A study of the diagnostic and prognostic role of enhancer of zeste homolog 2 and BRCA1-associated protein 1 expression in different prostatic lesions (an immunohistochemical study)

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Aim
To ascertain the applicability of enhancer of zeste homolog 2 (EZH2) and breast cancer gene 1 (BRCA1)-associated protein 1 (BAP-1) in the diagnosis of prostatic adenocarcinoma (PCa) as well as their correlation with different clinicopathological characteristics of PCa cases and the patients' disease-free survival.
Patient and methods
This study included 10 cases of benign prostatic hyperplasia (BPH), 6 cases of high-grade prostatic intraepithelial neoplasm PIN (HGPIN), and 60 cases of PCa. Immunohistochemical staining techniques were used to evaluate the roles of EZH-2 and BAP-1 in PCa and their correlations to different clinicopathological data and patient survival.
Results
High nuclear positivity of EZH2 was detected in 53.3% of PCa, while 80% of BPH and 66.7% of HGPIN cases showed no/low expression. Conversely, BAP1 nuclear positivity was detected in 70% of BPH and 50% of HGPIN versus 48.3% of PCa cases. Using the receiver-operating characteristic curve, the EZH2 showed 60.2% sensitivity, 83.3% specificity, and 65% diagnostic accuracy compared with BAP1 that showed 86.7, 51.7, and 58.7%, respectively. However, the markers showed 70% sensitivity, 56.2% specificity, and 67.1% diagnostic accuracy when tested synchronously. A statistically significant inverse relationship between EZH 2 and BAP1 nuclear expression in the examined PCa cases was found. Furthermore, EZH-2 overexpression and BAP-1 nuclear loss are associated with unfavorable clinicopathological characteristics. Moreover, it was demonstrated that EZH-2 overexpression and low patient survival were statistically correlated.
Conclusions
These results suggest that both EZH2 and BAP1 can be added to the diagnostic panel of PCa and can serve as potential independent prognostic biomarkers for predicting the outcome of patients.

Keywords:
BRCA-associated protein-1, prostatic hyperplasia, enhancer of zeste homolog 2, prostate cancer, prostatic lesions, survival

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Introduction

With a predicted 1 414 000 new cases and 375 304 deaths from cancer in 2020, prostate cancer (PC) is the second most common cancer in the world to be diagnosed in males and the fifth greatest cause of cancer-related mortality in this age group.

According to data from Egypt's National Cancer Registry Program, PC is the fourth most prevalent cancer type among Egyptian men, with an incidence rate of ~4.5% (Sung *et al.*, 2021). Based on the most recent WHO data reported in 2020, 1,282 deaths in Egypt were related to prostate cancer, accounting for 0.24% of all deaths (Center *et al.*, 2012).

PC risk factors that are well-established include black ethnicity, advancing age, and family history. Meanwhile, various dietary and lifestyle factors, such as obesity, fitness levels, diabetes mellitus, food patterns, and vitamin E supplementation, have been investigated as potential risk factors for PC (Brookman-May *et al.*, 2019).

The entire spectrum of molecular alterations associated with PCa development is still unknown. Consequently, to predict PCa oncogenesis and prognosis, it is essential to look into PCa progression determinants and discover novel biomarkers (Alarcón-Zendejas *et al.*, 2022).

One of the three essential subunits of the polycomb repressive complex 2 (PRC2) with histone methyltransferase (MTase) activity is the enhancer of zeste homolog 2 (EZH2). Targeted genes are epigenetically silenced by EZH2 by inducing chromatin condensation and histone H3 lysine 27 trimethylation (H3K27me3) (Park *et al.*, 2021). In several types of solid tumors and hematological cancers, such as uterine cancer, breast cancer, and malignant mesothelioma, rising evidence over the last two decades supports the presence of EZH2 mutations and/or overexpression, where its expression and activity are correlated with the course of the disease (Kim, Roberts, 2016).

Deubiquitinating protein, breast cancer gene 1 (BRCA1)-associated protein 1 (BAP-1) interacts with the BRCA1/BARD1 tumor-suppressor heterodimer to control cell division and DNA damage. Numerous malignancies, such as uveal melanoma, malignant pleural mesothelioma, clear cell renal cell carcinoma, and cholangiocarcinoma, have been linked to BAP-1 loss-of-function mutations (Testa *et al.*, 2011, Wiesner *et al.*, 2011, Popova *et al.*, 2013, Klebe *et al.*, 2015).

To evaluate the diagnostic validity of EZH2 and BAP-1, both alone and in combination, in the diagnosis of PCa, the current study looks for immunohistochemical expression of these markers in various prostatic pathologies, such as benign prostatic hyperplasia (BPH), high-grade prostatic intraepithelial neoplasm (HGPIN), and PCa. Their link to the different clinicopathological characteristics of PCa cases as well as the disease-free survival of the cases under examination will also be investigated. In addition, the study looks into the relationship between the immunohistochemical expression of EZH2 and BAP1 in PCa cases.

Patient and methods

This investigation, which is retrospective, selective, and uncontrolled, was carried out on 76 prostatic lesion cases. Archival formalin-fixed, paraffin-embedded blocks produced between 2016 and 2018 from the Pathology Department of the Benha Faculty of Medicine were among the cases under study.

The Research Ethics Committee at Benha University's Faculty of Medicine in Egypt gave its approval to the study (RC 8-12-2023).

