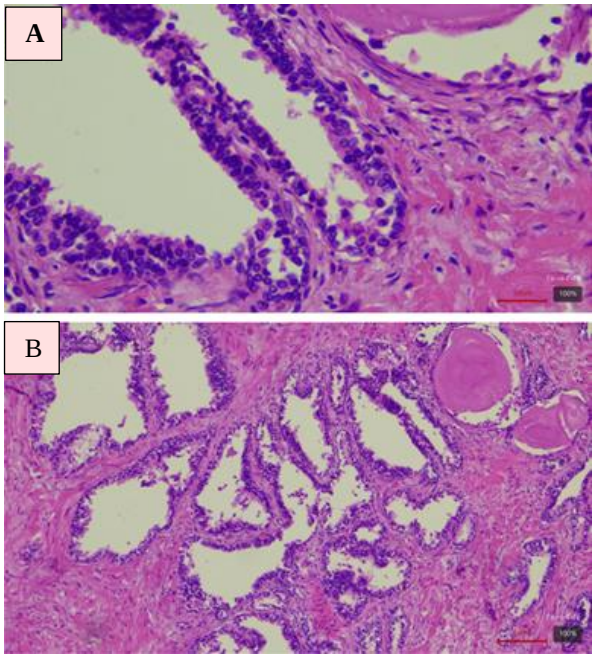


Figure 2: Benign prostatic hyperplasia BPH, H&E, power10x (A), power 40x (B): glandular and stromal hyperplasia with eosinophilic secretions (corpora amylacea) seen at the right upper corner.

While BPH is not considered a direct risk factor for prostate cancer, it is common for patients with PCa to also have histological evidence of BPH; over 80% of PCa patients exhibit histo-anatomical findings of BPH. The precise interaction between these two common disease states remains incompletely understood. Interestingly, some clinical studies have reported an inverse relationship between prostate/BPH size and the incidence and aggressiveness of PCa. This observation has led to the hypothesis that the expanding BPH zone might physically or biologically impede the development or progression of PCa in the peripheral zone, suggesting a potential protective effect [1]. This complex interplay highlights the need for continued research into the dynamic interactions between different prostate zones. Additionally, chronic inflammation, specifically histological prostatitis (HP), is frequently detected in the prostate tissue of BPH patients and is increasingly recognized as a potential contributor to BPH development and progression. This inflammatory process may influence the prostatic immune response and contribute to prostatic enlargement [17].

Role of Immunohistochemistry in Prostate Pathology

Immunohistochemistry (IHC) stands as a cornerstone in modern diagnostic pathology, offering a powerful method to selectively identify and localize specific antigens within cells and tissues. This technique leverages the highly specific binding of antibodies to their corresponding antigens, enabling pathologists to visualize otherwise invisible molecular markers under a microscope [18]. In prostate pathology, IHC provides invaluable insights beyond conventional hematoxylin and eosin (H&E) staining. It is routinely employed to aid in the diagnosis of prostate cancer, to determine the specific type and subtype of malignancy, to predict a tumor's response to therapy, and to assess its likely prognosis [15]. Its utility is particularly pronounced in challenging diagnostic scenarios, such as equivocal cases where standard histopathological examination alone may not yield a definitive diagnosis. By revealing the presence or absence of specific molecular markers, IHC significantly enhances diagnostic precision, guiding clinicians toward appropriate treatment strategies and ultimately improving patient outcomes [12].

Advantages and Considerations of Using FFPE Blocks for IHC

Formalin-fixed paraffin-embedded (FFPE) tissue blocks are the most common type of tissue preparation used in diagnostic pathology and for IHC due to several distinct advantages. FFPE tissues offer excellent preservation of tissue architecture and cellular morphology, which is crucial for accurate histopathological assessment. Furthermore, paraffin blocks allow for long-term storage, enabling retrospective studies and archival purposes for many years. They are also easily obtainable for research and are generally cost-effective. Despite these benefits, there are important considerations. The process of paraffin infiltration and the chemical nature of formalin fixation can lead to the degradation of DNA and RNA, making it challenging to extract high-quality nucleic acids for molecular applications like next-generation sequencing. The preparation process itself, from fixation to embedding, is time-consuming. Most significantly for IHC, formalin fixation can cause the masking of antigenic epitopes due to cross-linking, necessitating the critical step of antigen retrieval to unmask these sites and allow antibody binding [19-21].

Materials and methods

Between January and March 2025, 40 paraffin-embedded tissue blocks of prostate cancer and 30 of benign prostatic tumors, along with the data related to them, this retrospective cross-sectional study based on archived tissue samples collected between 2022 and 2024 from the Ghazi Hariri Hospital for Surgical Specialization/Medical City and the National Center for Educational Laboratories/Medical City. All patients, aged 50 to 80, presented with malignant cases. Furthermore, 15 healthy prostate autopsy from individuals aged 50 to 80 were obtained as a control group from the Forensic Medicine Institute in Baghdad. The biopsies were preserved in 10% formalin prior to a sequence of tissue processing procedures that produced paraffin blocks, as referenced in [22]. Cases were selected based on histological confirmation and availability of complete pathology and clinical records. Patients with prior hormone therapy were excluded, the pathological report and the medical records of the studied patients supplied the clinical data, encompassing age, grade, scoring, and histological type of tumor. Immunohistochemical analysis was conducted for the Nestin and AMACR markers (Wuhan Fine Biotech Co., China). IHC scoring for the markers often involves two components combined to get a final score, commonly ranging from 0 to 300 [22].

Sample Processing for the Immunohistochemistry Staining Technique

1- The histology blocks were sectioned into 5 µm thick slices, subsequently immersed in a 37 °C water bath for several seconds to facilitate relaxation and ensure adherence of the paraffin to the glass. The specimens were placed on positively charged slides and allowed to dry at ambient temperature overnight.

2- The slides were placed in a hot air oven at 65°C for one hour.

3- The histology sections were immersed in xylene at ambient temperature for ten minutes on three distinct occasions to remove the paraffin.

4- The specimens were immersed in 100%, 90%, 70%, and 50% ethanol for five minutes each, followed by a five-minute immersion in distilled water.

