

Immunohistochemical Study of Tissue Expression of Nucleophosmin Protein and its association with histopathological parameters in Prostatic Carcinoma in Suez Canal University Hospital

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Abstract

Background: Prostate cancer is the second most common cancer in males. Nucleophosmin (NPM1) is a multifunctional nucleolar protein that plays a role in many cellular processes and in the development of several types of cancer, including prostate cancer. **Aim:** To investigate the immunohistochemical expression of NPM1 Protein in prostatic carcinoma (PCa) tissues and determine its association with the histopathological parameters of PCa. **Subjects and Methods:** The current work is a cross-sectional descriptive study, conducted in the pathology laboratory of Suez Canal University Hospital on 30 PCa specimens. The specimens included 25 TRUS needle biopsies, 3 prostatectomies and 2 TURP. They were gathered by convenience sampling from records and formalin fixed paraffin embedded tissue blocks during the interval between 2013 and 2019. NPM1 expression was assessed in PCa tissues using immunohistochemistry. **Results:** NPM1 expression showed nuclear positivity in luminal prostatic epithelial cells in all studied PCa specimens, and the median percentage of expression was 90%. Moreover, strong NPM1 expressions were found in 22 specimens that included 19 TRUS needle biopsies, 2 prostatectomies and 1 TURP. Moderate expressions were found in only 8 specimens. NPM1 expression showed a weak positive correlation with patients' age ($r=0.103$) and Gleason Grade ($r=0.142$). NPM1 expression showed a non-statistically significant association with patients' age, specimen characteristics, prostatic adenocarcinoma growth patterns, Gleason Grade, comedo necrosis, lymphovascular and perineural invasion ($p\text{-value} > 0.05$). **Conclusions:** NPM1 is a potential immunohistochemical marker that may be added to the immunohistochemical diagnostic panel of prostatic carcinoma.

Keywords: Prostate, Cancer, immunohistochemistry

Introduction

Prostate cancer is the second most common cancer in males. In 2020, according to GLOBOCAN estimates, 1,276,106 new cases were detected worldwide ⁽¹⁾ and 358,989 men of these new cases died from this cancer ⁽²⁾. GLOBOCAN also estimated in December 2020 that prostate cancer is the fifth most prevalent cancer in Egypt and the fifth highly incident cancer among Egyptian males (4767 new

prostate cancer cases among a total of 66542 new cancer cases in Egyptian males) ⁽³⁾.

Screening of prostate cancer is carried out by ultrasonography, routine measuring of prostate-specific antigen (PSA) serum level accompanied by digital rectal examination. However, Diagnostic confirmation is performed only by histopathological examination of H&E and immunohistochemically stained prostatic biopsies. So, we are always in urgent need to study new

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immunohistochemical markers to reach accurate diagnosis^(4,5).

Nucleophosmin (NPM1) is a multifunctional nucleolar protein that plays a role in a variety of cellular processes, including ribosome assembly, DNA repair, regulation of gene expression by mRNA processing, binding to histones and transferring them to DNA to help nucleosome assembly and chromatin remodeling⁽⁶⁻⁸⁾.

Nucleophosmin is overexpressed in many hematologic malignancies as non-Hodgkin's lymphomas, acute myeloid leukemia and many solid cancers as glioblastoma, non-small cell lung cancers hepatocellular carcinoma, colon cancer, ovarian cancer, bladder cancer, thyroid cancer and endometrial carcinoma. Generally, NPM1 overexpression correlates with high grade tumors and poor outcome⁽⁹⁻¹³⁾.

Several studies on prostatic carcinomas have shown the role of nucleolar proteins in prostate carcinogenesis. This is explained by the fact that nucleoli are the site of protein synthesis and DNA regulation. These processes are tightly regulated by nucleolar proto-oncoproteins. So, mutations affecting nucleolar proteins can lead to prostatic malignant transformation and increased tumor proliferation^(14,15).

The role of nucleolar proteins in prostate carcinogenesis is evidenced by nucleolar prominence, one of the histopathological criteria of prostatic carcinomas. However, the expression, localization, and functional importance of specific nucleolar proteins in the progression of PCa have not been yet studied as potential markers^(14,16).

This raised the need to study the immunohistochemical expression of the nucleolar protein NPM1 in prostatic carcinoma tissue specimens as it might help as a potential diagnostic immunohistochemical marker or it could

be a promising target for new prostate cancer therapy regimens.

Subjects and Methods

Study setting and Study population:

The current work is a cross-sectional descriptive study, conducted in the pathology laboratory of Suez Canal University Hospital on 30 PCa specimens. The specimens included 25 TRUS needle biopsies, 3 prostatectomies and 2 TURP. They were gathered by convenience sampling from records and formalin fixed paraffin embedded tissue blocks during the interval between 2013 and 2019.

Inclusion Criteria:

1- Specimens of PCa with histopathological data including patient's age, tumor histologic type, Gleason grade, estimated approximate percentage of prostate involved by tumor in examined slides, lymphovascular and perineural invasion.

2- Available paraffin blocks of TRUS needle biopsy specimens, TURP and prostatectomy specimens.

Exclusion Criteria:

Specimens with unfit remaining tumor tissue

Histopathological evaluation, staining and scoring:

PCa were collected from stored specimens. Histopathological data of samples were retrieved from specimen records and included: patient's age, site of specimen in prostate, tumor histologic type, Gleason scoring, WHO/ISUP grade grouping, estimated approximate percentage of prostate involved by tumor in examined slides and the state of lymphovascular and perineural invasion. Sections were prepared from the paraffin embedded tissue blocks of PCa specimens at 5 µm thickness and were stained with H&E stains. Other sections were picked on positively charged slides for staining with NPM1 immunohistochemical marker.

Positive control slides were obtained from normal colonic tissue (**Figure 1**) and negative control slides were obtained from the same specimens by omitting the primary antibodies during the IHC staining protocol. Hematoxylin & eosin slides were initially assessed for tumor type, Gleason grade, estimated approximate percentage of prostate involved by tumor in examined slides, presence of lymphovascular and perineural invasion and other histopathological features according to the WHO 2022 classification of prostatic tumors.

