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Research Article

Cyclin D1 Immunohistochemical Expression in Sudanese Patients Affected with Prostatic Carcinoma in Khartoum State

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Abstract

Prostatic carcinoma is a common public health problem in aging people. Cyclin D1 proto-oncogene is an important regulator of G1 to S phase progression in many different cell types. It is believed to play an important role in both tumorigenesis and grading of many cancers including prostatic carcinoma; high levels of these proteins have been reported in certain human malignancies and have been implicated in aberrant cell division and dysregulated tumor growth. The aims of this study was to examine the immunohistochemical expression of Cyclin D1 in prostatic carcinoma and to demonstrate the association or relation between Cyclin D1 expressions and to determine the aggressiveness of the malignant tumors by Gleason Score. In this study, 50 samples, 25 cases of prostatic cancer and 25 cases of benign prostatic tissues, were studied for Cyclin D1 expression using an immunohistochemical technique which was performed on routinely processed, formalin-fixed, and paraffin-embedded tissues; the tissues were then sectioned into thickness of (3–5 µm) with rotary microtome instrument, and immunohistochemical expression of Cyclin D1 was evaluated in all cases. All of the primary human prostatic cancer samples revealed in different ranges of intensity from weak (+1), moderate (+2) to strongly positive nuclear staining (+3) for Cyclin D1. In this study, we revealed no nuclear staining in the benign prostatic hyperplasia (PBH) disease (+0) in 21 cases (84%), and 4 cases (16%) were ranged in different color intensity; 3 (12%) were weak (1+) and 1 (4%) was moderate (2+), while prostatic cancer cases were also evaluated in different color intensity; 13 cases (52%) were (+3), 7 (28%) were (+2), 3 were (12%) were (+1), and only two (8%) were negative. There was no significance correlation between Gleason's score and the intensity of Cyclin D1 expression. Conclusively, it can be said that Cyclin D1 may be helpful in the differentiation between BPH and prostate cancer, the correlation between the intensity of Cyclin D1 expression and prostatic diseases was statistically highly significant (p-value = 0.00). The authors recommend to use Cyclin D1 as a tumor marker to prostatic carcinoma.

Keywords: prostatic carcinoma, Cyclin D1, immunohistochemistry

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1. Introduction

Prostate cancer (PC) is currently the most common cause of cancer death in men. The exact cause of developing PC are not known though ageing, ethnicity, and heredity are important factors involved in the initiation and development of this cancer. Ageing is considered the most prominent risk factor, with majority of cases being diagnosed in men between 60 and 70 years of age (Shen and Abate-Shen 2010). Prostate cancer is the most common type of cancer among the Sudanese men. The first National Population-based Cancer Registry (NCR) was established in Sudan in 2009. During 2009–2010, 6771 new cancer cases were registered in Khartoum state. Of those, 3646 (53.8%) cases were of women and 3125 (46.2%) were of men. The most commonly diagnosed cancer among women was breast cancer, followed by leukemia, cervix, and ovary, and among men, it was prostate cancer, followed by leukemia, lymphoma, oral, colorectal, and liver (Saeed et al., 2014). The development of PC occurs through the accumulation of genetic and epigenetic changes, leading to an inactivation of tumor suppressor genes and activation of oncogenes (De Marzo et al., 2007). These alterations most likely take several decades, and cancer development can be considered as a continuous transformation from benign cells, cancer precursors, and malignant cells (Murphy et al., 1994). Cyclin D1 proto-oncogene is an important regulator of G1 to S phase progression in many different cell types. It is believed to play an important role in both tumorigenesis and grading of many cancers including prostatic carcinoma; high levels of these proteins have been reported in certain human malignancies and have been implicated in aberrant cell division and dysregulated tumor growth (He et al., 2007).

2. Aim of the Study

Because the role of Cyclin D1 in PC is unobvious, we studied the expression of Cyclin D1 in prostatic carcinoma and benign prostatic hyperplasia, relationship between Cyclin D1 expression and different Gleason Score.