5. Following the application of the antigen retrieval solution to the tissue sections, they were submerged in a water bath and heated to 98°C for a duration of up to ten minutes. The slides were extracted from the antigen retrieval solution and submerged in wash buffer for three minutes.

The process of IHC staining

Pap pens were utilized to outline tissue sections and avert solution leakage between slides.

1. Thoroughly drain and absorb the sample to eliminate any residual moisture. Following a 10-minute incubation at room temperature in a humid environment, a sufficient quantity of peroxidase blocking reagent was applied to entirely envelop the tissue. Subsequently, phosphate-buffered saline was employed to meticulously cleanse the slides for five minutes.

2. Primary antibodies were used. The slices were incubated in humid chambers at 4°C overnight with the appropriate concentrations of primary antibody. Following cleaning with buffer, the slide was immersed in a fresh buffer solution for five minutes. Subsequent to a further rinse with buffer, the slides were meticulously drained and dried.

3-The slices were preserved at 37°C for 20 minutes in a humidified environment following the application of the secondary antibody. Subsequently, the slides were washed for five minutes with a continuous flow of buffer from a washing bottle.

4. Subsequently, they were placed into a fresh buffer for an additional five minutes.

5-Putting streptavidin reagent drops evenly on the slice for the horse reddish peroxidase (HRP) combination. Then, leave it in a damp place at 37°C for 20 minutes. Wash thrice with buffer.

6-The tissue samples were submerged in DAB substrate chromogen solution for five minutes and subsequently rinsed three times with buffer.

7-. For nuclear counterstaining, the slides were immersed in haematoxylin for 30 seconds at ambient temperature. Following two minutes of moderate cleansing with tap water, the slides were drained and dried.

8- Two drops of DPX were applied to the tissue sections, followed by the placement of cover slips atop them. The slides were thereafter allowed to dry.

Imaging and counting for IHC assessment

A brown-colored reaction in the cytoplasm or nucleus was noted as a favorable response. The intensity of immunostaining was assessed using a customized technique that utilized a digital camera linked to a standard light microscope (40X magnification) to collect photos of each segment. The Image J program (version 1.46r, National Institutes of Health, USA) was employed to analyze each image and quantify the tissue markers on the cells. IHC scoring for the markers often involves two components combined to get a final score, commonly ranging from 0 to 300 [22]:

1. Staining Intensity: 0 = No staining, 1 = Weak staining, 2 = Moderate staining, 3 = Strong staining

2. Percentage of Positive Cells: The percentage of tumor or tissue cells that stain positive (0–100%).

Calculation:

Final score = (% of positive cells) × (intensity score).

Statistical Examination

The Statistical Analysis System SPSS-22 (Statistical Packages for Social Sciences—Version 22) was utilized to assess the influence of different factors on the study parameters. Essential metrics like percentage, frequency, mean, standard deviation, and range (minimum to maximum values) were employed to represent the data. The LSD (Least Significant Difference) and Duncan's test were utilized to compare means substantially. The Chi-Square test was utilized to meaningfully compare percentages in this study, also the Pearson correlation coefficient (r) was used to assess the strength and direction of the linear [23].

Ethical approval

The tissue was collected as part of routine clinical practice. Moreover, the study design was approved by the Research Ethical Committee in Biology department/ College of science / University of Baghdad

Results and discussion

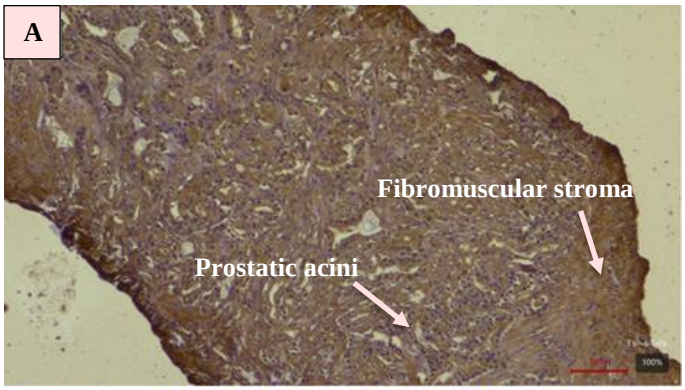
Immunohistochemical expression of Nestin

The score of the nestin marker is ranged between 0-300, the study data show that Nestin is significantly expressed among malignant, while the benign and control group show low expression of this marker (P-value = 0.0001) (Table 1). The marker has high expression in all malignant cases (figures 3 A, B, C, D).

Table 1: Comparison between difference groups in Nestin h-score.

Group	Means ±SE
	Nestin
Malignant	122.88 ±12.98 a
Benign	31.33 ±6.55 b
Control	21.33 ±6.38 b
L.S.D.	35.253 **
P-value	0.0001

Means having with the different letters in same column differed significantly. ** (P≤0.01).



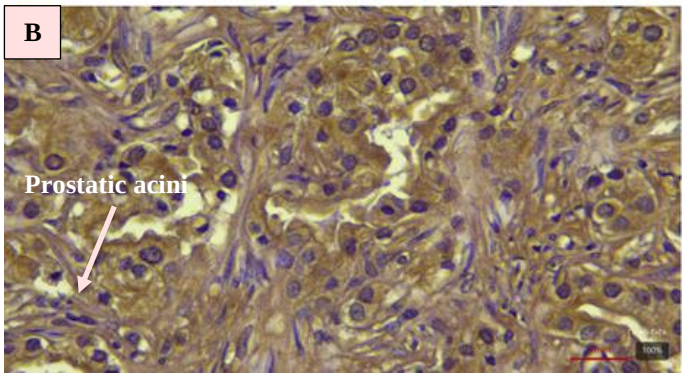


Figure 3. A: NESTIN IHC in prostatic adenocarcinoma power 10x (A) power 40x (B).