Indirect immunohistochemical staining technique was manually performed using the peroxidase anti-peroxidase method. PCa sections stained by NPM1 marker

were microscopically examined at high power magnification, for nuclear staining intensity and the proportion of stained tumor cells. The expression of NPM1 was scored as negative if percentage of stained tumor cell-nuclei <10% and was scored as positive if percentage of stained tumor cells is 11-100%. NPM1 positive cells were categorized according to extent of staining in tissue into three subgroups of intensity of reaction: +3 (strong), +2 (moderate), +1 (weak) (**Table 1**). This semi quantitative scoring system was utilized according to the protocol used by Leal, M.F., Mazzotti, T.K.F., Calcagno, D.Q. et al. to quantify NPM1 immunoreactivity in gastric cancer⁽¹⁷⁾.

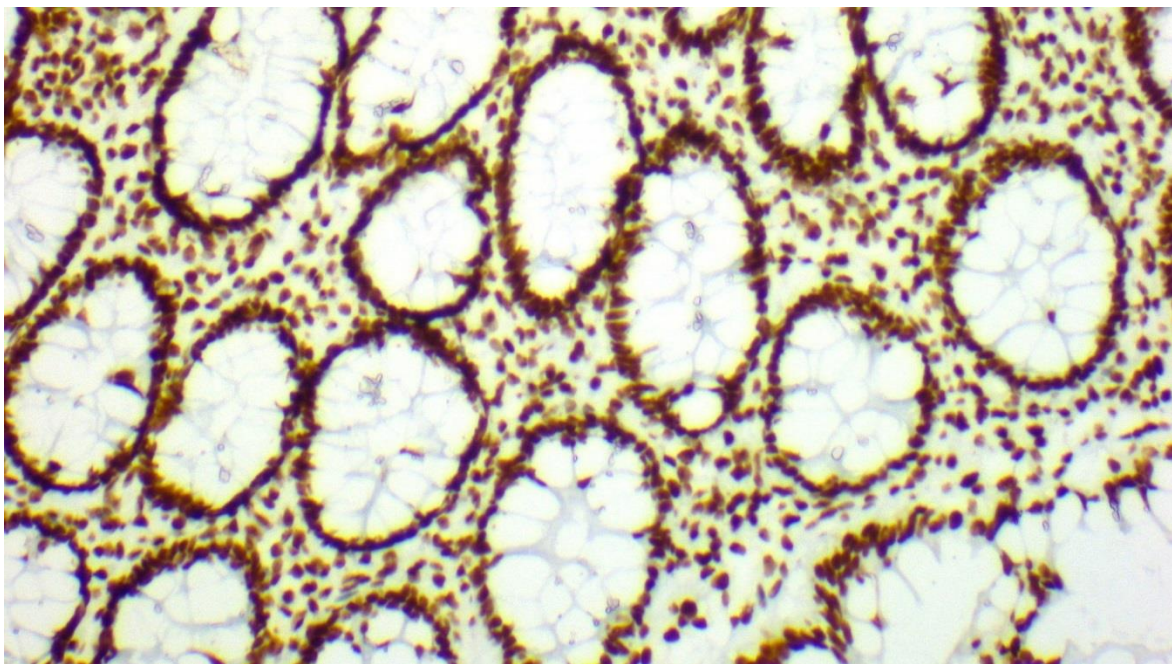


Figure 1 Positive strong NPM1 nuclear staining (score 3) in normal colonic mucosa positive control, original magnification x200.

Table 1 NPM1 Protein Expression Evaluation Criteria.				
NPM1 expression	Negative (0)	Positive		
Percentage of nuclear stained tumor cells	0-10%	11-100%		
Intensity of nuclear stained tumor cells	negative to minimal	+1 (weak)	+2 (moderate)	+3 (strong)

Results

Age of patients and specimen characteristics

A total of 30 prostatic carcinoma specimens were included in the present study. The mean age of patients to which specimens belong was 69 years (n = 30). Twenty two patients were younger than

75 years and 8 patients were older than 75 years. The specimens included 25 TRUS needle biopsies, 3 prostatectomies and 2 TURP. As regards TRUS needle biopsies; 12 specimens were obtained from base, 7 from apex and 6 specimens were obtained from mid gland (**Table 2**).

Table 2 Patients' age and Specimens' Characteristics in PCa study Group.

Characteristics	PCa (n = 30)
Age (years)	
Mean $\pm$ SD	69.0 $\pm$ 8.8
Range	51 - 83
Age Groups	
Younger (<75 years)	22 (73.3%)
Older ($\geq$ 75 years)	8 (26.7%)
Type of Specimen	
TRUS needle biopsy	25 (83.3%)
TURP	2 (6.7%)
Prostatectomy	3 (10.0%)
Site of Biopsy in TRUS needle biopsies	
Base	12 (48%)
Mid-gland	6 (24.0%)
Apex	7 (28%)

TRUS: Trans Rectal Ultrasound Scan, TURP: Transurethral Resection of the Prostate, * statistically significant p-value at $p < 0.05$, ^F. Fischer's exact test, ^t. student t test.

Microscopic Features of PCa Specimens

Thirty specimens showed prostatic carcinoma. Most PCas were acinar adenocarcinomas (n=28), that were present in 23 TRUS needle biopsy specimens, 2 TURP specimens and 3 prostatectomies.

Acinar adenocarcinomas showed small crowded back to back glands with oval lumens lined by malignant epithelial cells showing enlarged hyperchromatic nuclei, prominent nucleoli and absent basal cell layer (**figure 2-4**). Some specimens showed surrounding foci of benign prostatic hyperplasia and prostatitis.

Ductal adenocarcinoma was found in only 2 TRUS needle biopsy specimens and showed complex papillae with fibrovascular cores and glands lined by tall pseudostratified columnar cells with abundant pale eosinophilic cytoplasm.

Regarding Gleason grading, 18 specimens were Gleason grade group 5 that appeared in form of solid nests, sheets and cords of malignant cells with no gland formation (**figure 4**), followed by Gleason grade group 4 (8 specimens) that showed fused, cribriform and ill-defined poorly formed glands with slit-like and punched out lumens (**figure 3**) and 4 specimens were Gleason grade group 3 that revealed discrete variable sized small crowded glands with regular contours (**figure 2**).