Inclusion criteria

Cases with available clinicopathological data regarding age, grade, stage, depth of invasion, lymph node status, and lympho-vascular space invasion (LVSI) were included.

(1) Follow-up data and survival outcomes for carcinoma.

Exclusion Criteria were

(a) Cases with other histology such as mixed carcinomas.
(b) Patients whose clinical data were not available.
(c) Carcinoma cases without available follow-up data.

Histopathological evaluation: Two pathologists examined all of the cases' hematoxylin and eosin-stained slides to verify the diagnosis. The cases were as follows: there were 60 PCa cases, 6 HGPIN cases, and 10 BPH cases. Every case of pooled carcinoma exhibited prostatic histological characteristics. The Gleason grade group and the 2014 modified Gleason grading system were used to assign grades to all cases of cancer. Cases of cancer were staged following FIGO staging (Kench *et al.*, 2022).

The patient file had the following clinicopathological parameters: age, prostateic-specific antigen (PSA) level, tumor size, lymph node metastasis, distant metastases, capsular invasion, lymphovascular invasion, perineural invasion, and patient survival.

Immunohistochemical evaluation: Formalin-fixed, paraffin-embedded tissue blocks on coated slides were sectioned into 4 μm sections. Using a standard labeled streptavidin–biotin system (Dako Cytomation A/S, Glostrup, Denmark), the manufacturer's instructions were adhered to. Using 10 mmol/L citrate monohydrate buffers (pH 6.0), antigen retrieval was carried out and microwaved for 15 min. The slides of both markers then were incubated overnight at 4°C with optimal dilutions of EZH2 antibody (rabbit polyclonal antibody, dilution 1: 250, ab 137110, Abcam, Cambridge, UK) and anti-BAP-1(rabbit monoclonal antibody, 1: 100 dilution; Code ab92307, Abcam, USA) and immunoreaction was visualized by adding 3,3'-diaminobenzidine (DAB) as a chromogen. An external positive control for EZH2 was a section of positive breast duct cancer. A portion of healthy human pancreatic tissue served as BAP-1's external positive control. Saline or phosphate buffer (PB) was used in place of primary antibodies during the staining process as negative controls.

Interpretation of immunostaining

Assessment of Enhancer of zeste homolog 2 (EZH2) expression

For EZH2, nuclear staining was defined as immunoreactions that were visible at 4×magnification. Two categories based on EZH2 immunoreactivity were

Table 1 Immunohistochemical expression of enhancer of zeste homolog 2 and BRCA1-associated protein 1 in different prostatic lesions

Studied cases	Number	EZH2 expression			BAP1 Expression		
		No/low expression [n (%)]	High expression [n (%)]	*P* value	Negative [n (%)]	Positive [n (%)]	*P* value
BPH	10	8 (80)	2 (20)	0.002**	3 (30)	7 (70)	0.003**
HGPIN	6	4 (66.7)	2 (33.3)		3 (50)	3 (50%)	
PCa	60	28 (46.7)	32 (53.3)		31 (51.7)	29 (48.3)	

** Correlation is highly significant at the 0.01 level

Abbreviations: BPH, benign prostatic hyperplasia; HGPIN, high-grade prostatic intraepithelial neoplasm; PCa, prostatic adenocarcinoma

identified: those with no or low expression (percentage of cells <50%) and those with a strong expression (proportion of cells ≥50%) (Song *et al.*, 2023).

Assessment of BRCA1-associated protein 1 (BAP-1) expression

For BAP-1, nuclear staining only was defined as immunoreactions, and cytoplasmic staining was regarded as negative. The percentage of positive cells was used to interpret BAP-1 IHC staining. If the nuclear staining intensity of BAP-1 was greater than 10% of the tumor cells, it was considered positive; if it was less than 10% of the tumor cells, it was considered negative (Shah *et al.*, 2013).

Statistical analysis

Version 22 of the Statistical Package for the Social Science (SPSS) program was used to tabulate and examine the collected data (SPSS Inc, Chicago, ILL Company). There were two sorts of statistics performed: analytical statistics, which comprise the following tests: ANOVA, chi-square, *t*-test, Pearson's correlation test (r-test), Fisher's exact test (FET), and descriptive statistics, such as percentage (%). Differences were considered statistically significant (S) when (*P* value <0.05) and highly significant (HS) when (*P* value<0.001). To determine the sensitivity, specificity, positive predictive value, and negative predictive value of both markers at various cutoff points for PCa diagnosis, the receiver-operating characteristic (ROC) curve was employed. The area under the curve (AUC): the greater the area, the more accurate is the curve; the total area is 1.0. A rough guide for classifying the accuracy of a diagnostic test is the traditional academic point system:

0.90–1=excellent, 0.80–0.90 good, 0.70–0.80= fair, 0.60–0.70=poor, 0.05–0.60=fail. Specificity: The ability of the test to detect the true negative cases with minimal false positives. Sensitivity: The ability of the test to detect true positive cases with minimal false negatives. Negative predictive value (NPV): Probability that an individual with a negative test result does not have the condition. Positive predictive value (PPV): Possibility that an individual with a positive test result has the condition.

Kaplan–Meier curves were used to display the survival data, and the log-rank test was used to assess the data's statistical significance.