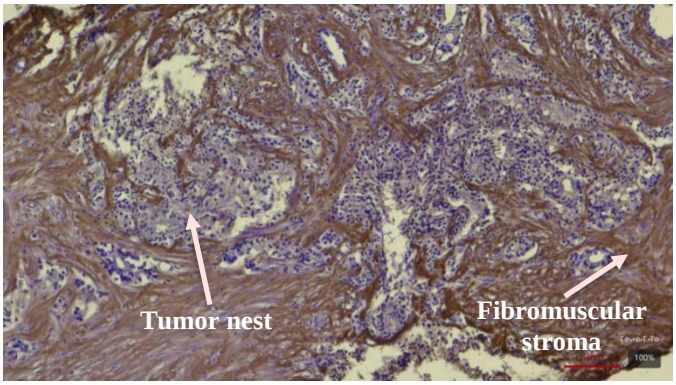


Figure 3. B: NESTIN IHC in prostatic adenocarcinoma power 10x: negative staining or low in the tumor cells while positive in the surrounding stroma.

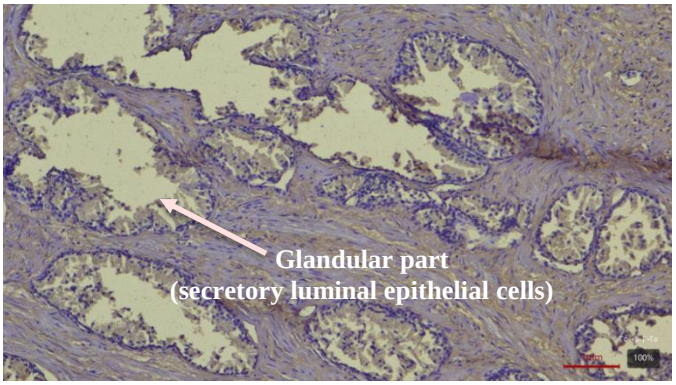


Figure 3. C: BPH NSE IHC power 10x: the glandular epithelium is negative while the surrounding stroma are positive.

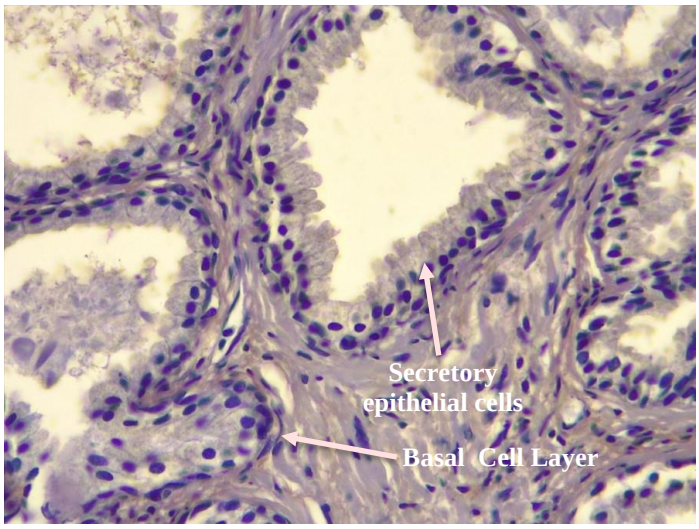


Figure 3. D: normal prostate tissue (control), NES IHC at power 40x shows the glandular epithelium is negative while the surrounding stroma are weak positive.

The significant increase of Nestin in malignant prostate tissue revealed in this study may support its recognized function as a marker of tumor-associated angiogenesis and progenitor cell activity. Nestin, a class VI intermediate filament protein, is re-expressed in pathological situations and has been associated with the proliferative potential of neoplastic vasculature in several cancer types, including prostate carcinoma. High levels of Nestin have also been linked to higher microvessel density, which is a reliable sign of how well a solid tumor will do, and to aggressive clinical behavior [24,25].

A study conducted by [26] on a cohort of lethally metastatic, androgen-deprived prostate tumors revealed the presence of Nestin protein in 75% of cases, with a median of 52% Nestin-positive cells per lesion, nestin seems to have significantly greater semiquantitative values than other stem- or tumor-associated markers in prostate cancer significant difference between their lack of expression in early disease and the high H-scores in malignant tissues of this study supports the idea that Nestin up-regulation is related to treatment resistance and advanced tumor biology.

For instance, Nectin-4, a cell-adhesion molecule recently assessed in a substantial tissue microarray (TMA) of 302 prostatic adenocarcinomas, exhibited a mean H-score of 90 in malignant tissue, which is markedly lower than the mean Nestin H-score recorded in this study [27]. This indicates that, among novel IHC indicators of prostate tumor aggressiveness, Nestin may offer superior differentiation between malignant and non-malignant conditions.

In contrast to the findings of this study, [28] showed extremely weak or absent Nestin staining in both prostate adenocarcinoma (n = 38) and non-cancerous tissues, with no significant difference between the groups ($P > 0.05$), This could be due to variances in antibody clones, antigen-retrieval techniques, and

semiquantitative scoring criteria. It's vital to note that their group mostly consisted of low-grade core-biopsy specimens.

Nestin is not only a useful marker for predicting the future, but it has also been demonstrated to actively control the invasion of prostate cancer cells. [28] used shRNA knock-down in AT6.3 prostate cancer cells to show that losing Nestin changes where focal adhesion kinase (FAK) is located, makes integrin α5β1 cluster more, and greatly reduces the cells' ability to move and invade ($P < 0.0001$). The observation of elevated Nestin H-scores in malignant tissues in this study aligns with a functional paradigm wherein Nestin enhances both adhesion dynamics and metastatic capability.

Association of Nestin expression with clinicopathological features of PCa and BPH patients

Association of Nestin expression with age

The study results revealed that no significant difference in the results, H-scores did not differ significantly across age groups in all cases as shown in tables 2 A, B, C. This suggests that, in this cohort, Nestin expression by IHC is not associated with patient age category Score among age groups.

Table 2 A: H-score within the age group of malignant cases.

Age group (year)	Means ±SE
	Nestein
<60 yr.	38.75 ±14.32
60-70 yr.	27.85 ±12.95
>70 yr.	30.00 ±12.95
F -statistic	0.19
P-value	0.83

Table 2 B: H-score within the age group of benign cases.

Age group (year)	Means ±SE
	Nestein
<60 yr.	95.00 ±21.51
60-70 yr.	134.28 ±19.54
>70 yr.	127.22 ±26.47
F -statistic	0.78
P-value	0.46

Table 2 C: H-score within the age group of control cases.