The approximate percentage of prostate involved by tumor in examined slides was more than 80% in most PCa specimens (n=20). Associated prostatitis and lymphoid aggregates were present in 12 PCa specimens. Lymphovascular invasion was found in 5 specimens & perineural invasion was found in 9 specimens (**Table 3**).

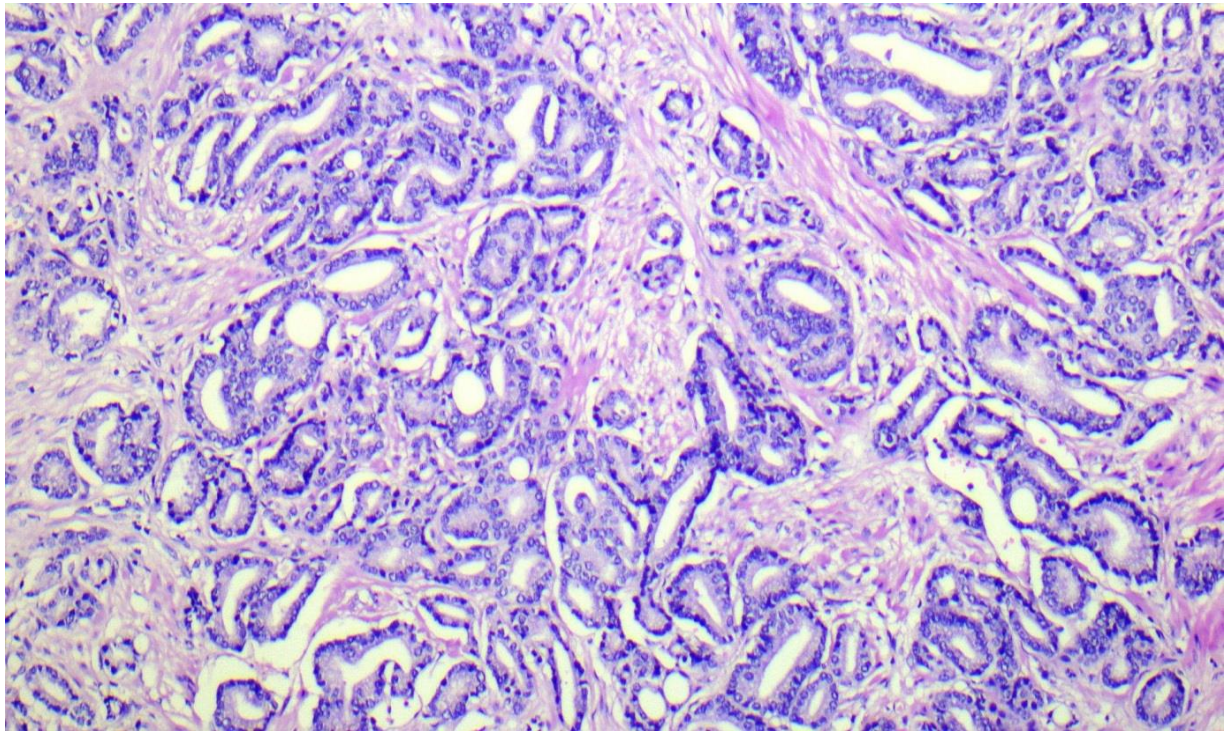


Figure 2 Prostatic acinar adenocarcinoma, Gleason grade (4+3), grade group 3, original magnification x100.

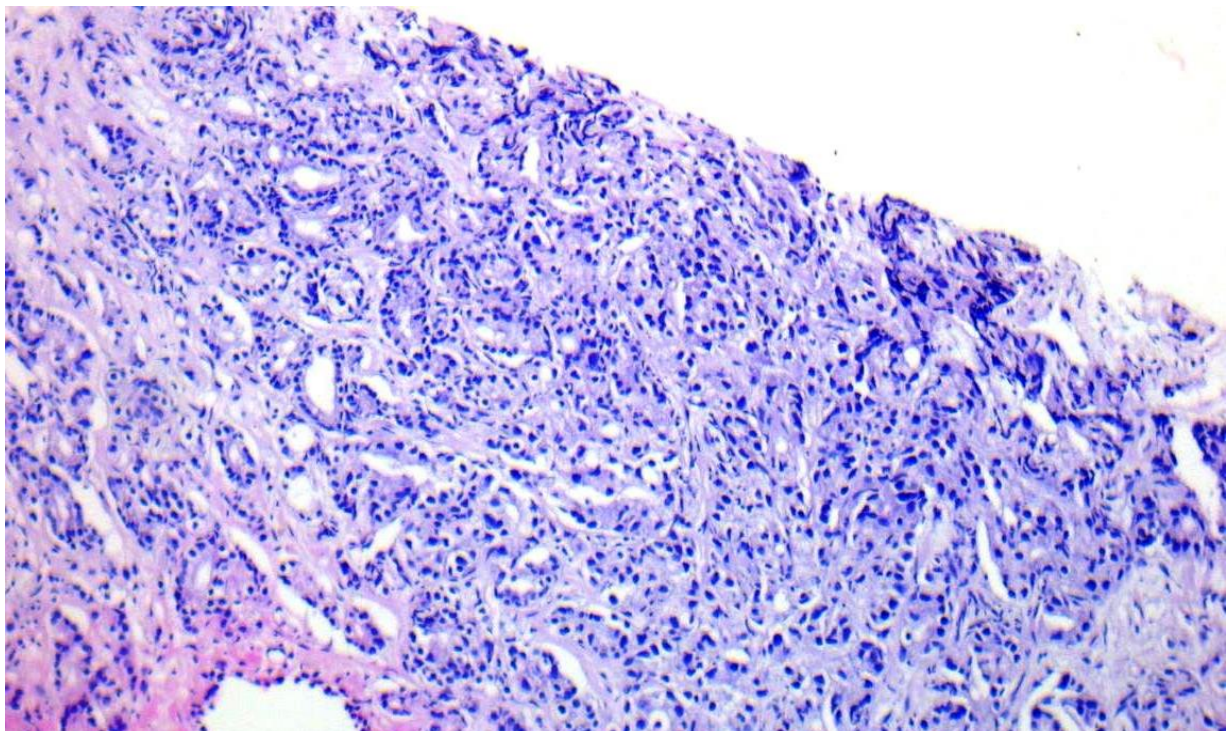


Figure 3 Prostatic acinar adenocarcinoma, Gleason grade (4+4), grade group 4, original magnification x100.