3. Material and Methods

3.1. Samples

Tissue blocks were obtained from 50 specimen of prostatic tissues, 50% of the cases were previously diagnosed as malignant prostate tissues and 50% were diagnosed as benign tumor. The specimens were obtained from histopathology section, Al-tyseer Medical Centre, during the period of April 2017 to May 2018. Patients' identification information such as their age, histopathology diagnosis, and malignant tumor grade, were obtained from patients files.

3.2. Immunohistochemical techniques

The immunohistochemical procedure was done as follows: one section (3–5 μm) from formalin-fixed, paraffin-embedded tumors were cut and mounted onto adhesion microscope slides (super-frosted slides, pre-cleaned 1.1 mm-thick 25.75 mm made in Citotest Labware Manufacturing Co., Ltd., China). Following deparaffinization in xylene, slides were rehydrated through a graded series of alcohol and were placed in distilled water. Samples were steamed for antigen retrieval for Cyclin D1 using high PH (9) by water bath at 95C for 30 min. After washing with PBS for 3 min Endogenous peroxides activity were blocked with 3% hydrogen peroxide and methanol for 10 min, and after washing with PBS for 3 min the Slides were incubated with 20 μL of Polyclonal antibody (anti-human Cyclin D1 PhosphoThr286 IMMUNOTAG) for 40 min at room temperature in a moisture chamber washed with PBS and adding secondary antibody for 20 min. After washing with PBS for 3 min, binding of antibodies will be detected, followed by adding 3, 3 di amino benzidine tetra hydrochloride (DAB) as a chromogen to produce the characteristic brown stain for the visualization of the antibody/enzyme complex for up to 5 min. After washing with distal water for 3 min, Slides were counterstained with hematoxylin (MAYER'S) for one min and then washed in running tap water for several minutes 7–10 (bluing), then dehydrated and cleaned, mount in DBX. Each slide was evaluated with investigator, and the results were confirmed by the consultant histopathologist.

3.3. Histopathological evaluation of cyclin D1 expression

Cyclin D1 expression was graded on the basis of the intensity of staining within the tumor cells. Nuclear staining was considered positive, and cytoplasmic staining

in tumor of prostatic tissue. The scoring system used for evaluation of Cyclin D1 expression (Raju et al., 2005) was as follows:

grade 0+ Negative (no staining)

grade1+ Weak (nuclear staining of < 10% cells)

grade2+ Moderate (staining of 20–40% cells)

grade 3+ Strong (staining of ≥ 50–40% cells).

3.4. Data management analysis

Statistical analysis was done using SPSS (statistical package for social sciences), version 16.0, computer program. Frequencies mean and Chi-square Test values were calculated.

4. Result

Among the 50 studied cases, 25 (50%) were previously diagnosed as prostatic carcinoma and 25 (50%) were diagnosed as BPH based on hematoxylin and eosin-stained sections.

A total number of 50 cases of prostatic adenocarcinoma removed by radical prostatectomy procedure and benign prostatic hyperplasia were studied. The age of the patients ranged between 40 and 100 years with mean age of 65 years. Most patients with prostatic carcinoma, that is, 39 (78%) of them, were between the age of 61 and 80 years, 6 (12%) were between 40 and 60 years, and 5 (10%) were between 81 and 100, as indicated in Table 1.

Table 1: Risk age group.

Age group	Frequency	%
40–60	6	12
61–80	39	78
81–100	5	10
Total	50	100%

The Gleason scoring of the studied prostatic carcinoma cases ranged between 2 and 10. The most prevalent score in the studied cases was Gleason score 4 (24%) as showed in Table 2.

Table 2: Frequency of prostatic carcinoma cases according to Gleason score.