Age group (year)	Means ±SE
	Nestein
<60 yr.	38.75 ±14.32
60-70 yr.	27.85 ±12.95
>70 yr.	30.00 ±12.95
F -statistic	0.16
P-value	0.85

Nestin, an intermediate filament protein, is extensively researched as a marker for progenitor or stem-like cells and neovascularization in diverse tumors. In prostate cancer, Nestin expression is associated with tumor cell motility and invasiveness, correlating with more aggressive or androgen-independent disease stages [26,29].

Published research on Nestin in prostate cancer predominantly examines its influence on tumor aggressiveness, metastasis, or prognosis, rather than considering patient age as a factor in expression levels. The study's discovery of no age-related variation supports the idea that Nestin overexpression is influenced by tumor biological properties (e.g., hypoxia, stem-like phenotypes, angiogenic signals) rather than by chronological age [26].

Also, Numerous previous investigations have established that Nestin is significantly upregulated in prostate cancer and high-grade premalignant lesions in contrast of benign lesions. Nestin is undetectable in confined, hormone-naïve prostate tumors but is expressed in up to 75% of fatal, androgen-independent carcinomas. Functional studies have further implicated Nestin in metastatic behavior [24]. A study by [30] demonstrated that Nestin enhances prostate cancer cell invasion through the control of FAK and integrin dynamics, and that the knockdown of Nestin significantly diminishes metastatic colonization in vivo.

Numerous studies have indicated that Nestin expression is more prevalent in younger breast cancer patients. A systematic review and meta-analysis aggregating data from several research revealed that Nestin positive was substantially correlated with an earlier age at onset.[31], This indicates that Nestin-related progenitor/stem-like characteristics may be more prominent in breast tumors occurring in younger patients. Nonetheless, the biology of breast cancer and its hormonal environment significantly diverge from that of prostate cancer.

A study of Nestin in gliomas reported no statistical correlation between Nestin expression and patient age or sex (among other parameters) [32].

Association of Nestin expression with histological types

The finding of this study shows that all the samples of malignant cases where from the type of acini adenocarcinoma and the benign cases where form the type prostatic hyperplasia, group comparisons aren't possible for both types. However, a significant dereferences is demonstrated in Nestin expression between malignant, benign and control groups as showing in table 1.

The findings of this study align with research conducted at the Al-Hussein Cancer Center in Karbala, Iraq, which involved 265 participants diagnosed with prostate cancer. The predominant histology seen was adenocarcinoma, present in 95.85% of individuals [8]. A separate research conducted by [33] at Medical City. The data were acquired from the medical records of patients diagnosed with prostate cancer. The predominant histological type observed among patients was acinar adenocarcinoma in all instances. This study's findings are analogous. A study was conducted at Nanakali Hospital for Blood Diseases and Cancer and the Oncology Department of Rizgary Teaching Hospital in

Erbil, Kurdistan region, Iraq. The trial included 212 individuals, 208 of whom were diagnosed with histopathologically confirmed prostate cancer. The findings reveal that 203 individuals have histological features of adenocarcinoma [34].

Other study conducted by [35] which collocated a one hundred postmortem specimens of whole prostates were acquired from the Institute of Forensic Medicine in Baghdad. All autopsy cases over a period of five decades resulted from causes unrelated to prostatic disease. The data reveal that benign prostatic hyperplasia was the primary pathological finding (92%). BPH is indicated as the most common condition among males over 50 years of age. A retrospective analysis conducted at the Department of Pathology, Baghdad Medical City, Al-Emamain Al-Kadhmain Medical, indicates that the predominant lesions were benign prostatic hyperplasia [36]. This observation is consistent with the results of this investigation.

Association of Nestin expression and tumor grade

The results are summarized in table 3 and figure 4. A Pearson correlation study between Gleason score and Nestin H-score revealed r = 0.3430 (p = 0.0303), demonstrating a moderate yet statistically significant positive correlation: elevated Gleason scores are associated with increased Nestin expression levels, despite large diversity within groups.

Table 3: correlation between H-score and Gleason score.

Gleason score	Count	H-score Means ±SD
5	4	137.50 ± 88.84
6	9	63.33 ± 47.70
7	11	120.91 ± 98.33
8	7	121.43 ± 67.93
9	4	192.50 ± 76.32
10	5	169.00 ± 64.27
Pearson correlation (r)	0.3430	
p-value	0.0303	

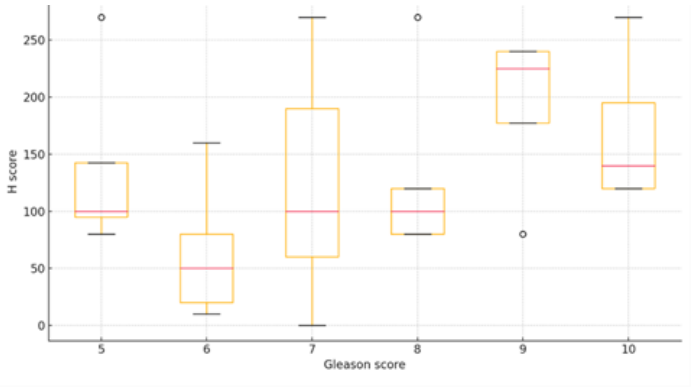


Figure 4: boxplot of h-score by Gleason grad.

A study by [28]. investigated Nestin among several stem cell markers in prostate carcinogenesis, observing its overexpression in advanced stages of the illness. Studies on vascular proliferation utilizing Nestin/Ki-67 expression have underscored the significance of Nestin in identifying young tumor vasculature; heightened vascular proliferation is associated with worse outcomes in localized prostate cancer [37].

The unexpected elevation in the mean at Gleason 5 compared to Gleason 6 may be attributed to a limited sample size (4 cases at Gleason 5) and a significant standard deviation; sampling variability could affect the averages. Elevated Gleason scores of 9 and 10 correspond to more aggressive, poorly differentiated tumors that may display increased Nestin expression due to stem-like characteristics and/or active angiogenesis. The expression of Nestin in cancer cells may signify a subpopulation characterized by self-renewal and resistance to therapy. Nestin in endothelial cells indicates young vasculature; high-grade tumors frequently have enhanced microvascular proliferation. Nestin may promote cell migration and invasion in advanced tumors.