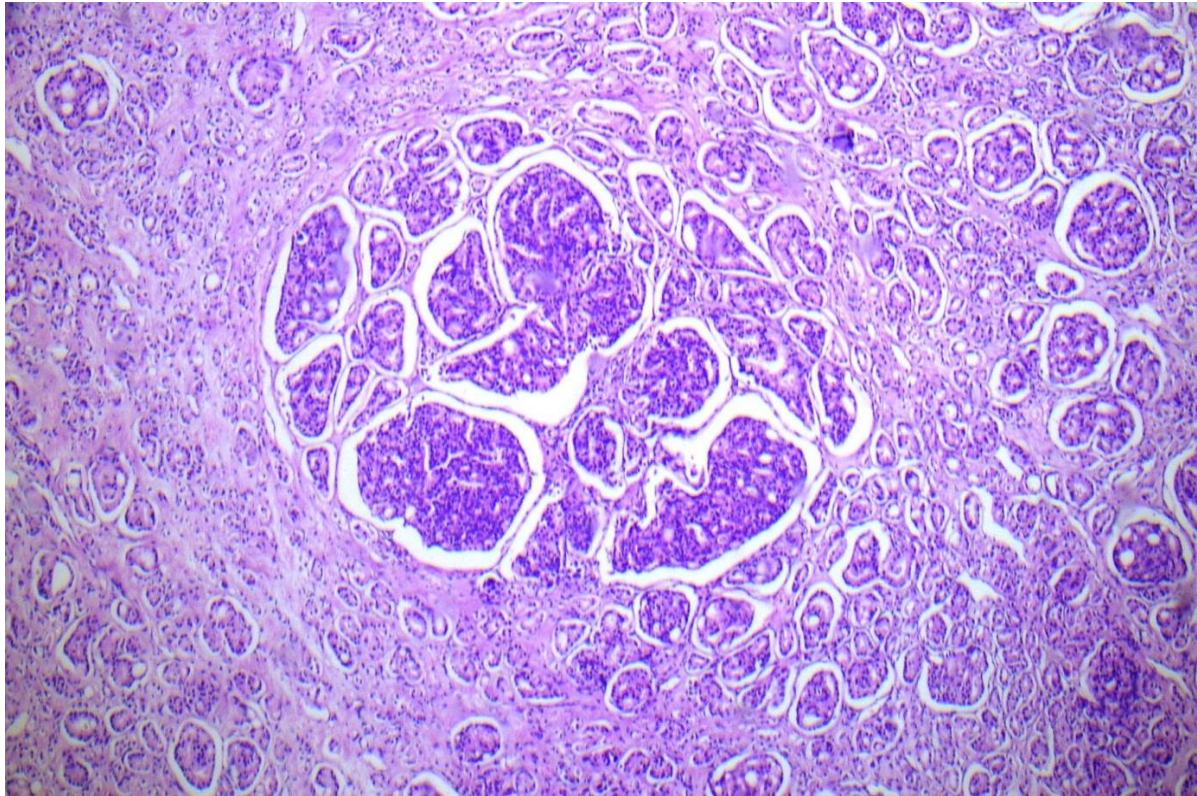


Figure 4 Prostatic acinar adenocarcinoma, Gleason grade (4+5), grade group 5, original magnification x40.

Table 3 Morphological Features of PCa Specimens.	
Characteristics	PCa (n = 30)
Dominant Growth Pattern	
Acinar	28 (93.3%)
Ductal	2 (6.7%)
Cribriform Pattern	
No	5 (16.7%)
Yes	25 (83.3%)
Gleason Grade	
Grade group 3	4 (13.3%)
Grade group 4	8 (26.7%)
Grade group 5	18 (60.0%)
Gleason Score	
Median	9.0 (8.0 – 9.0)
Approximate percentage of prostate involved by tumor in examined slides	
Median	90.0 (70.0 – 100.0)
Less than 80%	10 (33.3%)
80 – 99%	8 (26.7%)
100%	12 (40.0%)
Comedo necrosis	
None	13 (43.3%)
Focal	10 (33.3%)
Diffuse	7 (23.3%)
Lymphocytic infiltration	

Negative	18 (60.0%)
Positive	12 (40.0%)
Lymphovascular Invasion (LVI)	
No	25 (83.3%)
Yes	5 (16.7%)
Perineural Invasion (PNI)	
No	21 (70.0%)
Yes	9 (30.0%)

NPM1 expression in prostatic carcinoma specimens

NPM1 expression showed nuclear positivity in luminal prostatic epithelial cells in all studied PCa specimens and the median percentage of NPM1 positivity NPM1 expression was found in 22 PCa

specimens (**figure 6-8**) and moderate expression was found in only 8 specimens (**figure 5**) (**Table 4**). was 90% (**figure 5-8**). Moreover, strong

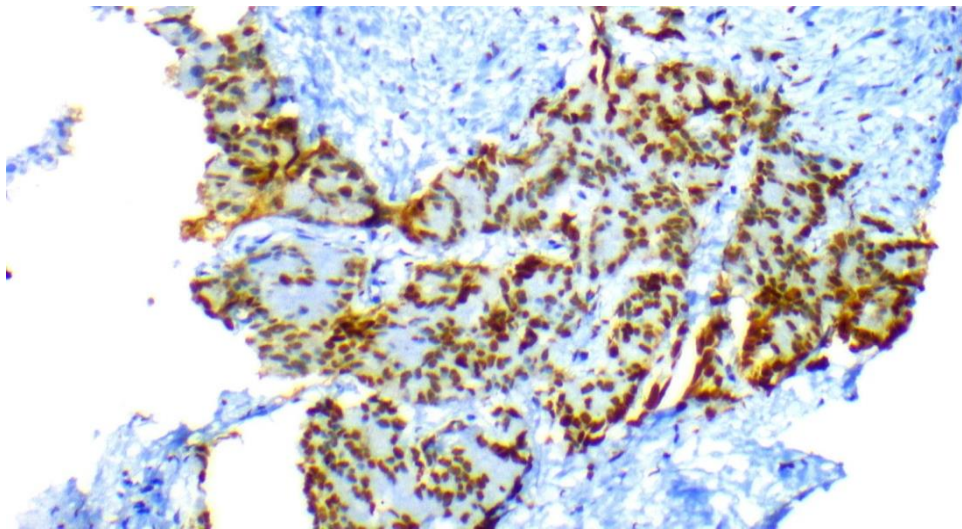


Figure 5 Positive moderate NPM1 nuclear staining (score 2) in prostatic acinar adenocarcinoma, Gleason grade (4+3), Gleason grade group 3, original magnification x100.

