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Immunohistochemical Expression of Claspin and TopBP1 in Prostatic Adenocarcinoma: Correlation with Clinicopathological Parameters and Prognostic Significance

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ABSTRACT

Background: Understanding the molecular basis of prostate cancer development is essential to improve the therapeutic efficacy and to determine the clinical behavior of different cases. Genome protective mechanisms investigations is a mainstay of cancer research.

Objective: To study the immunohistochemical expression of Claspin and TopBP1 in cases of prostate cancer to assess their relationship with clinicopathological and prognostic features of the disease.

Patients and Methods: Claspin and TopBP1 expression was assessed immunohistochemically in eighty-four (84) prostate cancer patients and their prognostic significance was evaluated.

Results: Positive Claspin expression and high TopBP1 score were strongly correlated with high pre-treatment level of PSA, groups of Gleason grade, higher Gleason score and advanced stage of tumor (P<0.001) for each. Positive Claspin expression was correlated with LN and distant metastasis (P<0.001). High TopBP1 score was strongly correlated with distant and LN metastasis (P=0.002, P=0.007 respectively). No association was found between both markers and age. A significant association was found between higher TopBP1 expression score & positive Claspin expression (P<0.001) and also shorter progression free survival. Upregulation of Claspin was associated with worse overall survival (p=0.045), but no association was detected among the expression of TopBP1 and overall survival (P=0.355).

Conclusion: Claspin and TopBP1 expression have an important prognostic value in prostate cancer. Using them may help to identify high risk patients who will benefit the most from treatment. Besides, both markers can aid in the creation of improved methods that are useful against the therapy-resistant cancer.

Keywords: Claspin, TopBP1, Prostate cancer, Genomic instability.

INTRODUCTION

Prostate cancer (PCa) is a common solid neoplasm among males in the developed nations. In 2019, PCa was the cause of about 20% of the newly diagnosed cancers and about 10% of cancer related death in American men [(1)]. The frequency and death of PCa in developing countries also increasing[(2)].

Prostate cancer is a multifactorial neoplasm with variable clinical spectrum and prognosis. Unfortunately, the important factors in prognosis including Gleason score and TNM staging, don't clarify the variability in the treatment outcomes [(3)]. Furthermore, stratification of PCa patients into prognostic subgroups according to PSA kinetics cannot sufficiently differentiate indolent from aggressive cases, there are still key questions to answer [(4)].

A cornerstone of neoplastic transformation is instability of the genome that occurs due to deficiencies in maintenance mechanisms of genome integrity, including the checkpoints of the cell cycle, DNA repair and DNA proliferation control. Accordingly, a mainstay of cancer research is genome protective mechanisms investigations [(5)]. Throughout proliferation stress, to keep the integrity of the genome, the proliferation checkpoint pathways ATR-Claspin-Chk1 is triggered [(6)].

After DNA damage, checkpoint activation gives cells a period to repair before continuing the cell cycle. Otherwise, checkpoints are disabled and cells undergo apoptosis. Chk1 is a chief mediators of checkpoint stimulation [(7)]. Claspin is essential for Chk1 stimulation after DNA damage, facilitating phosphorylation of Chk1 Via ATR, Claspin upregulation in neoplastic cells guards them from stress of proliferation in a checkpoint-independent mode [(8)]. Claspin upregulation is triggering proliferation of many neoplasms such as carcinomas of the stomach and kidney [(9, 10)].

Interestingly, Claspin may have neoplasm suppressive behavior [(11)]. In PCa, the biological behavior and function of Claspin is unclear and need further clarification [(12)]. An important triggering of Ataxia telangiectasia and Rad3 related (ATR) is Topoisomerase II-binding protein one (TopBP1) [(13)]. Suppressors of ATR, a cheif protein kinases working in DNA damage response (DDR), are striking as sensitizers in treatment with chemotherapy [(14)]. TopBP1 upregulation is related with genome instability in neoplasia [(13)].

In vitro, TopBP1 encourages the cellular replication of PCa by inhibition of apoptosis mediated via ATR-CHK1. Still, the exact clinical behavior of TopBP1 in PCa is obscured [(15)]. In response to

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genotoxic stress, the adaptor proteins TopBP1 and Claspin make it easier for Chk1 to be phosphorylated by the ATR kinase. Therefore, downregulation of Claspin and TopBP1 mimicked Chk1 inactivation by inducing spontaneous DNA damage [13, 16].

The aim of the present research is to investigate the immunohistochemical reactivity of Claspin and TopBP1 in PCa patients and to assess their relationship to clinicopathologic features of the disease to distinguish between aggressive and indolent cases of PCa.

MATERIAL AND METHODS

Patients and tissue selection:

Eighty four (84) Paraffin blocks were collected retrospectively from the archive of Pathology Department, Zagazig University Hospitals in the period between November 2017 till December 2019.