Immunohistochemical expression of AMACR

The score of the AMACR marker is ranged between 0-300, the study data show that AMACR is significantly expressed among malignant, while the benign and control group low or neglectable expression (P-value = 0.0001) (table 4 and figure 5). the marker has high expression in all malignant cases figures 6 A, B, C.

Table 4: Comparison between difference groups in AMACR h-score.

Group	Means ±SE
	AMACR
Malignant	210.0 ±8.366 a
Benign	23.00±2.000 b
Control	24.333±2.213 b
L.S.D.	14.415 **
P-value	0.0001

Means having with the different letters in same column differed significantly. ** (P≤0.01).

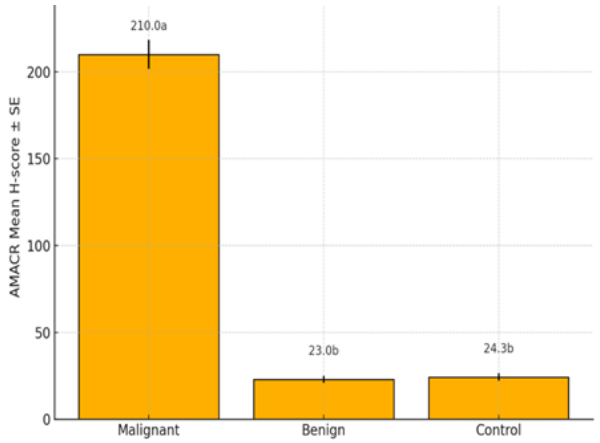


Figure 5: comparison of AMACR mean h-score across groups mean with different letters, different significant (P≤0.01).

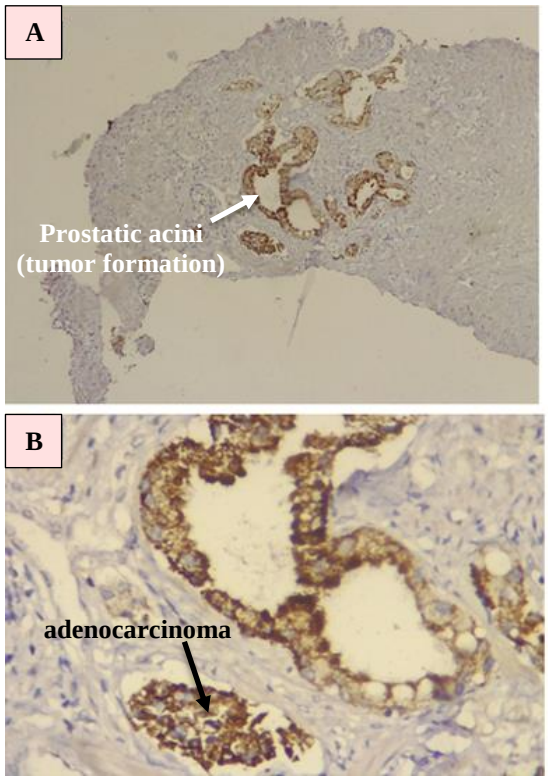


Figure 6 A: AMACR IHC in prostatic adenocarcinoma, power 10x (A), power 40x (B): strong diffuse positivity in the tumor cells, high expression.

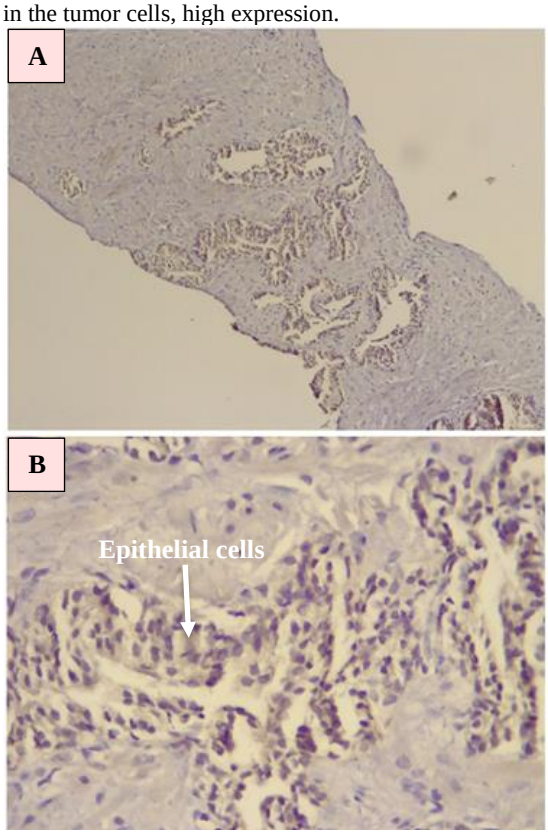


Figure 6 B: AMACR IHC in BPH, power 10x (A), power 40x (B) negative staining in the glandular epithelium.

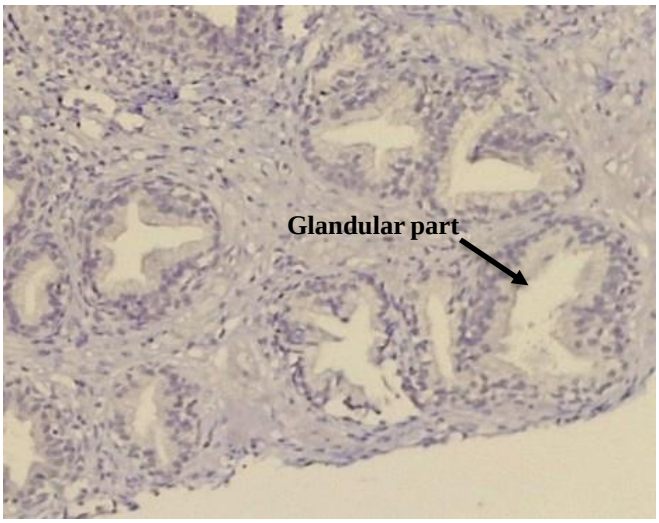


Figure 6 C: normal prostate tissue (control) IHC stain with AMACR at power 40x shows negative staining in glandular tissue.