Gleason Score	Frequency	%
3/10	4	16.0
2/10	2	8.0
4/10	6	24.0
6/10	3	12.0
8/10	5	20.0
10/10	3	12.0
9/10	1	4.0
7/10	1	4.0
Total	25	100%

The Gleason score of the studied prostatic carcinoma cases ranged between 2–5 and 6–10 (Table 3). The most prevalent score in the studied cases was Gleason score 6–10 (52%).

Table 3: Gleason score group.

Gleason Score	Percentage
2–5	48.0
6–10	52.0

The statistically significant difference in Cyclin D1 expression among prostatic adenocarcinoma, BPH, and BPH+ chronic prostatitis cases with higher expression in carcinoma cases as shown in Table 4, Pearson chi-square, value = 36.6, df = 6, and *p*-value = 0.00 (highly significant).

Table 4: Cyclin D1 expression among prostatic adenocarcinoma, BPH, and BPH+ chronic prostatitis cases.

Diagnosis		Intensity of Cyclin D1 staining				Total
		Negative	Weak	Moderate	Strong	
Type	Prostatic adenocarcinoma	2	3	7	13	25
	BPH	10	0	0	0	10
	BPH+ chronic prostatitis	11	3	1	0	15
Total		23	6	8	13	50

Cyclin D1 expression in 25 cases, in prostate adenocarcinoma and according to intensity of Cyclin D1 staining, Strong 3+, moderate 2+, weak 1+, and negative 0, and highest grade 3+ as shown in Table 5.

Table 5: Frequency of Cyclin D1 staining intensity in prostatic carcinoma.

Grade	Frequency	%
+3	13	52.0
2+	7	28.0
1+	3	12.0
0	2	8.0
Total	25	100

There was a negative significant correlation between Gleason score and staining intensity for Cyclin D1 (–0.135) and p.v no significant association (0.519), as shown in Table 6.

Table 6: Correlation between intensity of cyclin D1 expression and Gleason score.

		Gleason score	Intensity Cyclin D1 expression
Gleason score	Pearson Correlation	1	–0.135
	Sig. (2-tailed)		0.519
intensity of Cyclin D1 expression	Pearson Correlation	–0.135	1
	Sig. (2-tailed)	0.519	

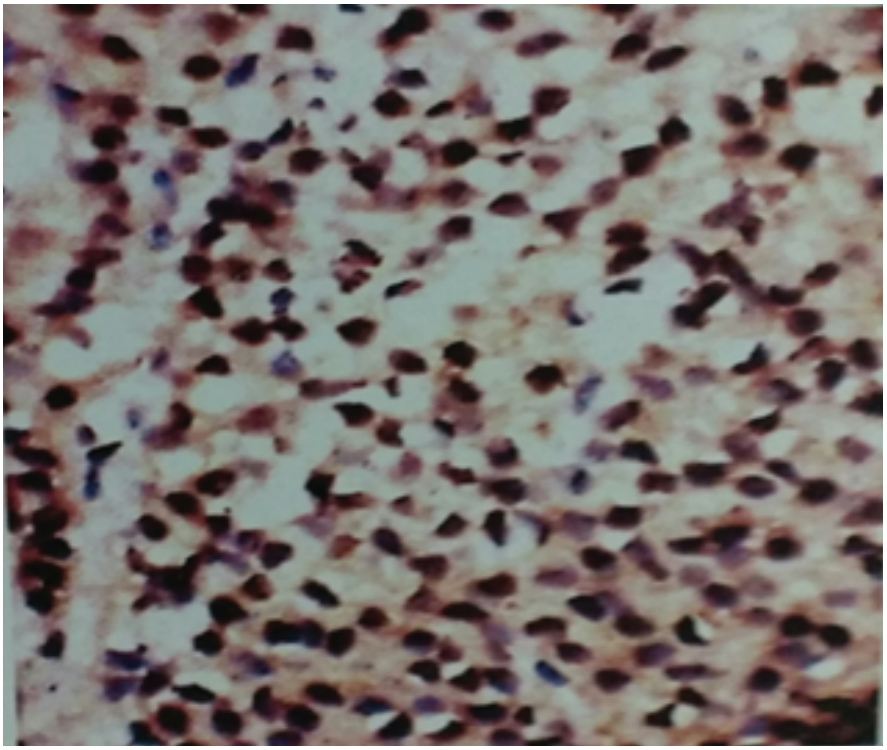

Figure 1: Clear cell carcinoma, showing strong nuclear cyclin D1 staining, grade 3+ (cyclin D1 Olympus digital camera).