Clinical and follow-up data were obtained retrospectively from the archives of Urology and Medical Oncology Departments of the same institute. Distant metastasis was established by radio-isotopic bone scan and pan CT or PSMA PET/CT. None of the cases underwent androgen ablation before surgery. Every follow-up included a serum chemistry study that include PSA. Biochemical progression was assessed as rise of serum PSA level of 2 ng/mL or more above the baseline PSA.

Histopathological Examination:

To confirm the diagnosis, slides stained with Hematoxylin and eosin of all PCa were reviewed. Gleason scoring was done according to the guidelines of International Society of Urological Pathology (ISUP), 2019 [17]. Staging of PCa was done based on the TNM system of the American Joint Committee on Cancer (AJCC), 2017 [18].

Immunohistochemistry:

Immunohistochemistry was performed by streptavidin–biotin immunoperoxidase. Paraffin blocks were sectioned, deparaffinized and rehydrated. Retrieval of the epitope was done, and endogenous activity of the peroxidase was blocked. The Tissue sections of all PCa were incubated with a rabbit polyclonal anti-Claspin Ab (1:20000, clone ab3720; Abcam) and a rabbit polyclonal anti-TopBP1 Ab (1:100, clone ab2402, Abcam Co. Ltd., UK), overnight at room temperature. Immune complexes were recognized using a peroxidase reaction with DAB as a chromogen. Each batch of slides contained both positive and negative controls.

Interpretation of immunohistochemistry:

For Claspin IHC, the nuclear staining was expressed positive or negative. Immunostaining was considered positive if >5% of PCa cells showed nuclear immunoreactivity [19].

For TopBP1 IHC, The intensity of cytoplasmic immunoreactivity was scored as: [negative =0 point, weak = one point, moderate =two points, and strong =three points]. The extent of staining was scored as: [<5% (0 point), 6-25% (one point), and 26-50% (two points), and 51-75% (three points), and more than 75% (four points)]. The final scores were calculated by adding points of the level of intensity and the points of staining percentage. The staining scores of > 4 was interpreted as high immunehistochemical expression[15].

Ethical approval:

The study was approved by the Ethics Board of Zagazig University (ZU-IRB 9845).

Statistical analysis:

Analysis of our collected data was done by IBM SPSS 23.0 for windows (SPSS Inc., Chicago, IL, USA). Quantitative data were analyzed as Mean ± standard deviation (SD) and qualitative data were analyzed as frequency and percentage. The following tests were done: for data that was not distributed normally, independent sample t test & Mann-whitney test were used, while fisher exact & chi-square were used for qualitative data. Analysis of the survival was done using Kaplan-mayer test. Probability (P-value): less than 0.05 was regarded significant.

RESULTS

Clinicopathological results (Table 1)

Our research included 84 tissue samples from PCa patients. The mean age of cancer patients at the diagnosis was 64.4 ± 9.28 years. Mean serum PSA level was 281.1 ± 355.3 ng/ml. Overall, 29.8% of the cases were grade group IV and 23.8% were grade group V. Moreover, 39 (46.4%) of the cases were Gleason score ≤7 and 45 (53.6%) were Gleason score >7. Eight cases (9.5%), 23 cases (27.4 %), 24 cases (28.6 %) & 29 (34.5%) were stage I, II, III, and IV respectively. Metastasis in LN was detected in 21 cases (25 %) and distant metastasis was in 23 cases (27.4%) with 20 patients had bone metastasis. Progression was noted in 41 patients (48.8 %), of them 17 patients had biochemical recurrence.

Table (1): General characteristics of the studied PCa cases.

		Studied group N=84	
		N	%
Age	Mean ±SD	64.4 ± 9.28	
PSA (ng\ml)	Mean ±SD	281.1 ± 355.3 (5-1235)	
Gleason score	≤7 >7	39 (46.4%) 45 (53.6%)	
Gleason grade group	I II III IV V	17 (20.2%) 5 (6.0) 17 (20.2%) 25 (29.8%) 20 (23.8%)	
Stage	I II III IV	8 23 24 29	9.5 27.4 28.6 34.5
LN metastasis		21	25.0
Distant metastasis		23	27.4
CTH		14	16.7
RTH		41	48.8
Treated by surgery		14	16.7
Hormonal treatment		57	67.9
Relapse		41	48.8
Mortality	Died	5	6.0

Immunohistochemical expression of Claspin and TopBP1 (Fig 1, 2) and association of expression with patients' clinicopathological features (Table 2, 3)

Positive Claspin expression was detected as nuclear immunoreactivity in 44% (37/84) of cases, while cytoplasmic TopBP1 immunoreactivity with high expression detected in 65.5% (55/84). Positive Claspin expression and high TopBP1 score was associated strongly with, Gleason grade groups, higher Gleason score, high PSA level and advanced stage (P<0.001). Positive Claspin immunoreactivity was correlated with distant metastasis & LN metastasis (P<0.001). High TopBP1 score was strongly correlated with LN (P=0.007) & distant metastasis (p=0.002).