The study's results correspond with several recent investigations indicating that AMACR immunoreactivity exhibits great sensitivity and specificity for prostate cancer. The [38] conducted a study revealing an AMACR sensitivity of 95.8% and specificity of 96.5% in a prospective analysis of prostate cancer compared to benign prostatic hyperplasia tissues. Similarity [39] found a sensitivity of 94% and a specificity of 96% for AMACR in differentiating prostate cancer from benign lesions and controls.

Accurate interpretation of AMACR staining requires an understanding of staining patterns together the morphological context and basal cell markers. Current best-practice guidelines stipulate that AMACR positivity should be deemed significant solely when the staining is circumferential, robust, and cytoplasmic (granular), and distinctly more intense than that of adjacent benign glands, with validation through basal cell markers (e.g., p63, 34βE12) to prevent false negatives or positives [39,40]. The near-zero mean H-scores in benign/control groups may indicate low background staining using standardized methods.

AMACR (α-methylacyl-CoA racemase) is a peroxisomal enzyme that helps break down branched-chain fatty acids. It is highly overexpressed in prostate cancer, which shows that the tumor is changing its metabolism to support growth and survival. In contrast, normal or benign prostate epithelium does not need this enzyme and keeps AMACR levels low [41]. Immunohistochemically, AMACR demonstrates robust, circumferential cytoplasmic staining in over 90% of prostate adenocarcinomas, while benign prostatic hyperplasia and normal glands display either absent or minimal/focal labelling beneath diagnostic thresholds, resulting in a sensitivity of approximately 94–96% and specificity of about 95–98% when used in conjunction with basal cell markers [38]. AMACR upregulation seems to be influenced by oncogenic and metabolic signals that are independent of androgen signaling, aligning with evidence that its knockdown hinders cancer cell proliferation and that its expression continues in hormone-refractory environments.

Standardized immunohistochemistry techniques (antibody clone, antigen retrieval, scoring) and the application of basal cell markers (e.g., p63, 34βE12) reduce background staining in benign tissue [38,42,43].

Association of AMACR expression with clinicopathological features of PCa and BPH patients

Association of AMACR expression with age

The results indicated no statistically significant difference among the three age groups (F= 0.9016, p = 0.4146) in the malignant cases as shown in Table 5. Figure 7 illustrates a little increase in the mean H-score for the 60–70 year cohort, however there is significant overlap in the interquartile ranges among all groups. In the benign and control cohorts, AMACR expression was minimal or absent in the majority of samples, regardless of age, resulting in consistent values and an ambiguous or non-informative outcome.

Table 5: Mean h-score of AMACR in different age groups of malignant cases**.**

Age group (year)	Means ±SE
	AMACR
<60 yr.	171.67 ± 16.37
60-70 yr.	199.47 ± 14.15
>70 yr.	181.11 ± 17.11
F statistic	0.9016
P-value	0.41464

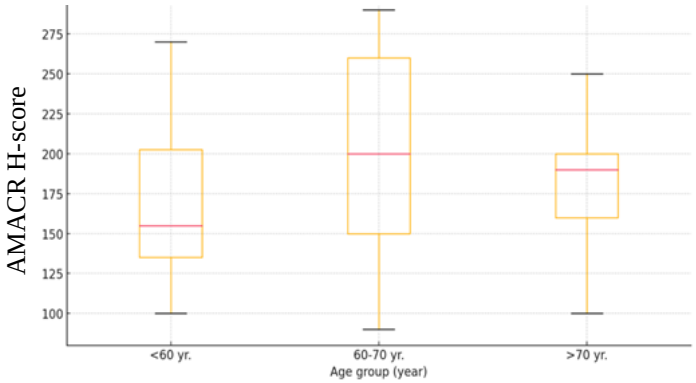


Figure 7: Boxplot of AMACR H-score by age groups of malignant cases.

The results indicate that AMACR overexpression is age-independent and robust once the prostate epithelium becomes malignant. This aligns with the findings of [44], who reported that the AMACR immunoreactivity was consistently elevated in prostate cancer cases, with no correlation to patient age (mean age ≈ 66 yr.; Kruskal–Wallis $p > 0.05$). Similarly, [39] discovered that the intensity of AMACR staining did not vary among prostate cancer patients aged 50–89 years (ANOVA $p > 0.1$), thereby confirming the marker's stability throughout the adult lifespan.

From a mechanistic perspective, these age-invariant patterns are likely indicative of AMACR's function as a lipid-metabolism enzyme that is up-regulated by oncogenic signaling pathways rather than by senescence-related metabolic shifts. The sustained reliability of AMACR staining in the diagnosis of carcinoma in older patients is supported by the absence of age-related decline in clinical settings. This minimizes false-negative interpretations, even in septuagenarians. However, pathologists should always ensure that their immunohistochemical findings are correlated with histomorphology in order to avoid the "pitfall" of rare focal AMACR positivity in benign peri-urethral glands, which can mimic early malignancy [38].

Association of AMACR expression with tumor grade

The results indicate that the intensity of AMACR immunostaining does not vary systematically with tumor grade in this cohort. In practical terms, patients with higher Gleason scores did not consistently demonstrate higher (or lower) H-scores for AMACR (tables 6, 7 and figure 8).

Table 6: AMCR Expression with Gleason Grade.

Gleason Score	NO. of cases	AMACR-negative	AMACR-positive
5	4	0 (0%)	4 (100%)
6	9	0 (0%)	9 (100%)
7	11	0 (0%)	11 (100%)
8	7	0 (0%)	7 (100%)
9	4	0 (0%)	4 (100%)
10	5	0 (0%)	5 (100%)
Total	**40**	**40 (100%)**	**40 (100%)**

Table 7: comparison between h-score of AMACR by Gleason score of malignant cases.