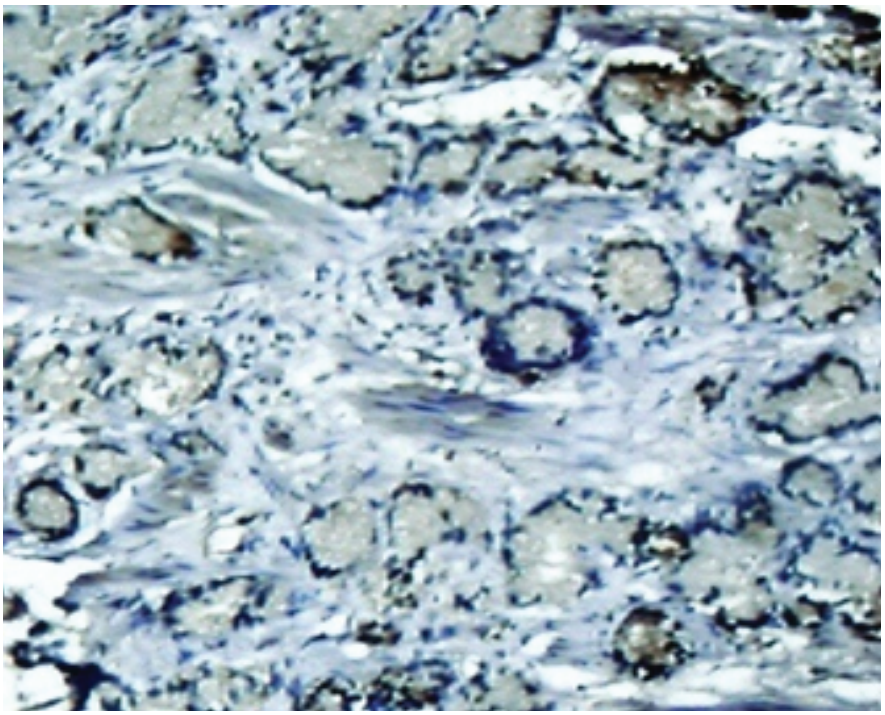

Figure 2: Prostatic adenocarcinoma with rounded glands showing strong nuclear Cyclin D1 staining, grade 3+.