Results

Clinicopathological characteristics

This study was conducted on 10 cases of BPH, whose ages ranged from 45 to 76 years; there were 6 cases of HGPIN whose ages ranged from 58 to 73 years, and 60 cases of prostatic adenocarcinoma (PCa) whose ages ranged from 45 to 84 years. The total serum PSA level ranged from 1.90 to 9.60 ng/ml in BPH cases and from 1.70 to 13.8 ng/ml in HGPIN cases, while it ranged in carcinoma cases from 4.90 to 41.50 ng/ml. Perineural invasion was detected in 45%; lymphovascular invasion was detected in 33.3%; and distant metastasis was detected in 18.3% of PCa cases studied.

Immunohistochemical staining results

Expression of both markers in different prostatic lesions including BPH, HGPIN, and Pca

A statistically highly significant difference was detected between BPH, HGPIN, and PCa cases regarding EZH2 expression in favor of PCa (*P* value =0.002) (Table 1), (Fig. 1).

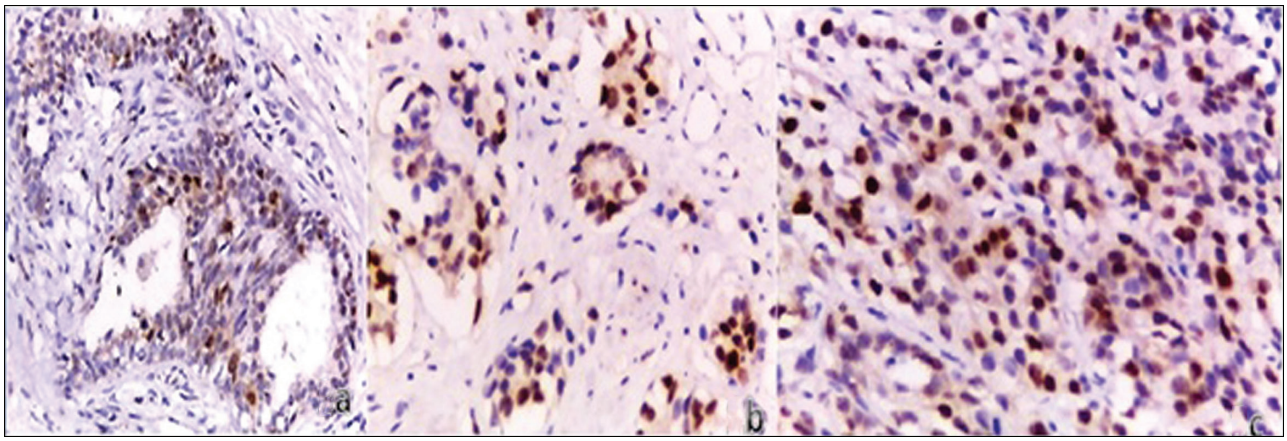
However, a statistically highly significant difference was detected between BPH, HGPIN, and PCa cases regarding BAP1 expression in favor of BPH and HGPIN (*P* value =0.003) (Table 1), (Fig. 2).

Diagnostic validity of EZH2 and BAP1 in diagnosing prostatic adenocarcinoma when each marker was used individually (ROC curve)

Using the ROC curve, when EZH2 is used individually, its diagnostic power in differentiating adenocarcinoma from noncarcinoma cases (BPH and HGPIN) at a cutoff point of 55% revealed 60.2% sensitivity, 83.3% specificity, 91.4% positive predictive value (PPV), and 32.2% negative predictive value (NPV). The diagnostic accuracy (DA) was 65% with an area under the curve (AUC) of 0.906 (Graph 1A).

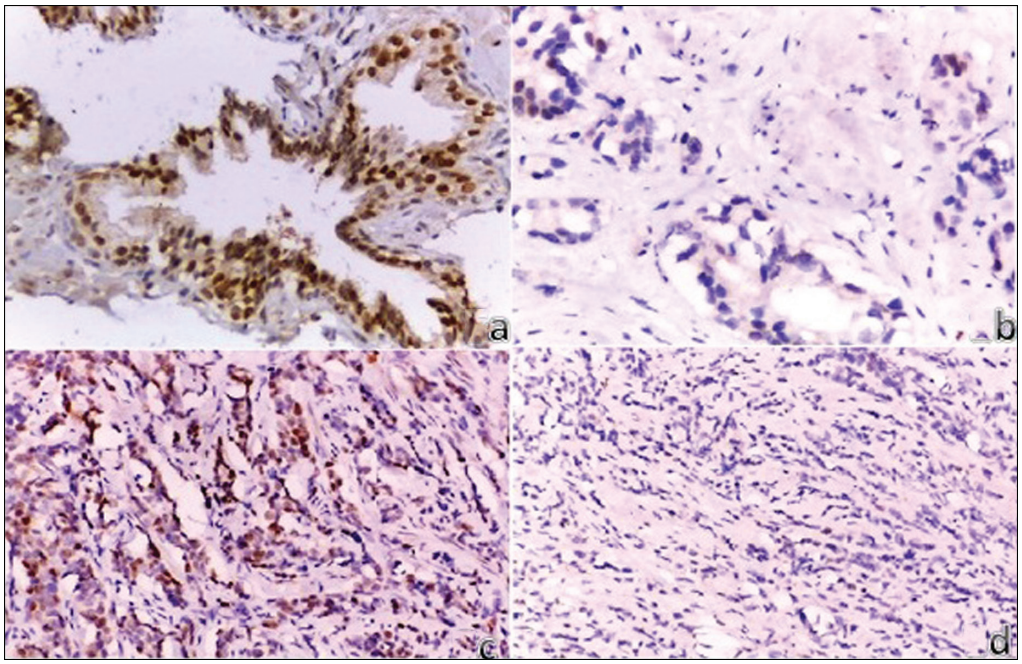
The diagnostic power of BAP1 in differentiating adenocarcinoma from noncarcinoma cases (BPH and