Gleason score	Count	H-score Means ±SD
5	4	202.50 ± 66.52
6	9	206.67 ± 75.33
7	11	168.18 ± 52.12
8	7	170.00 ± 43.59
9	4	207.50 ± 58.52
10	5	188.00 ± 53.57
Pearson correlation (r)	- 0.0707	
p-value	0.6648	

The results are consistent with a recent immunohistochemical investigation, which also found no significant association between AMACR expression and Gleason grade ($p > 0.05$) [38]. In contrast, numerous studies have demonstrated a positive correlation, with increased AMACR expression in more aggressive malignancies (Gleason ≥ 8) [45,46,47]. These discrepancies may be attributed to the size of the cohort, the type

of scoring used (manual versus digital), and the variability between laboratories.

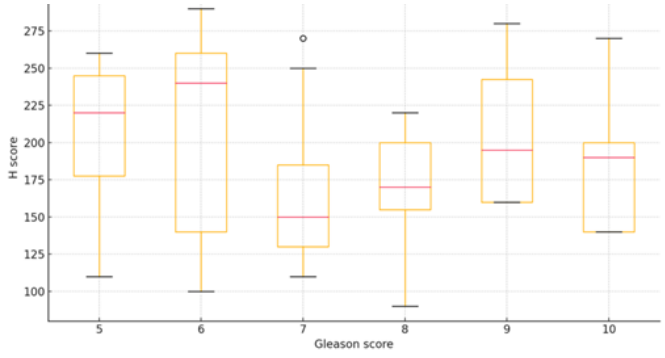


Figure 8: boxplot AMACR H-score by Gleason score.

Conclusion

Immunohistochemical analysis of Nestin and AMACR in Iraqi prostate samples highlights both markers' capacity to differentiate malignant from benign and control tissues. Nestin expression is elevated in tumors and exhibits a moderate positive correlation with Gleason score, supporting its diagnostic and prognostic potential linked to angiogenic and stem-like characteristics. AMACR shows high diagnostic accuracy, with sensitivity and specificity for cancer detection but lacks a consistent association with tumor grade, underscoring its role as a diagnostic, not prognostic, marker. Expression levels were independent of patient age, affirming stability across age groups. These findings support adding Nestin and AMACR to clinical IHC panels in equivocal cases and call for larger prospective regional multi-center studies to confirm prognostic value and standardize clinical protocols.

References

[1] Sellers J, Horne SNS, de Riese WT. The Origin of BPH and Prostate Cancer in Different Prostate Zones and the Impact on the Incidence of Prostate Cancer: A Systematic Review and Update of the Literature for Urologists and Clinicians. Expl Res and Hypo in Med **9**(1):39-46. (2024).

[2] Wasim, S., Lee, S.-Y. & Kim, J. Complexities of prostate cancer. *International journal of molecular sciences* **23**, 14257 (2022).

[3] Nasif, Z. N. Comparative Study of Genomic DNA Extraction Protocols from Whole Blood for P53 Gene Polymorphism in Persons with and without Prostate Cancer. *Baghdad Science Journal* **21**, 0975-0975 (2024).

[4] Langan, R. C. Benign prostatic hyperplasia. *Primary Care: Clinics in Office Practice* **46**, 223-232 (2019).

[5] Khosravi, P. *et al.* A deep learning approach to diagnostic classification of prostate cancer using pathology–radiology fusion. *Journal of Magnetic Resonance Imaging* **54**, 462-471 (2021).

[6] Al-qazzaz, A. & Altaie, A. F. The Role of Omentin-1 and Fibroblast Growth Factor-23 in Iraqi Patients with Prostate Cancer during Chemotherapy. *Journal of the Faculty of Medicine Baghdad* **66**, 254-259 (2024).

[7] Hou, Z., Huang, S. & Li, Z. Androgens in prostate cancer: A tale that never ends. *Cancer letters* **516**, 1-12 (2021).

[8] Mjali, A. *et al.* Pattern of Prostate Cancer in Karbala Province of Iraq: Data from Developing Country. *Asian Pacific Journal of Cancer Care* **10**, 11-15 (2025).

[9] El-Karak, F. *et al.* Prostate cancer across four countries in the Middle East: a multi-centre, observational, retrospective and prognostic study. *ecancermedicalscience* **18**, 1695 (2024).

[10] Ørsted, D. D. & Bojesen, S. E. The link between benign prostatic hyperplasia and prostate cancer. *Nature Reviews Urology* **10**, 49-54 (2013).

[11] Waly, A.-H. T. & Barraj, A. H. Prostate Cancer in Iraq: A Comprehensive Literature Review of Epidemiology, Clinical Landscape, Challenges, and Public Health Strategies. *Asian Pacific Journal of Cancer Care* **10**, 871-880 (2025).

[12] Ramatlho, P. *et al.* The Role of Immunohistochemistry for AMACR/p504s and p63 in Distinguishing Prostate Cancer from Benign Prostate Tissue Samples in Botswana. *Research and Reports in Urology*, 1-10 (2025).

[13] Malik, N., Maheshwari, V., Aijaz, M. & Afroz, N. Diagnostic significance of combined immunohistochemical panel of p63, High Molecular Weight Cytokeratin (34betaE12) and α-Methyl Acyl Co A Racemase (AMACR) in resolving suspicious foci in prostatic lesions. *Annals of Cytology and Pathology* **7**, 029-034 (2022).

[14] Humphrey, P. A. Histopathology of prostate cancer. *Cold Spring Harbor perspectives in medicine* **7**, a030411 (2017).

[15] Okoye, J. O., Nnatuanya, I. N. & Okoye, J. Immunohistochemistry: a revolutionary technique in laboratory medicine. *Clin Med Diagn* **5**, 60-69 (2015).

[16] Wilson, A. The prostate gland: a review of its anatomy, pathology, and treatment. *Jama* **312**, 562-562 (2014).

[17] Li, J. *et al.* The association between histological prostatitis and benign prostatic hyperplasia: a single-center retrospective study. *The Aging Male* **25**, 95-100 (2022).

[18] Coons, A. Basic principles of immunohistochemistry and epithelial immunohistochemical markers.

[19] EWoods, A. Laboratory Histopathology.

[20] Selves, J., Long-Mira, E., Mathieu, M.-C., Rochaix, P. & Ilié, M. Immunohistochemistry for diagnosis of metastatic carcinomas of unknown primary site. *Cancers* **10**, 108 (2018).