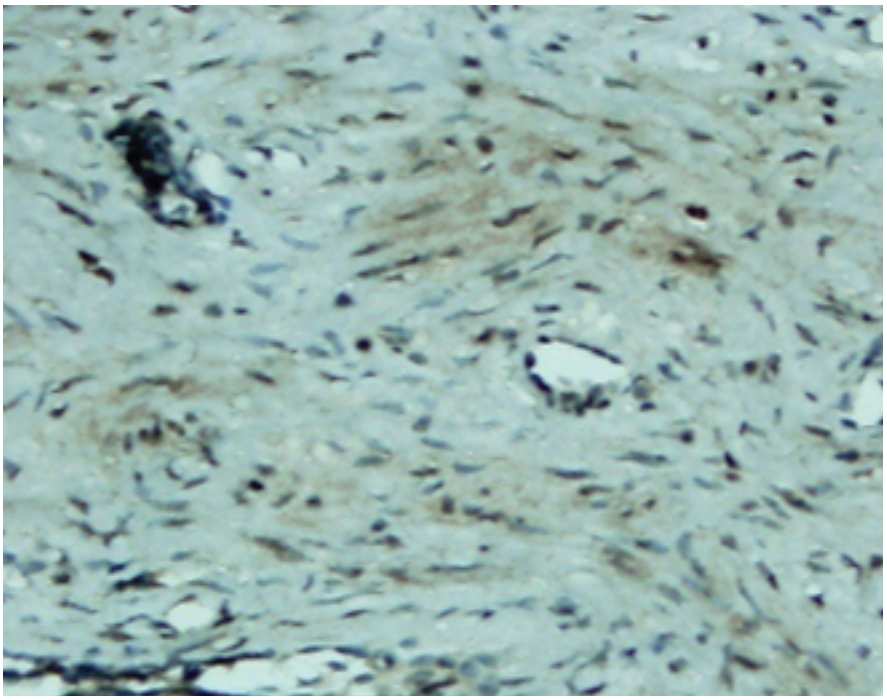

Figure 3: Prostatic adenocarcinoma, showing moderate nuclear Cyclin D1 staining, grade 2+.

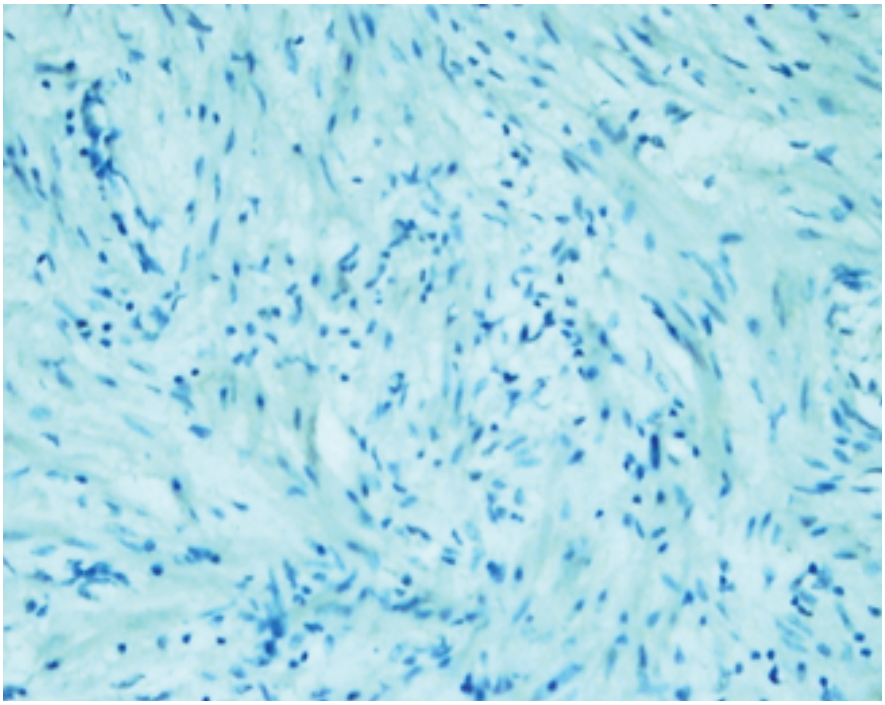

Figure 4: Cyclin D1 staining in benign prostate tissue, showing negative staining of the benign prostatic tissue and the absence of staning in the nucleus grade 0+.