Table 4 NPM1 Immunohistochemical Staining in PCa specimens.	
Parameters	PC (n = 30)
NPM1 Expression	
Negative	0
Positive	30 (100.0%)
Median percentage of NPM1 postivity	90.0 (80.0 – 90.0)
NPM1 Staining Intensity	
Moderate	8 (26.7%)
Strong	22 (73.3%)
BPH: Benign Prostatic Hyperplasia, PC: Prostatic Carcinoma, NPM1: Nucleophosmin 1, * Statistically significant p-value at $p < 0.05$, ^M . Mann Whitney test, ^C . Chi-square test.	

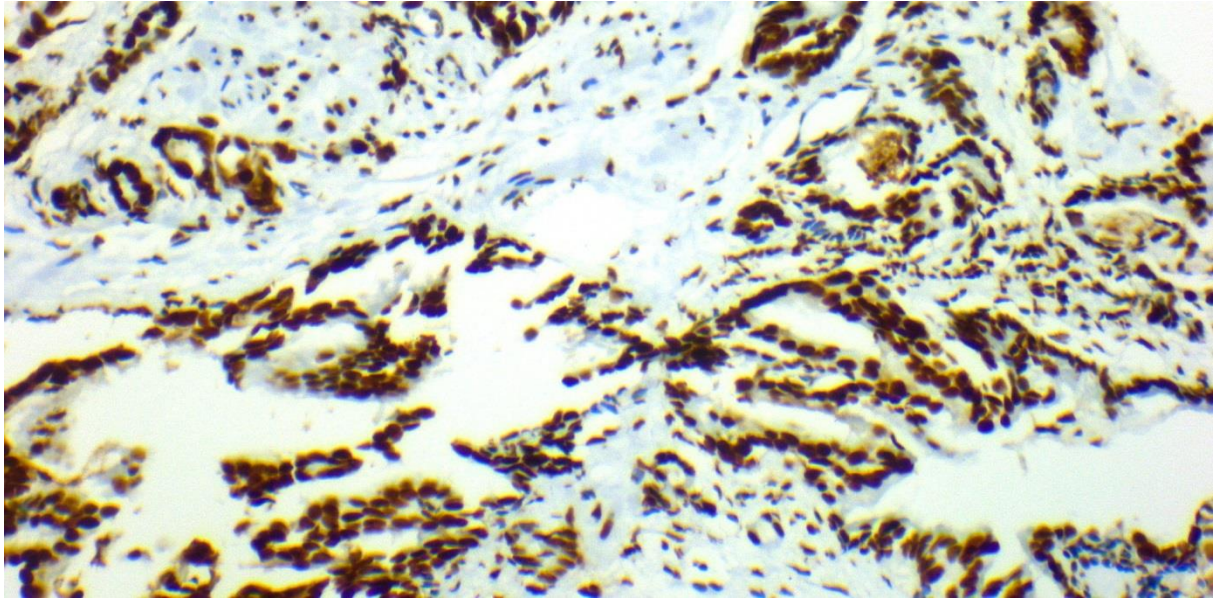


Figure 6 Positive strong NPM1 nuclear staining (score 3) in prostatic acinar adenocarcinoma, Gleason grade (4+4), Gleason grade group 5, original magnification x200.

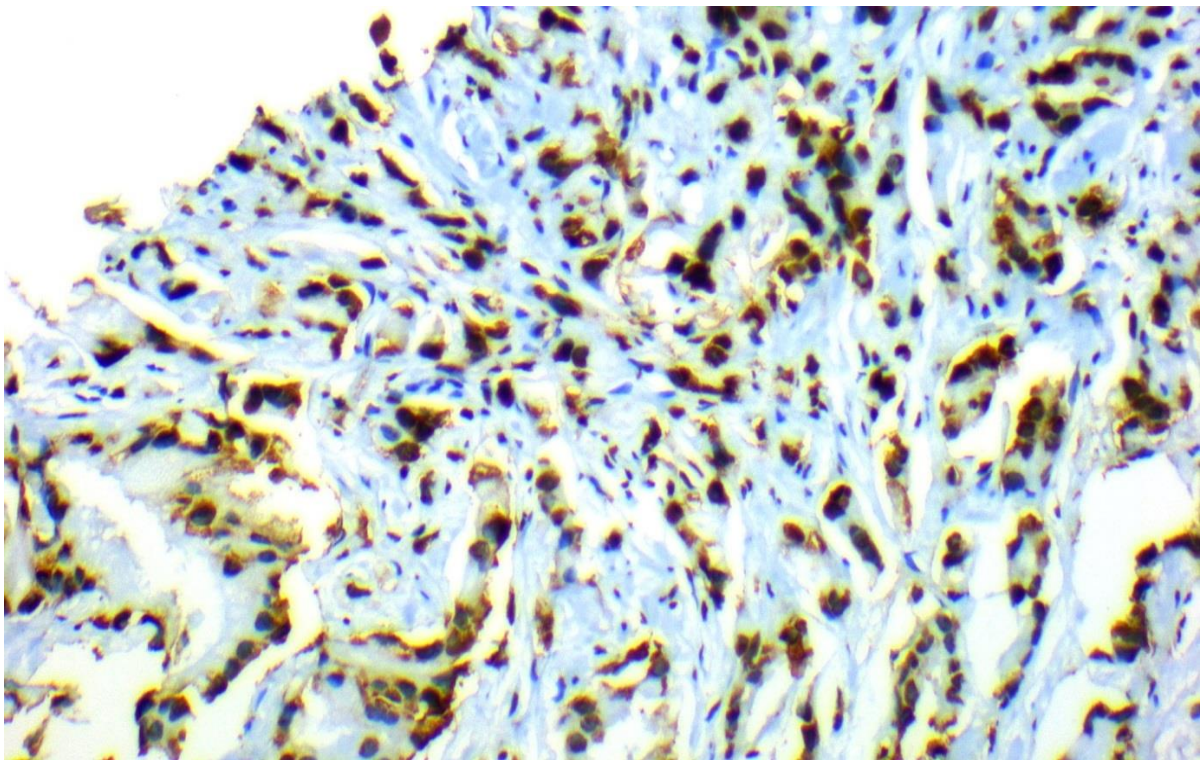


Figure 7 Positive strong NPM1 nuclear staining (score 3) in prostatic acinar adenocarcinoma, Gleason grade (4+5), Gleason grade group 5, original magnification x200.