Figure 1



Enhancer of zeste homolog 2 immunohistochemical expression, 1a: showing low nuclear expression (<50%) in benign prostatic hyperplasia ((IHC, ABC X400), 1b: showing high nuclear expression (≥50%) in prostatic adenocarcinoma, Gleason score 6 (3+3) (IHC, ABC X400), 1c: showing high nuclear expression (≥50%) in prostatic adenocarcinoma, Gleason score 9(4+5) (IHC, ABC X400).

Figure 2



b BRCA1-associated protein 1 immunohistochemical expression, 2a: showing positive nuclear expression (≥10% of tumor cells) in benign prostatic hyperplasia (IHC, ABC X400), 2b: showing negative nuclear expression (<10% of tumor cells) in prostatic adenocarcinoma, Gleason score 6(3+3) (IHC, ABC X400), 2c: showing positive nuclear (≥10% of tumor cells) in prostatic adenocarcinoma, Gleason score 8(5+3) (IHC, ABC X200), 2d: showing negative nuclear expression (<10% of tumor cells) in prostatic adenocarcinoma, Gleason score 9(5+4) (IHC, ABC X200).

HGPIN) at a cutoff point of 17.5% revealed 67.4% sensitivity, 60.1% specificity, 48.3% positive predictive value (PPV), and 12.5% negative predictive value (NPV). The DA was 41.3% with an area under the curve (AUC) of 0.879 (Graph 1B).

Diagnostic utility of combined BAP1 and EZH 2 in prostatic carcinoma

Sixty cases of prostatic adenocarcinoma included in this study were divided into BAP1-loss/EZH2-high (n=21, 65.6%), BAP1-loss/EZH2-low (n=10, 35.7%), BAP1-positive/EZH2-low (n=18, 64.3%), and BAP1-positive/EZH2-high (n=11, 34.4%) groups. There was a significant statistical association between high nuclear EZH2 IHC expression and BAP1 loss among the studied PCa cases (*P* value <0.05, χ^2test) as shown in Table 2. When the cases were assessed on the basis of combination of EZH2 and BAP1 expression, the sensitivity and negative predictive value were 70% and 33% respectively, whereas the

specificity and positive protective value were 56.2 and 85.7%, respectively.

Association between EZH2 and BAP1 expression and different clinicopathological parameters in PCa cases

In terms of the association between EZH2 expression and pathological features in PCa cases, a statistically significant direct relationship between high nuclear EZH2 IHC expression and total serum PSA level (*P* value <0.05), Gleason score (*P* value <0.01), Gleason groups (*P* value <0.01), perineural invasion (*P* value <0.05), size and extent of primary tumor (T) (*P* value <0.01) and tumor stage (*P* value <0.01) of the examined cases was found. However, the age of the patients, capsular invasion, lymph-node metastasis, and lympho-vascular invasion showed no significant relation with EZH2 expression (Table 3).

Regarding the association between BAP1 expression and pathological features in PCa cases, a statistically significant inverse relationship between BAP-1 positive expression and Gleason score (*P* value <0.01), Gleason groups (*P* value <0.01), capsular invasion (*P* value <0.05), lympho-vascular invasion (*P* value >0.05), tumor size and extent (T) (*P* value <0.05), lymph-node metastasis (*P* value <0.05), and tumor stage (*P* value <0.01) of the examined cases was found. However, the age of the patients, total serum PSA level, and perineural invasion showed no significant relationship with BAP-1expression (Table 3).

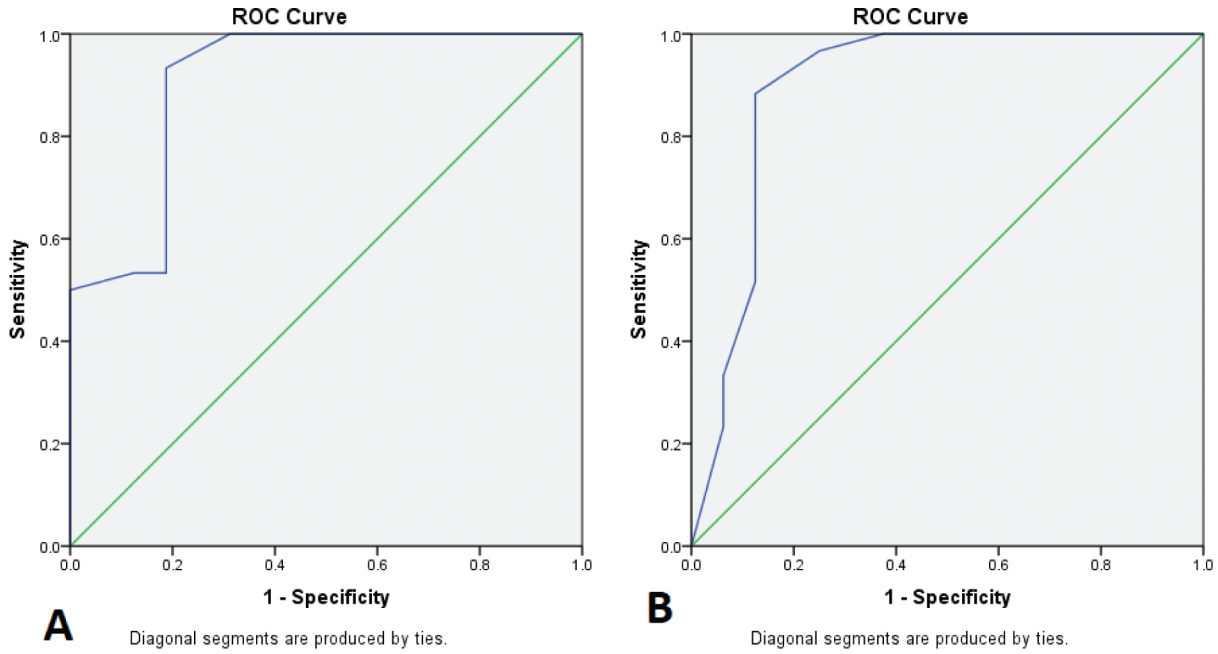
Kaplan–Meier survival analysis to test survival functions of both EZH2 and BAP1