Association between Claspin and TopBP1 expression (Table 3).

Positive Claspin expression and high TopBP1 expression were significantly associated (p <0.001).

Table (2): Relation between Claspin immunoreactivity and tumor characteristics of the studied groups.

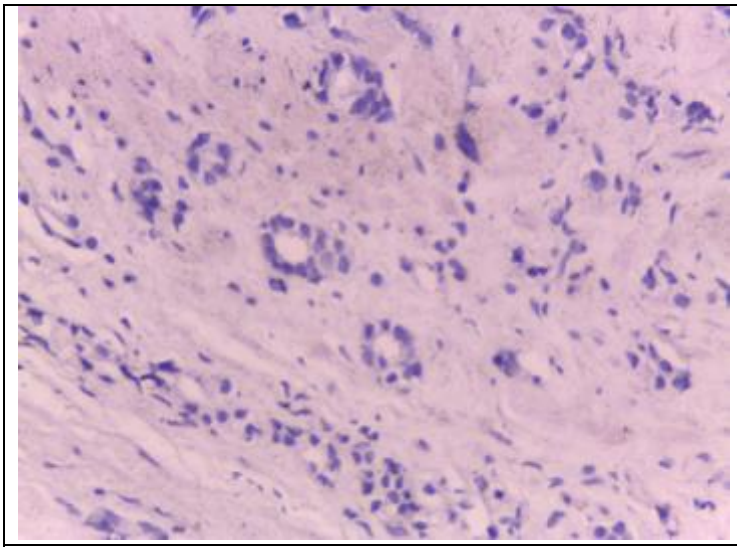
		Positive N=37		Negative N=47		Tests	P
		N	%	N	%		
Age	Mean ±SD	65.8±11.1		63.3 ±7.56		1.21*	0.23 NS
PSA (ng\ml)	Mean ±SD	421.7±385.8		170.3±287.8		4.25#	<0.001
	Median (Range)	320 (13-1235)		17 (5-1117)			HS
Gleason score	≤7	7 (18.9%)		32 (68.1%)		25.3	<0.001 HS
	>7	30 (81.1%)		15 (31.9%)			
Gleason grade group	I	0 (0.0%)		17 (36.2%)		25.4	<0.001 HS
	II	1 (2.7%)		4 (8.5%)			
	III	6 (16.2%)		11 (23.4%)			
	IV	15 (40.5%)		10 (21.3%)			
	V	15 (40.5%)		5 (10.6%)			
Stage	I	0	0.0	8	17.0	27.5$	<0.001
	II	3	8.1	20	42.6		HS
	III	12	32.4	12	25.5		
	IV	22	59.5	7	14.9		
LN metastasis		17	45.9	4	8.5	15.5$	<0.001 HS
Distant metastasis		18	48.6	5	10.6	15.1$	<0.001 HS
CTH		11	29.7	3	6.4	F	0.005 S
RTH		19	51.4	22	46.8	0.17 $	0.67 NS
Treated by surgery		0	0.0	14	29.8	F	<0.001 S
Hormonal treatment		36	97.3	21	44.7	F	<0.001 HS
Relapse		30	81.1	11	23.4	27.6$	<0.001 HS
Mortality	Died	4	10.8	1	2.1	F	0.163 NS

*Independent t-test #Mann-whitney test $Chi-square test F: Fisher exact test NS: p-value>0.05 is not significant S: P-value<0.05 is significant HS: p-value<0.001 is high significant

Table (3): Relation between Top Bp1 immunoreactivity and tumor characteristics of the studied PCa cases.

		High (N=55)		Low (N=29)		Tests	P
		N	%	N	%		
Age	Mean ±SD	64.9 ± 10.1		63.6 ±7.44		0.61*	0.53 NS
PSA (ng\ml)	Mean ±SD	398.1 ± 372.3		59.1 ± 169.8		5.85#	<0.001
	Median (Range)	320 (12-1235)		13 (5-889)			HS
Gleason score	≤7	15 (27.3%)		24 (82.8%)		43.5$	<0.001 HS
	>7	40 (72.7%)		5 (17.2%)			
Gleason grade group	I	0 (0.0%)		17 (58.7%)		45.3$	<0.001 HS
	II	3 (5.5%)		2 (6.9%)			
	III	12 (21.8%)		5 (17.2%)			
	IV	22 (40.0%)		3 (10.3%)			
	V	18 (32.7%)		2 (6.9%)			
Stage	I	0	0.0	8	27.6	48.5$	<0.001
	II	6	10.9	17	58.6		HS
	III	23	41.8	1	3.4		
	IV	26	47.3	3	10.3		
LN metastasis		19	34.5	2	6.9	F	0.007 S
Distant metastasis		21	38.2	2	6.9	F	0.002 S
CTH		14	25.5	0	0.0	F	0.002 S
RTH		32	58.2	9	31.0	5.57 $	0.01 S
Treated by surgery		2	3.6	12	41.4	F	<0.001 HS
Hormonal treatment		5	9.1	22	75.9	F	<0.001 HS
Positive claspin		37	67.3	0	0.0	F	<0.001 HS
Relapse		37	67.3	4	13.8	F	<0.001 HS
Mortality	Died	5	9.1	0	0.0	F	0.163 NS

*Independent t-test #Mann-whitney test $Chi-square test F: Fisher exact test NS: p-value>0.05 is not significant S: P-value<0.05 is significant HS: p-value<0.001 is high significant.