5. Discussion

Prostate cancer is a major public health problem in countries with aging population, and is now the most commonly diagnosed cancer in men as well as the second leading cause of male cancer deaths that occurs in 1 out of 6 men (Ferlay et al., 2008). Many studies have revealed the association of PC with certain abnormalities as Cyclin D1 proto-oncogene, which is an important regulator of G1 to S phase progression in many different cell types. It is believed to play an important role in both tumorigenesis and grading of many cancers including prostatic carcinoma (He et al., 2007). Previous studies have reported regarding the role of Cyclin D1 expression in PC, however, in the present study, all of the primary human PC samples revealed different ranges according to the regions of staining intensity from weak, moderate to strongly positive nuclear Cyclin D1 staining in 23 out of 25 cases (92%). This study is in contrary to those observed by Samia et al. who found that in PC, the Cyclin D1 was expressed in 23 of 25 (92%) cases (Samia et al., 2017). The results obtained in the present study was almost in agreement with the following studies: Ueda et al. in 2001 found that 84.6% of cases of prostatic carcinoma showed Cyclin D1 expression. These results indicated that increased expression of Cyclin D1 in malignant prostate tissue (Ueda et al, 2001) and also Alqahtani et al. in 2015 found that 90% of PC revealed positivity of Cyclin D1.

The correlation between Cyclin D1 and Gleason grades was not significant (Alqahtani et al., 2015). Gupta et al. reported that Cyclin D1 expression was seen with higher frequency in prostatic carcinoma and also observed focally and weak staining may be seen in benign cases but that had never reached a significant proportion as seen in carcinoma of the prostate. However, the study observed no significant correlation of Cyclin D1 expression with Gleason grade (Gupta et al., 2014). Chen et al. notice that overexpression of Cyclin D1 increases cell growth and tumorigenicity in human PC (Chen et al., 2004). Hosni et al., in 2013, found that all cases (100%) revealed foci (> 10% of cancer cells) with positive nuclear staining for Cyclin D1 with different grades ranging from moderate to strong, no significant correlation was found between the intensity of Cyclin D1 expression and Gleason's score (Hosni et al., 2013). ÖZBEK et al. (2000) reported more expression of Cyclin D1 in PC samples, but our study was different from previous study reported by Shiraishi et al. (1998), who noticed positive nuclear Cyclin D1 expression in 20 of 66 (30%) studied cases (Shiraishi et al., 1998), and in same contrast, Kallakury et al. in 1997 found Cyclin D1 positivity in 31 of 140 PC (22%) of studied cases and also different from that reported by Kallakury et al., 1997, and Han et al. in 1998 reported moderate to strongly positive staining for Cyclin D1 in 12 of the 50 primary PC samples (24%) (Han et al., 1998). We can't suggest a cause for this obvious discrepancy, other than difference in the diagnostic approaches. In the present study, we also observed no significant correlation between Cyclin D1 expression and Gleason score as shown in Table 6); so, our findings were quite similar to the results observed by Samia et al. (2017); Drobniak et al. (2000); Hosni et al. (2013); Fleischmann et al. (2011), and Nagela et al. (2016) and contrary to that of Comstock et al. (2007), who reported significant correlation between Cyclin D1 and Gleason score. In this study, BPH revealed no staining in the epithelial cells (84%), but there was low staining in benign prostatic hyperplasia with prostatitis to Cyclin D1 staining was congruent to study reported by Alqahtani et al. (2015), who reported that 84% of PBH showed negativity to Cyclin D1 and Samia et al. reported that in BPH group, only three cases (20%) showed positive nuclear Cyclin D1 expression (Samia et al. 2017), and also Comstock et al. (2007). But this study disagrees with Ueda et al. (2001) who found that 53.8% BPH cases were positive to Cyclin D1. In the present study, we also observed 1 case (4%) of cytoplasmic positive out of 25 cases; we disagree with Comstock et al. in 2007 who reported that most tumors showed cytoplasmic restriction of Cyclin D1, and Gupta et al. in 2014 found that carcinoma cases; 24 cases on the other hand showed both nuclear and cytoplasmic positivity, whereas 2 cases showed cytoplasmic and 4 cases showed nuclear positivity only to Cyclin D1 staining and another only nuclear Cyclin D1 staining.

6. Conclusion

Conclusively, we found that most Cyclin D1 expression in prostate carcinoma were 93% of cases, while in benign prostatic hyperplasia was 16%, and the correlation between Cyclin D1 and Gleason score showed negative significant.

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