Kaplan–Meier survival analysis showed that the 5 years' overall survival rate in the group of patients with low/no expression of EZH2 was significantly longer than that for patients with high expression with a statistically significant correlation (*P*=0.020*). However, a 5-year survival rate is more in PCa cases with a positive expression of BAP-1, although there was no statistically significant difference according to the log-rank test (*P* =0.103) (Graph 2).

Discussion

Worldwide, the diagnosis of PCa is a challenge. While screening programs for prostatic cancer that measure the total serum PSA level provide early diagnosis of the disease before clinical manifestation, not all counties participate in these programs. Furthermore, patients with any type of prostate pathology, whether benign or

Graph 1:



Receiver-operating characteristic curve for validity and predictivity of enhancer of zeste homolog 2 (A) and BRCA1-associated protein 1 (B) in the diagnosis of prostatic adenocarcinoma cases

Table 2 Association between enhancer of zeste homolog 2 and BRCA1-associated protein 1 in prostatic adenocarcinoma cases

	BAP-1 negative (*N*=60) [n (%)]	BAP-1 positive (*N*=60) [n (%)]	Total	*P* value
EZH-2 no/low expression	10 (35.7)	18 (64.3)	28	**< 0.05***
EZH-2 high expression	21 (65.6)	11 (34.4)	32	

Table 3 Association of both enhancer of zeste homolog 2 and BRCA1-associated protein 1 expression with different clinicopathological parameters in prostatic adenocarcinoma cases

Variables	EZH2		Total (*N*=60) [n (%)]	*P* value	BAP1		*P* value
	No/low expression (*N*=60) [n (%)]	High expression (*N*=60) [n (%)]			Negative (*N*=60) [n (%)]	Positive (*N*=60) [n (%)]	
Age							
<65	15 (25)	17 (28.3)	32 (53.3)	>0.05	17 (28.3)	15 (25)	>0.05
>=65	13 (21.7)	15 (25)	28 (46.7)		14 (23.3)	14 (23.3)	
PSA level							
< 4 ng/ml	1 (1.7)	3 (5)	4 (6.7)		1 (1.7)	4 (6.7)	>0.05
4-10 ng/ml	21 (35)	8 (13.3)	29 (48.3)	**<0.05***	13 (21.7)	16 (26.7)	
>10 ng/ml	6 (10)	21 (35)	27 (45)		17 (28.3)	10 (16.7)	
Gleason score							
Score 6	12 (20)	4 (6.7)	16 (26.7)		4 (6.7)	12 (20)	
Score 7	9 (15)	14 (23.3)	23 (38.3)		11 (18.3)	12 (20)	
Score 8	5 (8.3)	5 (8.3)	10 (16.7)	**<0.01****	8 (13.3)	2 (3.3)	**<0.01****
Score 9	2 (3.3)	9 (15)	11 (18.3)		8 (13.3)	3 (5)	
Gleason groups							
Group 1 (3+3)	12 (20)	4 (6.7)	16 (26.7)	**<0.01****	4 (6.7	12 (20)	**<0.01****
Group 2 (3+4)	7 (11.7)	5 (8.3)	12 (20)		6 (10)	6 (10)	
Group 3 (4+3)	2 (3.3)	9 (15)	11 (18.3)		5 (8.3)	6 (10)	
Group 4 (Score 8)	5 (8.3)	5 (8.3)	10 (16.7)		8 (13.3)	2 (3.3)	
Group 5 (Score 9)	2 (3.3)	9 (15)	11 (18.3)		8 (13.3)	3 (5)	
Capsular invasion							
Present	8 (13.3)	14 (23.3)	22 (36.7)	>0.05	16 (26.7)	6 (10)	**<0.05***
Absent	20 (33.3)	18 (30)	38 (63.3)		15 (25%)	23 (38.3)	
Lympho-vascular invasion							
Present	7 (11.7)	13 (21.7)	20 (33.3)	>0.05	14 (23.3)	6 (10)	**<0.05***
Absent	21 (35)	19 (31.7)	40 (66.7)		17 (28.3)	23 (38.3)	
Perineural invasion							
Present	8 (13.3)	19 (31.7)	27 (45)	**<0.05***	17 (28.3)	10 (16.7)	>0.05
Absent	20 (33.3)	13 (21.7)	33 (55)		14 (23.3)	19 (31.7)	
T							
T1	2 (3.3)	0	2 (3.3)	**<0.01****	0	2 (3.3)	**<0.05***
T2	22 (36.7)	15 (25)	37 (61.7)		15 (15)	22 (36.7)	
T3	2 (3.3)	14 (23.3)	16 (26.7)		13 (21.7)	3 (5)	
T4	2 (3.3)	3 (5)	5 (8.3)		3 (5)	2 (3.3)	
N							
Present	3 (5)	8 (13.3)	11 (18.3)	>0.05	9 (15)	2 (3.3)	**<0.05***
Absent	25 (41.7)	24 (40)	49 (81.7)		22 (36.7)	27 (45)	
Stage							
Stage 1	8 (13.8)	0	8 (13.3)		1 (1.7)	7 (11.7)	
Stage 2	18 (30)	11 (18.3)	29 (48.3)	**<0.01****	12 (20)	(28.3)17	**<0.01****
Stage 3	1 (1.7)	14 (23.3)	15 (25)		10 (16.7)	5 (8.3)	
Stage 4	1 (1.7)	7 (11.7)	8 (13.3)		8 (13.3)	0	