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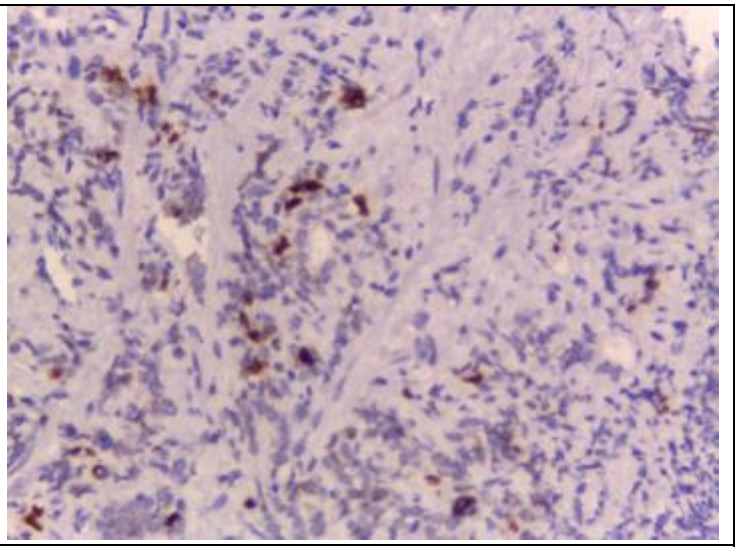

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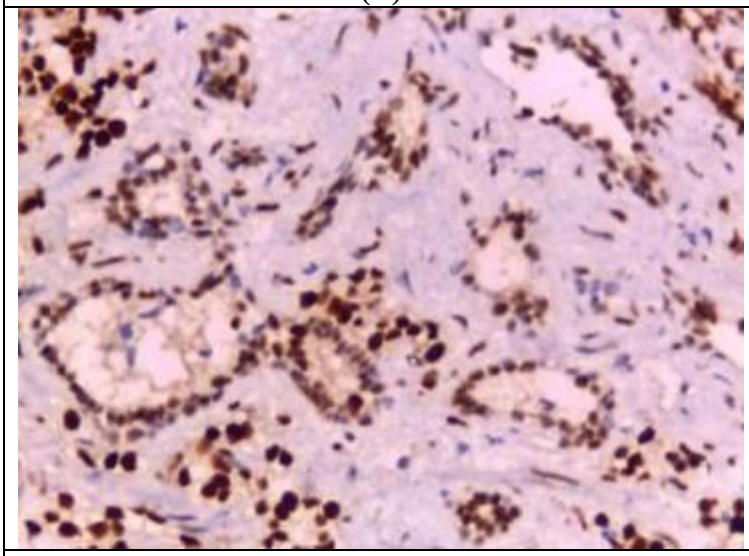

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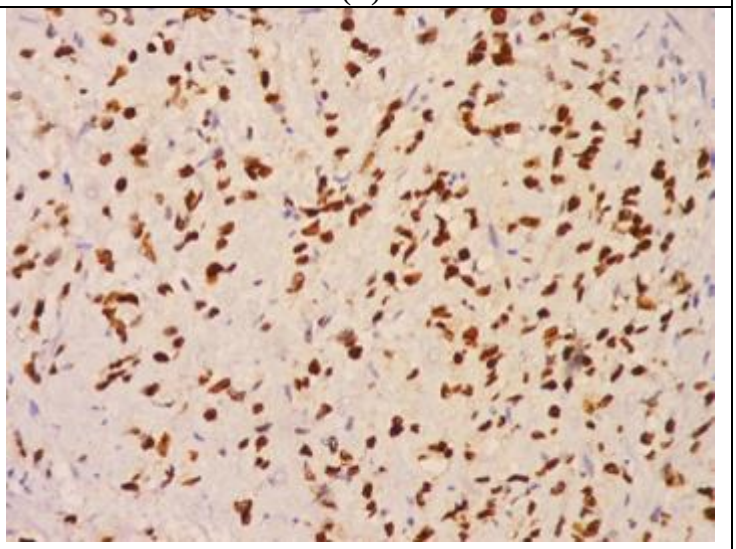


(D)

Fig. (1): Representative samples of Claspin Immunohistochemical expression in prostatic adenocarcinoma: (A) Negative Claspin immunoreactivity. (B) Negative Claspin expression with focal nuclear immunoreactvity in <5%. (C) & (D) Postive Claspin expression with diffuse nuclear immunoreactivity (x 400).