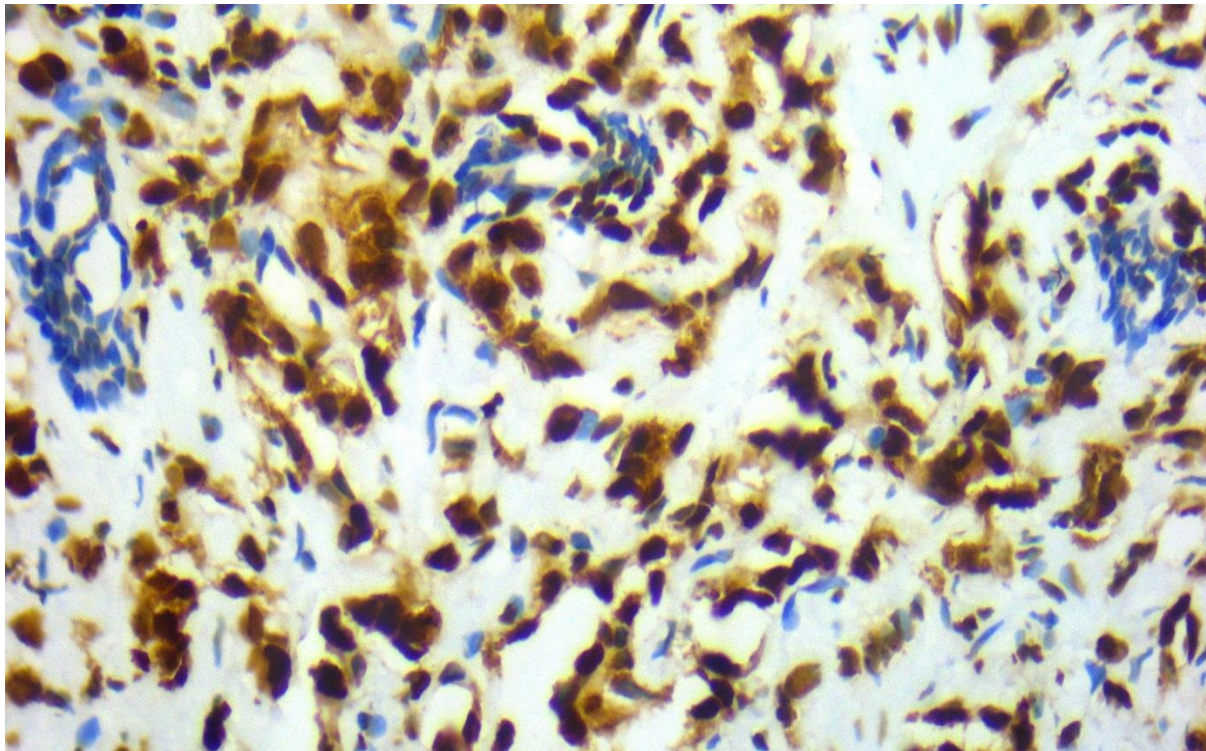


Figure 8 Positive strong NPM1 nuclear staining (score 3) in prostatic acinar adenocarcinoma, Gleason grade (5+5), Gleason grade group 5, original magnification x400.

Association and correlation between NPM1 immunohistochemical staining and age of prostatic carcinoma patients

Strong NPM1 staining was found in 16 out of 22 PCa specimens in the younger age group and in 6 out of 8 specimens in the older age group. However, no statistically significant association was found

between NPM1 expression and patients' age (p-value >0.05) (Table 5). In addition, positive weak correlation was found between NPM1 expression and patients age in the stained specimens ($r=0.103$), but this relationship wasn't statistically significant (Figure 9).

Table 5 Association between NPM1 Staining Intensity and Age in PCa Group (n = 30).

Parameters	n	NPM1 Staining Intensity		
		Moderate (n=8)	Strong (n=22)	p-value
Age (years), Median (IQR)	30	70.5 (65.0 – 74.0)	70.0 (62.0 – 75.0)	0.730 ^M
Age Groups				
Younger (<75 years)	22	6 (75.0%)	16 (72.7%)	1.00 ^F
Older (≥75 years)	8	2 (25.0%)	6 (27.3%)	