Abbreviations: BAP-1, BRCA1-associated protein-1; N, Lymph-node metastasis; PSA, prostate-specific antigen; T, size, and extent of primary tumor.

* significant.

** highly significant relation.

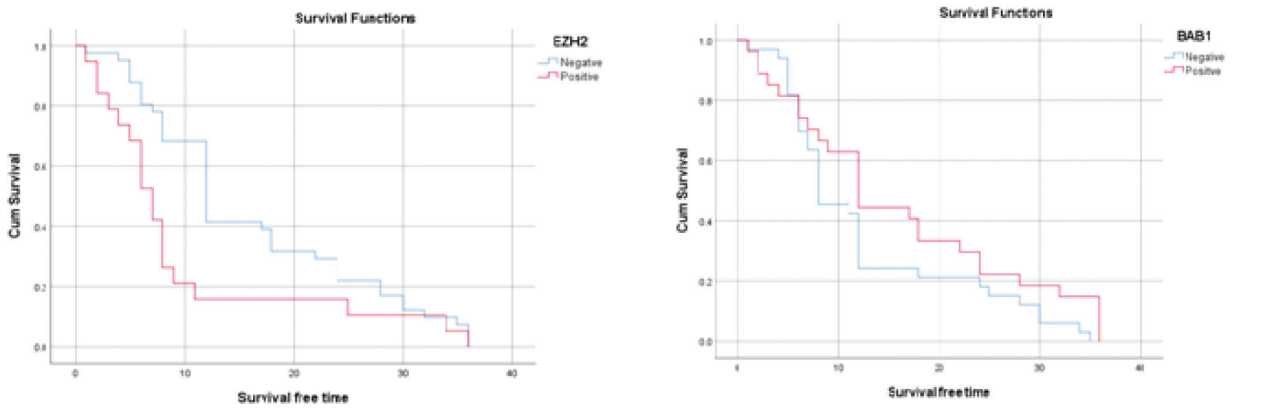
malignant, frequently have increased PSA level (Kench *et al.*, 2022).

Furthermore, there are no particular markers in PCa that can differentiate between tumors that behave aggressively and those that do not. The objective of this study was to assess and compare the immunohistochemical staining of EZH2 and BAP1 in various prostatic lesions to determine their diagnostic validity as well as their potential prognostic significance in PCa, a topic of ongoing discussion.

To the best of our knowledge, this is the first study to assess the prognostic and diagnostic utility of EZH2 and BAP1 immunohistochemistry expression in relation to each other in PCa.

In the current study, a highly significant difference was detected between noncarcinoma cases (BPH and

Graph 2:



Kaplan–Meier survival tables of prostatic adenocarcinoma patients with the expression of enhancer of zeste homolog 2 and BRCA1-associated protein-1.

HGPIN) and PCa regarding EZH2 expression (*P* value=0.002), where 80% of BPH cases and 66.7% of HGPIN cases showed no/low nuclear expression of EZH2, while a high nuclear EZH2 expression was detected in 53.3% of PCa cases. Moreover, ROC analysis has indicated that EZH2 was considerably overexpressed in examined PCa cases in relation to noncancerous lesions including BPH and HGPIN (*P* value = 0.001) by a sensitivity of 60.2% and a specificity of 83.3%. These findings are noteworthy because they imply that EZH2 is overexpressed in the nucleus during prostate carcinogenesis.