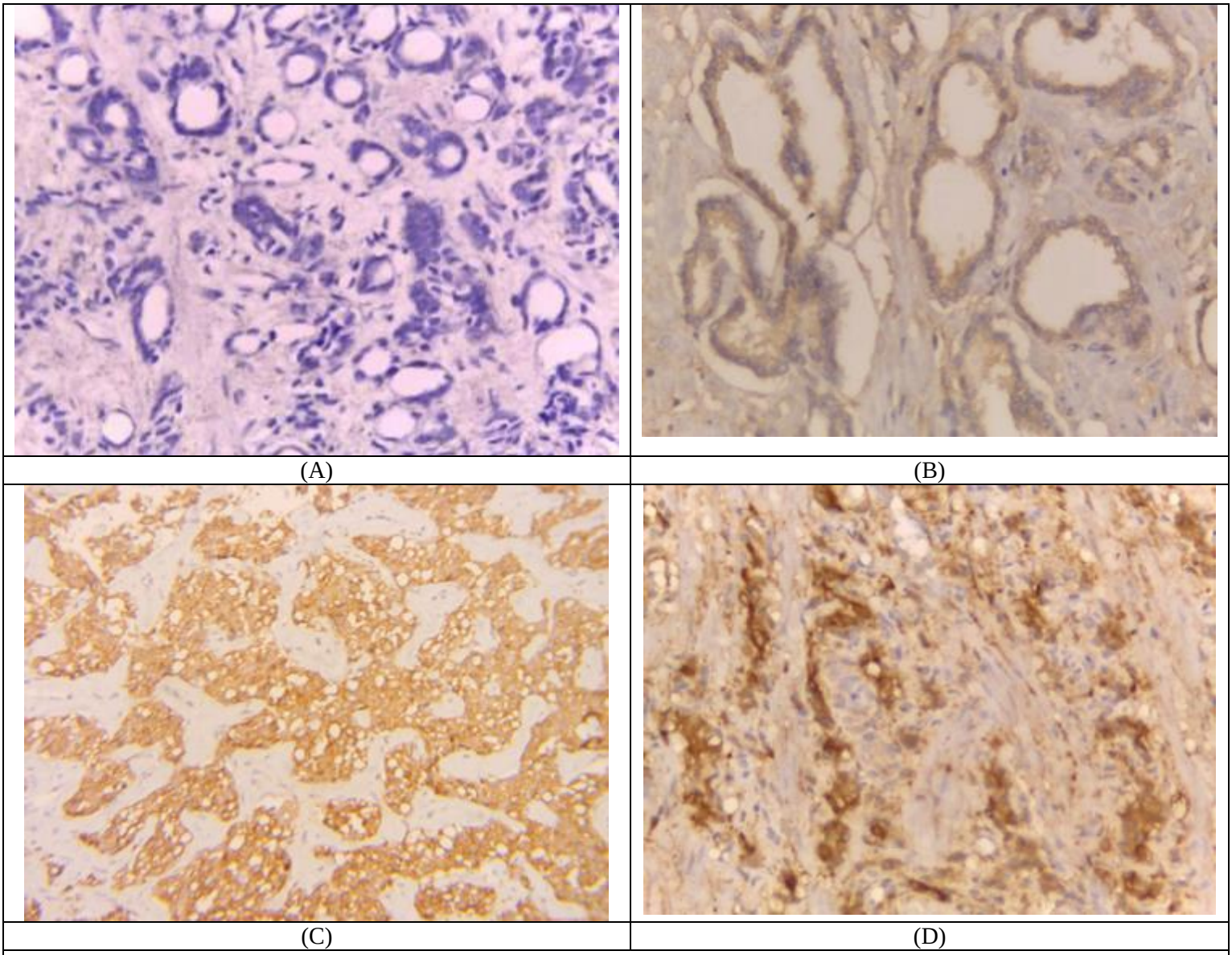


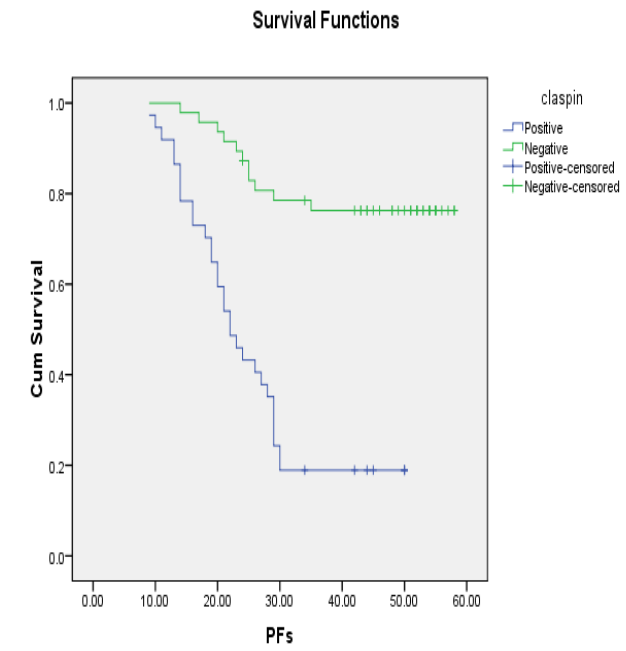
Fig. (2): Representative samples of TopBP1 Immunohistochemical expression in prostatic adenocarcinoma: (A) Negative TopBP1 immunoreactivity.(B) Postive TopBP1 immunoreativity with weak cytoplasmic expression. (C) Postive TopBP1 with moderate cytoplasmic immunoreativity. (D) Postive TopBP1 with strong cytoplasmic immunoreativity (x400).

Association of Claspin and TopBP1 expression with overall survival (Fig 3)

For negative Claspin cases, the survival mean time was 57.3 months, & 49.4 months for positive cases as median not reached. This means that chance of survival is more for negative Claspin cases, with significant statistically difference Log Rank test p-value (0.045). While the survival mean time for low Top BP1 patients was 56.8 months, and 53.8 months for high cases. This meaning that chance of survival is more for low Top BP1 cases, with no statistically significant difference Log Rank test p-value (0.355).