NPM1: Nucleophosmin 1, ^M. Mann Whitney test, ^F. Fischer's exact test

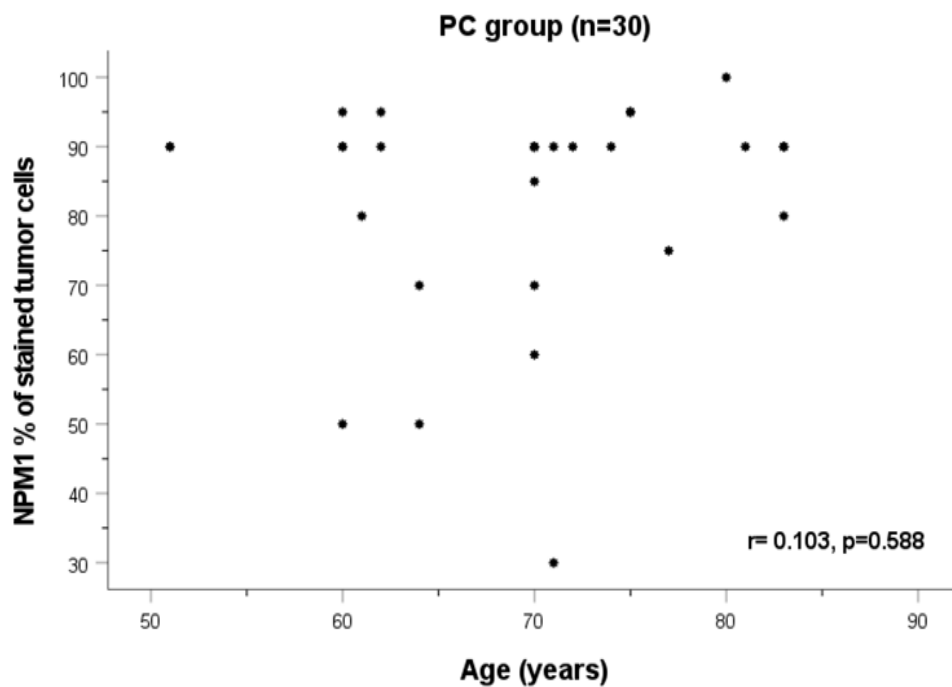


Figure 9 Scatter plot of the correlation between NPM1 expression and age of patients with PCa.

Association between NPM1 immunohistochemical staining and specimen characteristics

Regarding type of specimen and site of biopsy, strong NPM1 staining was found in 19 out of 25 TRUS needle biopsy specimens. Strong NPM1 staining was also found in 1 of 2 TURP specimens and 2 out of 3 prostatectomies. As regards

TRUS needle biopsies, strong NPM1 staining was found in 9 out of 12 biopsies obtained from base, 4 out of 6 biopsies obtained from mid-gland and 6 out of 7 biopsies obtained from apex. However, no statistically significant association was found between NPM1 expression and specimen characteristics (p-value >0.05) (Table 6).

Table 6 Association between NPM1 Staining Intensity and Specimen Characteristics in PCa Group (n = 30).

Parameters	N	NPM1 Staining Intensity		
		Moderate (n=8)	Strong (n=22)	p-value
Type of Specimen				
TRUS needle biopsy	25	6 (75.0%)	19 (86.4%)	0.569 ^F
TURP	2	1 (12.5%)	1 (4.5%)	
Prostatectomy	3	1 (12.5%)	2 (9.1%)	
TRUS needle biopsy site				
Base	12	3 (37.5%)	9 (40.9%)	0.908 ^F
Mid-gland	6	2 (25.0%)	4 (18.2%)	
Apex	7	1 (12.5%)	6 (27.3%)	

Association between NPM1 immunohistochemical staining and histopathological parameters of prostatic carcinoma specimens

Strong NPM1 expression was found in 2 ductal adenocarcinomas, 20 acinar adenocarcinomas and moderate NPM1 expression was found in only 8 acinar adenocarcinomas. NPM1 expression was strong in 18 out of 25 specimens showing cribriform pattern. Median Gleason score was higher in specimens with strong NPM1 staining (9.0) than in specimens showing moderate NPM1 staining (8.5) (Table 7).

Regarding Gleason grade, among 22 specimens with strong NPM1 expression, 14 specimens were Gleason grade group 5, 7 specimens were Gleason grade group 4 and only 1 specimen was

Gleason grade group 3. Moreover, weak positive correlation was found between NPM1 expression and Gleason Score ($r=0.142$) (Table 7) (Figure 10).

The approximate percentage of prostate involved by tumor in examined slides was more than 80% in 15 out of 22 specimens with strong NPM1 expression. Strong NPM1 expression was found in 13 out of 17 specimens showing comedo necrosis. NPM1 expression was also strong in 8 out of 12 specimens showing positive lymphocytic infiltration, in 4 out of 5 specimens demonstrating positive lymphovascular invasion and 7 out of 9 specimens with positive perineural invasion. But, No statistical significance was found between NPM1 expression and all these studied parameters (Table 7).

Table 7 Association between NPM1 Staining Intensity and Histopathological Parameters in PCa Group (n = 30)

Parameters	n	NPM1 Staining Intensity		
		Moderate (n=8)	Strong (n=22)	p-value
Dominant Growth Pattern				
Acinar	28	8 (100.0%)	20 (90.9%)	1.00 ^F
Ductal	2	0	2 (9.1%)	
Positive Cribriform Pattern	25	7 (87.5%)	18 (81.8%)	1.00 ^F
Gleason Grade				
Grade group 3	4	3 (37.5%)	1 (4.5%)	0.082 ^F
Grade group 4	8	1 (12.5%)	7 (31.8%)	
Grade group 5	18	4 (50.0%)	14 (63.6%)	
Gleason Score, Median	30	8.5 (7.0 – 9.0)	9.0 (8.0 – 9.0)	0.202 ^M
Approximate percentage of prostate involved by tumor in examined slides				
Less than 80%				1.00 ^F
80 – 99%	10	3 (37.5%)	7 (31.8%)	
100%	8	2 (25.0%)	6 (27.3%)	
	12	3 (37.5%)	9 (40.9%)	
Comedo necrosis				
None	13	4 (50.0%)	9 (40.9%)	0.761 ^F
Focal	10	3 (37.5%)	7 (31.8%)	
Diffuse	7	1 (12.5%)	6 (27.3%)	
Positive Lymphocytic infiltration	12	4 (50.0%)	8 (36.4%)	0.678 ^F
Positive Lymphovascular Invasion (LVI)	5	1 (12.5%)	4 (18.2%)	1.00 ^F
Positive Perineural Invasion	9	2 (25.0%)	7 (31.8%)	1.00 ^F

PCa: Prostatic Carcinoma, NPM1: Nucleophosmin 1, * statistically significant p-value at $p<0.05$, ^M. Mann Whitney test, ^F. Fischer's exact test