[21] Magaki, S., Hojat, S. A., Wei, B., So, A. & Yong, W. H. *An introduction to the performance of immunohistochemistry*. (Springer, 2019).

[22] Suvarna, S. K., Layton, C. & Bancroft, J. D. Theory and practice of histological techniqueseighth. *UK: Elsevier Health Sci* (2019).

[23] Sharma, B. Processing of data and analysis. *Biostatistics and Epidemiology International Journal* **1**, 3-5 (2018).

[24] Ishiwata, T., Matsuda, Y. & Naito, Z. Nestin in gastrointestinal and other cancers: effects on cells and tumor angiogenesis. *World journal of gastroenterology: WJG* **17**, 409 (2011).

[25] Szymańska-Chabowska, A., Świątkowski, F., Jankowska-Polańska, B., Mazur, G. & Chabowski, M. Nestin expression as a diagnostic and prognostic marker in colorectal cancer and other tumors. *Clinical Medicine Insights: Oncology* **15**, 11795549211038256 (2021).

[26] Kleeberger, W. *et al.* Roles for the stem cell–associated intermediate filament nestin in prostate cancer migration and metastasis. *Cancer research* **67**, 9199-9206 (2007).

[27] Baraban, E. G. *et al.* Nectin-4 Expression in Prostatic Adenocarcinoma: An Immunohistochemical Study. *The Prostate* (2025).
[28] Miyazawa, K., Tanaka, T., Nakai, D., Morita, N. & Suzuki, K. Immunohistochemical expression of four different stem cell markers in prostate cancer: High expression of NANOG in conjunction with hypoxia-inducible factor-1α expression is involved in prostate epithelial malignancy. *Oncology letters* **8**, 985-992 (2014).
[29] Hyder, C. L. *et al.* Nestin regulates prostate cancer cell invasion by influencing the localisation and functions of FAK and integrins. *Journal of cell science* **127**, 2161-2173 (2014).
[30] Zhang, Y. *et al.* Nuclear Nestin deficiency drives tumor senescence via lamin A/C-dependent nuclear deformation. *Nature communications* **9**, 3613 (2018).
[31] Zhang, X. *et al.* Clinicopathological and prognostic significance of nestin expression in patients with breast cancer: a systematic review and meta-analysis. *Cancer cell international* **20**, 1-17 (2020).
[32] Abdelkareem, R. M., Elnashar, A. T., Fadle, K. N. & Muhammad, E. M. Immunohistochemical expression of nestin as cancer stem cell marker in gliomas. *Journal of Neuroscience and Neurological Disorders* **3**, 162-166 (2019).
[33] Yaseen, W. K. Observational study of surgical treatment of prostate cancer in Iraq. *Onkologia i Radioterapia* **17** (2023).
[34] Sulaiman, L. R. Clinical and histopathological characteristics of prostate cancer in Erbil city/Iraq. *Zanco Journal of Medical Sciences (Zanco J Med Sci)* **28**, 489-500 (2024).
[35] Abid, A. H., Mohammad, E. J. & Abdulrazaq, A. A. Histopathological findings of prostatic autopsy in a sample of over 50 years old men in Baghdad, Iraq. *International Surgery Journal* **4**, 3606-3610 (2017).
[36] Hussain, A. G. & Khalid, H. Prostatic lesions: A histopathological study in Iraqi males. *World Journal of Pharmaceutical Research* **10**, 20768, doi:10.20959/wjpr20218-20768 (2021).
[37] Gravdal, K., Halvorsen, O. J., Haukaas, S. A. & Akslen, L. A. Proliferation of immature tumor vessels is a novel marker of clinical progression in prostate cancer. *Cancer research* **69**, 4708-4715 (2009).
[38] Stephen, N. & Badhe, B. A. Diagnostic utility of immunohistochemical markers alpha methyl acyl coA racemase (AMACR) and Ets related gene (ERG) in prostate cancer. *International Journal of Clinical and Experimental Pathology* **15**, 364 (2022).
[39] Sadeghifar, M. *et al.* Evaluation of the diagnostic value of immunohistochemistry staining for P63, Alpha-methyl Acyl-CoA Racemase, CK5/6, and 34βE12 in prostate carcinoma. *Clinical Cancer Investigation Journal* **10**, 214-218 (2021).
[40] Paner, G. P., Luthringer, D. J. & Amin, M. B. Best practice in diagnostic immunohistochemistry: prostate carcinoma and its mimics in needle core biopsies. *Archives of pathology & laboratory medicine* **132**, 1388-1396 (2008).
[41] Lasorsa, F. *et al.* Emerging hallmarks of metabolic reprogramming in prostate cancer. *International Journal of Molecular Sciences* **24**, 910 (2023).
[42] Xie, H. *et al.* Suppression of α-methylacyl-coenzyme A racemase by miR200c inhibits prostate adenocarcinoma cell proliferation and migration. *Experimental and Therapeutic Medicine* **19**, 1806-1816 (2020).
[43] Zha, S. *et al.* α-Methylacyl-CoA racemase as an androgen-independent growth modifier in prostate cancer. *Cancer research* **63**, 7365-7376 (2003).
[44] Gülhan, Ö. & Mahi, B. The role of AMACR, CD10, TMPRSS2-ERG, and p27 protein expression among different Gleason grades of prostatic adenocarcinoma on needle biopsy. *Clinical Medicine Insights: Oncology* **14**, 1179554920947322 (2020).
[45] Stopka-Farooqui, U. *et al.* Combining tissue biomarkers with mpMRI to diagnose clinically significant prostate cancer. Analysis of 21 biomarkers in the PICTURE study. *Prostate Cancer and Prostatic Diseases*, 1-12 (2024).
[46] Rubin, M. A. *et al.* α-Methylacyl–CoA Racemase as a Tissue Biomarker for Prostate Cancer. *JAMA* **287**, 1662-1670 (2002).
[47] Waly, A.-H. T. & Barraj, A. H. Prostate Cancer in Iraq: A Comprehensive Literature Review of Epidemiology, Clinical Landscape, Challenges, and Public Health Strategies. *Asian Pacific Journal of Cancer Care* **10**, 871-880 (2025).