Association of Claspin and TopBP1 expression with progression free survival (Fig 3).

In the period of the follow-up, 48.8% (41/84) of cases presented with diseases progression. Event free survival for negative Claspin patients was 76.6% during follow up with mean time of 49.8 months, and 18.9% for positive cases. Event free survival for low Top BP 1 patients was 86.2% during follow up with estimated mean time of 31.3 months, and 32.7% for high cases. Kaplan–Meier survival curve analysis for Claspin and TopBP1 expression revealed significant association between positive Claspin, higher TopBP1 expression (P <0.001) and shorter progression free survival.



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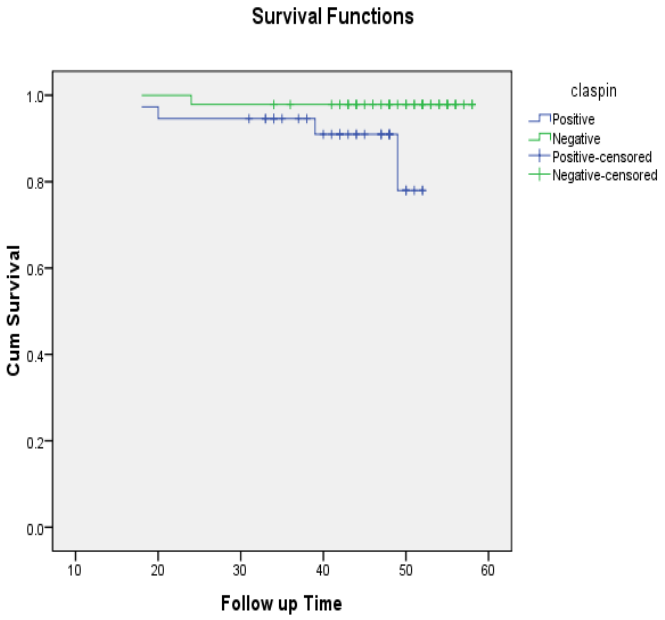

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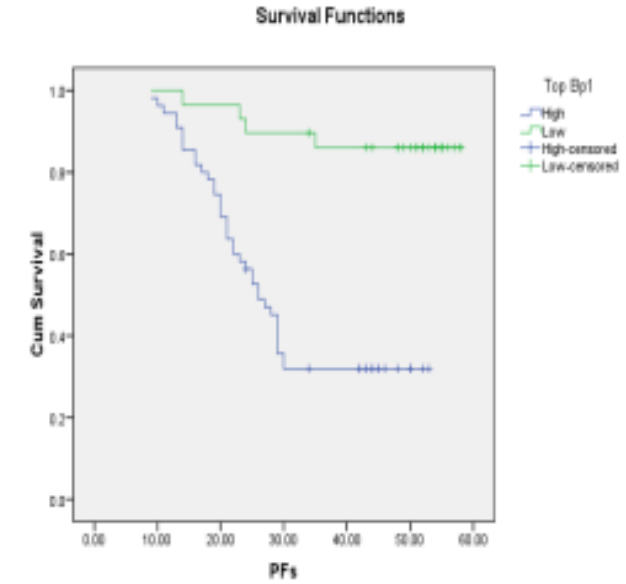