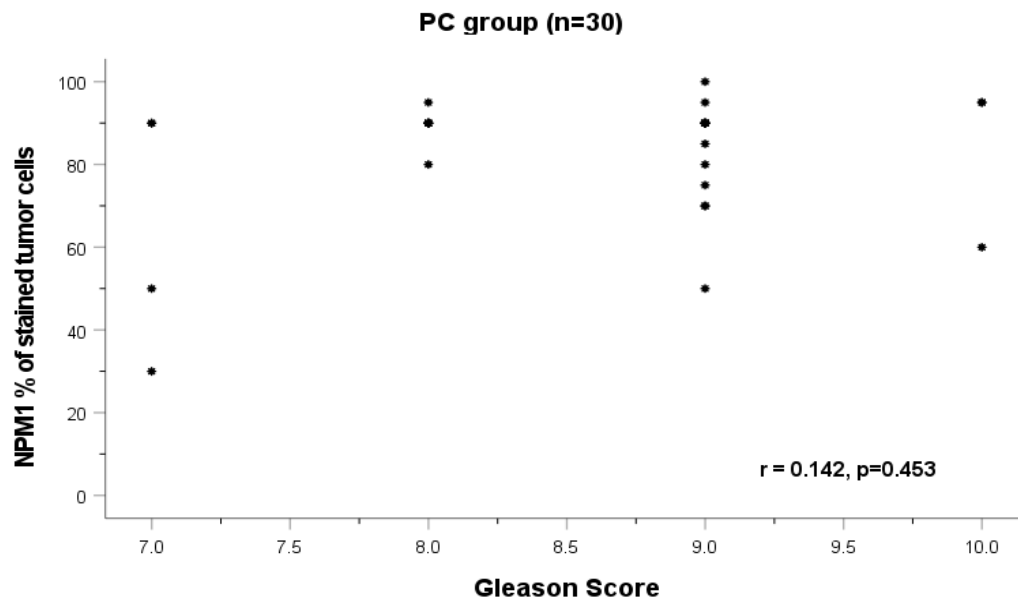


Figure 10 Scatter plot of the correlation between NPM1 expression and Gleason Score in patients with PCa

Discussion

The study included 30 specimens of prostatic carcinoma, selected by convenience sampling. This small sample size is considered a significant limitation and future multicenter studies are recommended. The age of prostatic carcinoma patients from whom specimens were obtained ranged from 51 to 83 years, including 22 men younger than 75 years and 8 men older than 75 years and with a mean age of 69 years.

Our findings regarding age of PCa patients differs from previous studies in which the mean age was only 64.2⁽¹⁸⁾ and 64.11⁽¹⁹⁾. However, our findings are matching with the Egyptian national data which showed that the highest incidence of prostatic carcinoma in Egypt was in males ranging from 70 to above 75 years old⁽²⁰⁾. These differences in age groups and mean age of PCa patients between studies may be related to more frequent screening and earlier diagnosis in developed countries.

This study included different types of prostatic biopsy specimens that were 25 TRUS needle biopsy specimens, 2 TURP and 3 radical prostatectomy specimens.

Previous immunohistochemical studies on NPM1 expression in prostate cancer only included radical prostatectomy specimens^(18,21,22). TRUS needle biopsy specimens in our study revealed that PCa is highly incident in base of gland (12 specimens). NPM1 showed positive nuclear staining in luminal prostatic epithelial cells in 100% of PCa specimens, in which strong NPM1 staining intensity was found in 22 specimens, moderate staining intensity in only 8 specimens and the median percentage of NPM1positivity was 90%. These findings are analogous to those found in all other research that studied NPM1 expression in malignant prostatic tumors^(18,21-23).

These previous findings are justifiable given the role of NPM1 in coactivating and regulating androgen functions by enhancing androgen binding affinity to its responsive elements resulting in increased expression of androgen target genes as PSA gene and increased proliferation of PCa cells. Additionally, NPM1 is the main protein that forms AgNOR that is proved to be involved in PCa initiation, proliferation and progression^(6,18).

NPM1 expression showed weak positive correlation with age ($r=0.103$), in which, strong NPM1 staining was found in sixteen out of 22 PCa specimens in the younger age group and in 6 out of 8 specimens in the older age group. However, p-value was greater than 0.05 and no statistically significant association was found between NPM1 expression and patients' age.

On studying the association between NPM1 expression and specimen characteristics, strong NPM1 staining was found in 19 TRUS needle biopsy specimens; including nine biopsies obtained from base, 6 from apex and 4 from mid-gland. Strong NPM1 staining was also found in 1 TURP specimen and 2 prostatectomies. However, no statistically significant association was found between NPM1 expression and specimen characteristics (p-value >0.05). This may be explained by the small available sample size.

NPM1 expression in our thesis showed weak positive correlation with Gleason grades ($r=0.142$), in which specimens with higher Gleason grades showed stronger NPM1 staining intensity than those with lower grades. Thus, among 22 specimens showing strong NPM1 expression, 14 specimens were Gleason grade group 5, 7 specimens were Gleason grade group 4 and only 1 specimen was Gleason grade group 3. These results are matching with NPM1 role in upregulation of protooncogenes transcription as c-Myc, EGF and EGFR complex. NPM1 also activates downstream growth factor signaling pathways as MAPK and AKT pathways, allowing tumor cell proliferation and promoting additional tumorigenic aggressive behavior of PCa cells^(6,22,24).

However, no statistically significant association was found between NPM1 staining intensity and Gleason grade (p-value >0.05).

Comparable result was found in two previous research^(21,23) and different result was revealed in another study⁽¹⁸⁾ that showed statistically significant association between NPM1 staining intensity and Gleason grade (p-value <0.05). That discrepancy between studies is due to differences in primary antibody provider and methods of statistical analysis.

To our knowledge, this is the first time in research to highlight the association of NPM1 with prostatic adenocarcinoma growth patterns, perineural, lymphovascular invasion and comedo necrosis. In the present thesis, strong NPM1 expression was found in 20 acinar and 2 ductal adenocarcinomas, 18 specimens showing cribriform pattern, 13 specimens showing comedo necrosis, 4 specimens demonstrating positive lymphovascular invasion and 7 specimens with positive perineural invasion. However, this positive association between NPM1 staining intensity and these studied parameters wasn't statistically significant.

These previous findings can be explained by the fact that NPM1 is a histone chaperone that increases assembly of AR containing regulatory protein complexes. So, NPM1 overexpression promote prostate carcinogenesis and tumor progression through activating AR signaling pathway^(6,21).

Conclusion

This study concluded that NPM1 is a potential immunohistochemical nucleolar protein, overexpressed in prostatic carcinomas and may be used as a promising therapeutic target. It is recommended to include NPM1 in the immunohistochemical diagnostic panel of prostatic carcinoma and further studies are needed to fully evaluate the prognostic significance of NPM1 expression in prostatic carcinomas.

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