The earlier findings aligned with those of Dunder *et al* (Dundr *et al.*, 2020), who observed that samples of PC expressed EZH2 at significantly higher levels than samples from adenomyomatous hyperplasia. These findings also coincided with those of a study by Kunju *et al* (Kunju *et al.*, 2011), which found that breast cancer had increased EZH2 expression in comparison to adjacent benign proliferative lesions and atypical ductal hyperplasia and Chen *et al* (Chen *et al.*, 2021), who demonstrated EZH2 significant upregulation in glioma tissues and cell lines. In addition, Chang *et al.* (Chang *et al.*, 2011) discovered that, in comparison to normal breast cell lines, cancer stem cell (CSC) populations have been reported to have elevated EZH2 levels. The ability of EZH2 to activate the RAF1-catenin signaling pathway, which promotes the development of tumor-initiating cells, has previously been used to explain these results (Dundr *et al.*, 2020). Also, an increased expression of EZH2 in vivo stimulates epithelial hyperplasia in mammary epithelial cells and promotes the development of mammary tumors produced by human epidermal growth factor 2/neu expression (Pourakbar *et al.*, 2017). These findings all point to the carcinogenic potential of EZH2. However, some research showed that EZH2 may function as a tumor suppressor gene in several malignancies, including colorectal carcinoma (Böhm *et al.*, 2019), malignant peripheral nerve sheath tumors (MPNSTs) (Lee *et al.*, 2014), human T-cell acute lymphoblastic leukemia (Simon *et al.*, 2012), and ovarian serous carcinoma (Naskou *et al.*, 2020).

Taken together, these findings reveal that EZH2's function depends on the cell environment, considering that in most solid tumors, EZH2 plays an oncogenic role (Yamaguchi and Hung, 2014). There may be a difference in the number of cases and approaches taken in this issue, but these differences may still need to be clarified and demonstrated. In this study, there was a significant association between high EZH2 nuclear expression and total serum PSA level (*P* value <0.05), Gleason score (*P* value <0.01), Gleason groups (*P* value <0.01), perineural invasion (*P* value <0.05), size and extent of primary tumor (T) (*P* value <0.01), tumor stage (*P* value <0.01) and short patient survival time, as demonstrated by Kaplan–Meier analysis, of the examined cases of PCa. These results are in line with earlier research by Dundr *et al* (Dundr *et al.*, 2020). and Schade *et al* (Schade *et al.*, 2023), which observed that in PCa, elevated expression of EZH2 mRNA exhibited a statistically significant positive correlation with stage, Gleason score, metastatic disease, and a short patient survival time. The findings presented here were also in agreement with those of Fan *et al* (Fan *et al.*, 2020), Huang *et al.*, (Huang *et al.*, 2019), Vantaku *et al.*, (Vantaku *et al.*, 2020), and Guo *et al.*, (Guo *et al.*, 2019), who concluded that EZH2 overexpression was associated with unfavorable clinicopathological features in non-small-cell lung carcinoma, colorectal carcinoma, bladder cancer, and hepatocellular carcinoma, respectively.

An increasing amount of research suggests that EZH2 influences many target genes, which are essential for many cancer-related characteristics, helping to

explain our findings. Transcriptional inhibition of the cell cycle suppressor (INK-ARF) by the polycomb repressive complex 2 (PRC2), including EZH2, was shown to promote cell cycle advancement, prevent cell senescence, and exhaust cancer stem cells. Furthermore, EZH2 represses the production of the epithelial marker E-cadherin (CDH1) and interacts with SNAIL to suppress E-cadherin expression. This downregulation of E-cadherin is essential for the epithelial–mesenchymal transition (EMT), a process connected to the development and spread of cancer (Huang *et al.*, 2022).

Moreover, EZH2 regulates Vasohibin 1 in tumor-associated endothelial cells, and this modulation contributes to the angiogenesis of tumors (Lu *et al.*, 2010). In addition, it has been demonstrated that a number of EZH2 target genes, such as cellular communication network factor 3/(nephroblastoma overexpressed) (CCN3/NOV) and disabled homolog 2-interacting protein (DAB2IP), are implicated in EZH2-driven cancer aggressiveness in PC (Xu *et al.*, 2019).

Several investigations have shown, in contrast to our results, that patients with myeloid malignancies, such as myelodysplastic syndrome and myeloproliferative neoplasms, have inactivating mutations of EZH2, and that these mutations are linked to poor patient survival (Nagata and Maciejewski, 2019).

In this study, BAP-1-positive nuclear expression was detected in 70% of BPH cases and 50% of HGPIN cases, while BAP1-negative nuclear immunohistochemical expression was detected in 51.7% of PCa cases, with a statistically highly significant difference (*P* value =0.003). Moreover, ROC analysis has indicated that BAP1 was considerably overexpressed in examined noncarcinoma cases including BPH and HGPIN in relation to PCa cases (*P* value = 0.001) by sensitivity (67.4%) and specificity (60.1%). These results agree with earlier research by Deng *et al* (Deng *et al.*, 2020), which found that BAP1's mRNA levels were downregulated in PC specimens when compared with specimens of normal tissue, indicating that BAP1 may have a tumor-suppressive role in PCa. In agreement with our research, Shinozaki-Ushiku *et al.* (Shinozaki-Ushiku *et al.*, 2017) observed that BAP1 expression was lost in 53% of cases of malignant mesothelioma, however not in any cases of benign mesothelial reactive lesions. Furthermore, a steady decrease in BAP1 protein levels was observed in lung cancer, breast cancer, and renal carcinoma cell lines as compared with normal cells (Joseph *et al.*, 2014 and Andrici *et al.*, 2016, respectively). Accordingly, aforementioned evidence suggested that BAP1 deletion or low expression may play a significant role in the carcinogenesis of various tumors. Results from research by Deng *et al* (Deng *et al.*, 2021) detailed how BAP1 may physically bind to and deubiquitinate PTEN in PCa cells may support this. This stabilizes PTEN protein and inhibits the development of PCa by preventing PTEN from being destroyed by ubiquitination. PTEN is one of the most significant tumor suppressors; it inhibits PI3K/Akt signaling, which is critical in the development of tumors and cancer metastasis (Huang *et al.*, 2012).