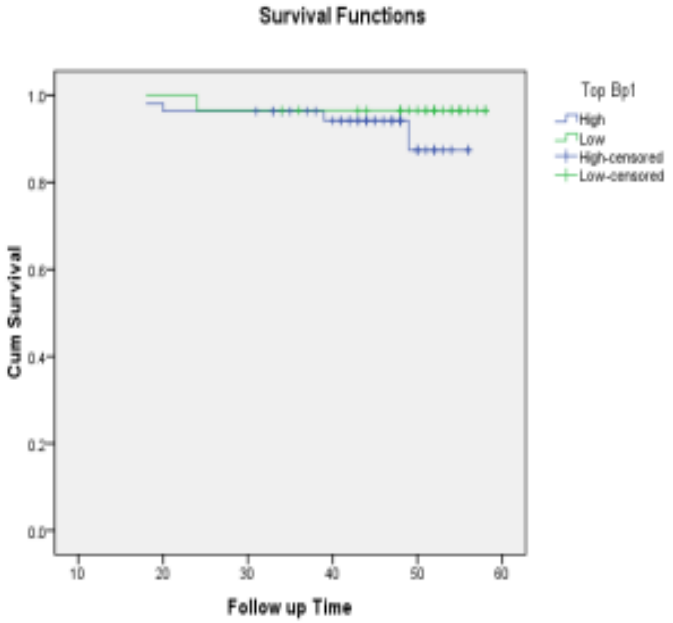

D

Fig. (3): Kaplan Meier Plot, Left Panel for event free survival; Right Panel for overall survival (OS); (A), (B) stratified by claspin IHC staining and (C), (D) stratified by Top BP1 IHC staining.

Predictive value of Claspin and TopBP1 expression for progression free survival in prostate cancer patients (Table 4).

Gleason scoring, Claspin, and TopBP1 all had independent predictive value for progression free survival in univariate and multivariate analysis ($P < 0.05$), while TNM staging had independent predictive value for progression free survival in univariate analysis ($P= 0.006$).

Table (4): Univariate and multivariate analysis Progression Free survival in PCa cases.

Variables	Event free survival	
Univariate	**HR (95% CI)**	**P value**
Age>60	1.85 (0.981-3.21)	0.231
Gleason score>7	2.65 (1.15-3.22)	0.007
Gleason grade (3-5)	2.25 (1.33-3.56)	0.02
Stage (III-IV)	5.25 (2.34-7.55)	0.006
LN metastasis	2.45 (0.95-3.63)	0.112
Distant metstasis	1.45 (0.85-3.12)	0.201
Top BP1(high)	4.63 (2.33-7.56)	<0.001
Claspin (high)	5.21 (3.15-8.25)	<0.001
Multivariate analysis		
Gleason score>7	2.15 (1.22-4.23)	0.02
Top BP1 (high)	3.22 (1.56-5.32)	0.003
Claspin (positive)	3.41 (2.12-5.33)	0.007

DISCUSSION

It has been suggested that improved cancer treatment therapy outcomes may be achieved through DDR targeting in cancer cells [19]. Therefore, most of oncology research have concentrated on responses of checkpoint like DDR, ATR and CHK1 [20].

In our research, the level of IHC expression of Claspin and TopBP1 in PCa was assessed to study their value in the development & pathogenesis, and to identify cases who are prone to develop an aggressive course and therefore require radical therapy and early intervention.

Claspin is a multifunctional crucial protein that are essential for the maintenance of genome stability[5]. Claspin has been constituted as a promising therapeutic target in neoplasms, specifically for chemo and radio- sensitization [20]. In PCa, upregulation of claspin provides docetaxel resistance[19].

In the present work, Claspin upregulation was observed in 44% of the studied PCa. In Babasaki et al study, Claspin upregulation was detected in (31%) of the studied prostate adenocarcinoma cases, they found no or very faint Claspin staining in the adjacent non-malignant prostatic tissue. They also stated that Claspin knockdown reduced cellular proliferation significantly and they detected more Claspin qRT-PCR expression PCa than in normal non neoplastic tissues [19]. Collectively, these results denote that Claspin is implicated in pathogenesis and progression of PCa.

In our research positive Claspin immunoreactivity was significantly correlated with tumor grade & Gleason score consisting with the previous report about its impact on the histological progression and cancer cell differentiation [12, 19]. In a prior in vitro research, deletion of Claspin caused cell cycle S and G2 arrest and slowed PCa cell proliferation. Indicating that Claspin encourages cell proliferation [12].

Analysis of Claspin immunoreactivity detected a significant association of positive reactivity with staging, LN and distant metastases of PCa, but was not associated with age. This is consistent with previous similar studies [12, 19]. The clarification of the positive impact of Claspin expression and disease aggressiveness and metastasis is that higher expression levels of Claspin guards neoplastic cells against oncogene- prompted proliferation stress in a checkpoint-nondependent mode [8]. Knockout of Claspin promotes apoptosis and diminishes cellular replication and invasiveness of PCa [12].

On the other side, Claspin, might have a role as a tumor suppressor factor. A previous study in gastric adenocarcinoma reported that high Claspin protein expression was associated with better outcome through deubiquitinase, USP20 stabilization [11].

The varied actions of Claspin rely on which pathway tumour cells are more reliant on, which may help to explain these conflicting effects. In contrast to its function in DNA replication, Claspin contains DNA repair pathways like repair of nucleotide excision [21], homologous recombination and mismatch repair [22]. Therefore, this dual effect of Claspin in carcinogenesis require further investigation [5].

In the current research, high TopBP1 immunoreactivity was observed among 65.5% of PCa. This is consistent with findings of **Li *et al.*** [15] who noted high TopBP1 in 62% of PCa. Moreover, a significantly positive association was detected between TopBP1 immunoreactivity with Gleason score, grading and staging of the PCa but was not associated with age as previously reported [15, 23].

These findings were highly consistent with previous related research, that found in vitro suppression of TopBP1 expression in PCa cells increases the sub-G1 cell population, indicating a link between TopBP1 and the proliferation of PCa cells [24], in addition, Li *et al.*, reported that high TopBP1 expression encourages the cellular replication of PCa in vitro by inhibiting apoptosis via ATR-CHK1 pathway. Besides, knocking down of TopBP1 markedly decrease migration capability of prostate cancer cells [15]. Overall, these results confirm the crucial role of TopBP1 protein in the promotion of carcinogenesis and progression.

DNA strand breakage permits TopBP1 to encourage AR-Chk1 facilitated checkpoint stimulation and repair of DNA [25]. **Li *et al.*** [15] reported that the key role of TopBP1 in PCa is avoiding damage of DNA, instead of encouraging DNA proliferation via TopBP1-ATR-Chk1 axis. In addition; TopBP1 also inhibit the transcriptional function of p53 through binding its DNA-binding domain (DBD) [26, 27]; supporting that TopBP1 can be a predictor for PCa prognosis [15].

In this study, analysis of Kaplan–Meier was performed in order to show the relationship between prognostic parameters, Claspin and TopBP1

expression with progression free survival in PCa. An association with significant level between initial level of PSA, Gleason scoring, and staging with progression free survival was detected as previously reported [28]. Moreover, early progression was more predominant in patients with Claspin expression and high TopBP1 level.

Regarding Claspin protein expression and progression free survival, a significant association was found. Related research have found that Claspin expression was associated with poor outcome [12, 19]. In research of Babasaki *et al.*, Claspin was approved to be linked with recurrence of high PSA level in PCa [19]. In addition, the level of Claspin mRNA was known as a factor of poor outcome of DFS and progression free survival in GSE21036 and TCGA [12]. Several researchers found that Claspin worsen response to many therapies in ovarian and lung malignancies [29, 30].

In a previous in vitro study, knockdown of Claspin improved phosphorylation of CHK1 and sensitivity of DTX in cellular line of PCa. Claspin may be an essential protein to improve response to chemotherapy based on DTX and may be a hopeful target in PCa therapy [19]. These findings about Claspin may aid in identifying a novel treatment target to slow PC development.

Regarding TopBP1, PCa with high immunoreactivity showed shorter progression free survival and so bad outcome. This data is consistent with Li *et al.*, who documented progression free survival that was better in little than high expression of TopBP1in PCa with a significant level [15]. In addition; **Choi *et al.* [35],** found that the levels of TopBP1 were more in radio-resistant lung cancer cell lines and strongly associated with numerous metastases in the brain and shorter progression-free survival. Moreover, TopBP1 overexpression is associated with a shorter survival in cancer breast [27, 31]. These data suggest that TopBP1 may be a useful measure for predicting the prognosis of PCa because it is an independent predictor of a worse recurrence-free survival.

Abnormality of DDR improve the response to treatment with DNA-damaging agents [32]. In human cancers, heat is one of the most effective ionizing radiation sensitizers. Protein structural changes brought on by hyperthermia have an impact on how the body responds to DNA damage [33]. So, heat stress has been combined with other treatments to improve the clinical outcome [34]. Stimulation of pathway of ATR-Chk1 by hyperthermia is mostly dependent on many cellular promoters including Rad17, n Rad9, Claspin or TopBP1 that are required in heat tolerance. Therefore, ATR or Chk1 selective inhibitors improved apoptosis induced by hyperthermia. They found that knockdown of TopBP1 and Claspin inhibited hyperthermia-induced Chk1 phosphorylation and increased heat cytotoxicity in human HeLa cells[35]. Therefore, understanding the role of TopBP1 and Claspin as Checkpoint adaptors in DDR response will aid in the creation of more effective schemes that are promising in therapy-resistant cancer.

CONCLUSION

Claspin and TopBP1 expression have an important prognostic value in prostate cancer. Using them may help to identify high risk patients who will benefit the most from treatment. Besides, both markers can aid in the creation of improved methods that are useful against the therapy-resistant cancer.

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