Regarding the predictive value of BAP1, the current study revealed that according to Kaplan–Meier analysis, patients with PCa who had negative nuclear BAP-1 expression had a significantly higher Gleason score, a higher Gleason group, capsular invasion, lympho-vascular invasion, lymph node metastases, a higher T-stage, and a shorter patient 5-year survival time. This agrees with the study by Deng *et al.* (Deng *et al.*, 2021).

In addition, in colorectal cancer (Tang *et al.*, 2013), gastric adenocarcinoma (Yan *et al.*, 2016), non-small-cell lung cancer (Shen *et al.*, 2016), gall bladder cancer (Hirosawa *et al.*, 2018), clear cell renal cell carcinoma (Joseph *et al.*, 2014), and uveal melanoma (Masoomian *et al.*, 2018) reduced BAP1 expression has been associated with a poor prognosis and unfavorable tumor characteristics.

The findings of Barnett *et al.* (2023) provide an explanation for our findings, as they reported that BAP-1 binds to the BRCA1 RING finger motif and increases the BRCA1-mediated cellular growth suppressor activity by deubiquitination. Also, nuclear-localized BAP-1 functions as an independent inhibitor in cell proliferation and as a regulator of apoptosis (Masclef *et al.*, 2021). Other authors suggested that the interaction between BAP1 and PTEN could be the mechanism of better prognosis (Chen *et al.*, 2021).

However, Park *et al* (Park *et al.*, 2020). found that BAP1 expression was low in normal prostate cell lines but high in tumorigenic and metastatic cell lines. Similarly, Streuer *et al* (Steurer *et al.*, 2019) reported that BAP1 expression was typically upregulated in cancers compared with adjacent normal prostatic glands,. A strong BAP1 staining correlated to advanced tumor stage ($P<0.0001$), high classical and quantitative Gleason grade ($P<0.0001$), lymph node metastasis ($P<0.0001$), a positive surgical margin ($P=0.0019$), and early biochemical recurrence ($P<0.0001$). This discrepancy might be attributable to a different cohort or a small number. To draw definitive conclusions, a study with a large cohort or a meta-analysis is required.

Furthermore, there is growing evidence that BAP1 can stimulate the formation of tumors when it is overexpressed in specific molecular settings. For instance, mutant ATRX in myeloid neoplasms (Asada *et al.*, 2018) and Krüppel-like factor 5 (KLF5) in basal-like breast malignancies (Qin *et al.*, 2015), which are both stabilized by BAP1 and subsequently accelerate tumor growth, are target genes of BAP1 deubiquitination.

Furthermore, earlier studies have suggested that BAP-1-mediated cell proliferation depends on the connection between BAP-1 and HCF-1. HCF-1, a cell cycle modulator, BAP-1 regulates cell proliferation by deubiquitinating HCF-1N, which promotes G1-phase progression and S-phase entry (Carbone *et al.*, 2020). When considered collectively, these results suggest that BAP-1 might have a dual role in cellular growth, both preventing excessive growth and ensuring appropriate cell growth (Oh *et al.*, 2020).

There was a significant association between high nuclear EZH2 IHC expression and BAP-1-negative IHC expression among the studied PCa cases (P value <0.05) with 70% sensitivity, 56.2% specificity, and 67.1% DA when tested synchronously. Although no comparable prior research has indicated the relationship between the two markers and PCa, Hakim *et al* (Hakim and Abou Gabal, 2021) reported an inverse relationship between BAP-1 loss and EZH2 overexpression, which could potentially be applied to differentiate between reactive mesothelial hyperplasia and pleural epithelioid mesothelioma. Their close relationship to the tumor suppressor PTEN might assist to explain this. This gene has been revealed to be an EZH2 target, which means that EZH2 can bind H3K27me3 to the PTEN promoter and inhibit PTEN transcription (Yang *et al.*, 2023), while BAP1 stabilizing PTEN as previously discussed. Furthermore, Lafava *et al.* (LaFave *et al.*, 2015) demonstrated that BAP1 loss in mice results in elevated EZH2 expression and Yoshimura *et al.* (Yoshimura *et al.*, 2019) observed enrichment for BAP1 at the EZH2 locus; these data suggested that BAP1 interact and occupy the EZH2 locus consequently. BAP1 loss leads to increased EZH2 transcriptional output. An advantage of BAP1 and EZH2 IHC is that they can stain the nuclei of tumor cells. However, most of known markers, such as PSA and AMACR, exhibit variable intensity cytoplasmic staining, which can occasionally be challenging to distinguish from nonspecific staining.

Conclusions

Testing for BAP-1 and EZH2 can be added to the diagnostic panel of Pca and may help in the early detection of Pca in asymptomatic high-risk men. A possible method for treating cancer that targets tumor cells and tumor stem cells involves blocking EZH2 expression or activating BAP-1. Additional investigations using a bigger sample size, other types of prostatic carcinomas and in-depth in vivo and in vitro experiments are required to confirm their role.

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Conflicts of interest

The authors declare that they have no competing interest.

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