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Immunohistochemical Expression of Cyclin D1 in Egyptian Patients with Prostatic Carcinoma

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Abstract: Prostatic carcinoma is a common and growing public health problem. Cyclin D1 is a cell regulatory protein, which is believed to play an important role in both tumorgenesis and grading of many cancers. The role of Cyclin D1 as a prognostic factor in cancer prostate is controversial. The present study was done on a total of forty cases of prostatic carcinoma removed by radical prostatectomy. Immunohistochemical expression of Cyclin D1 was evaluated in all cases. Correlation between the intensity of Cyclin D1 expression and patient's age, serum PSA level, PIN, Gleason grades, Gleason scores and stages of prostatic carcinoma was evaluated. All cases (100%) revealed foci (>10 % of cancer cells) with positive nuclear staining for Cyclin D1 with different grades of intensity ranging from moderate to strong, while positive Cyclin D1 expression was observed in the nuclei of PIN of 30 cases with grades of intensity ranging from weak to strong. No significant correlation was found between the intensity of Cyclin D1 expression and patient's age, PIN, Gleason grades, Gleason scores or stages of prostatic carcinoma, while a significant correlation between intensity of expression of Cyclin D1 and preoperative serum PSA level was observed. Cyclin D1 expression might affect PSA expression, which is considered an important tumor marker. Cyclin D1 plays an important role in the pathogenesis and evolution of prostate cancer rather than the prognosis, thus Cyclin D 1 is not a reliable prognostic factor in cancer prostate.

Key words: Immunohistochemical · Cyclin D1-Egyptian · Prostatic carcinoma

INTRODUCTION

Prostate cancer is a common and growing public health problem. The etiology of this cancer is not fully understood [1]. Prostate cancer occurs when cells of the prostate mutate and begin to grow out of control. Prognostic criteria currently in use cannot fully predict tumor behavior. The search for better prognostic markers is now focused on the molecular mechanisms such as altered cell cycle progression, apoptosis, neuroendocrine differentiation and angiogenesis, which underlay tumor behavior [2]. Cyclin D_1, a cell regulatory protein, considered a product of Cyclin D_1 protoncogene is an important regulator of G_1 to S-phase transition of the cell cycle. It is believed to play an important role in both tumorgenesis and grading of many cancers including prostatic carcinoma if its expression is deregulated, mainly over expressed [3]. Despite the influence of D-type Cyclins on prostate cancer proliferation, few studies have examined the expression of Cyclin D_1 in localized tumors or challenged its relevance to disease progression [4]. Moreover, the variation in the results of previous researches that studied the relationship between Cyclin D_1 and prostatic carcinoma was both variable and valuable. No correlation was found between Cyclin D_1 overexpression and either Gleason score, neoadjuvant hormone treatment or prostatic-specific antigen [5]. Overexpression of Cyclin D_1 rarely occurs in human prostate tumors and when it does it may identify a subset of tumor with a different molecular biology [6]. There was a relationship between Gleason grade and staining for Cyclin D_1 [7]. Cyclin D_1 expression levels are elevated in malignant human prostatic epithelial cell lines and its overexpression in benign prostatic hyperplasia cells can increase cell proliferation rate, migration and invasive ability [3]. The relevance of altered Cyclin D_1 status was observed; differential Cyclin D_1 status may influence clinico-pathological parameters and reveal new insight as to the regulation and potential consequence of Cyclin D_1 expression in prostate cancer. Tumors with predominantly cytoplasmic Cyclin D_1 showed the lowest ki-67 index, whereas nuclear Cyclin D_1 was associated with higher

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grade and elevated ki-67 [4]. The increased expression of Cyclin D1 in prostate cancer samples suggests that further studies on the expression of this gene may be of interest in understanding the pathogenesis of prostate cancer, moreover the positive correlation between Gleason grade and protein expression may be used as a prognostic marker in prostate cancer [7].

The aim of this study is to investigate the immunohistochemical expression of Cyclin D1 in prostatic carcinoma in Egyptian patients and to find the relationship between Cyclin D1 expression and clinical data (e.g. age and serum PSA level), PIN, histopathological features, different Gleason grades and stages of prostatic carcinoma. Also to prove or disprove an association or relation between Cyclin D1 expression and different Gleason grades and stages of prostatic carcinoma, thus allowing the use of Cyclin D1 as a prognostic marker for prostatic carcinoma.

MATERIALS AND METHODS

Case Selection: A total of forty cases of radical prostatectomy specimens were obtained from the Department of Pathology, Faculty of Medicine, Cairo University, Egypt and from other private laboratories, in the period from September 2008 up to May 2009. For all cases, some clinical data were available on the computer files including age of the patient which ranged from 52 years up to 76 years with a median age of 65 years and preoperative serum PSA which was available in 34 out of 40 cases and it ranged from 7ng/dl up to 42.2ng/dl with a mean 20ng/dl.

Histopathological Evaluation: Two serial sections were prepared from each tissue block and were cut at 5 microns thickness. One section was stained by Hematoxyline and Eosin (H&E), for histopathological evaluation and the other was m ounted on poly-L-lysine-coated slides (super frost slides) for Cyclin D1 immunohistochemical staining. Slides were examined for lesions of prostatic carcinoma and lesions of high grade PIN. Benign glandular lesions were also identified. Each case was graded according to the Gleason grading system [8] and cases were distributed according to their Gleason score into two groups (≤score 6) or (>score 6) and staged according to the AJCC-UICC TNM classification system. Cases were distributed according to their pathological stage into two groups; organ confined (≤T2) or extension outside capsule (>T2) [9].

Immunohistochemical Staining: Immunohistochemical staining was performed on routinely processed, formalin-fixed, paraffin-embedded tissue. Tissue sections were cut at 5 microns and mounted on poly-L-lysine-coated slides (super frost slides).

Evaluation of Imunohistochemisty: Cyclin D1 expression was graded on the basis of the intensity of staining within the tumor cells. Only nuclear staining was considered to be positive expression and isolated cytoplasmic staining whether in tumor tissue or few foci of benign prostatic tissue found adjacent to tumor was ignored [7]. The intensity of nuclear staining was graded from 0 to 3 as follows [10]:

- Grade 0: Absence of staining.
- Grade 1: Faint nuclear staining (requiring high power assessment).
- Grade 2: Moderate nuclear staining (easily appreciated at low power).
- Grade 3: Staining for intense nuclear staining.

Statistical Analysis: Statistical analysis was done to evaluate the significance of association between different grades of intensity of Cyclin D1 expression and other clinicopahtological values using the Chi square test of independence and the Fisher exact test. Correlation between Cyclin D1and other clinicopahtological values was done using Spearman's rho test. T test was used to estimate difference in quantitative variables. For quantitative variable, mean and median (as a measure of central tendency), standard deviation minimum and maximum (as a measure of variability) were estimated. Frequency and percentage were presented for qualitative variables. Computer software Statistical Package for the Social science (SPSS) version 15 was used in the analysis. A p-value of <0.05 was considered statistically significant.

RESULTS

A total number of forty cases of prostatic adenocarcinoma removed by radical prostatectomy procedure were studied. The age of the patients ranged from 52 years up to 76 years with a median age 65 years. The highest frequency of cases (60%) occurs between 60-69 years. Cases were statistically distributed according to their age into two groups (≤65 years) or (>65 years), age of the patients below 65 years constitutes (55%), while (45%) o f the studied cases above the age of

65 years. The preoperative serum PSA was available in 34 out of 40 cases and it ranged from 7ng/dl up to 42.2ng/dl with a mean 20ng/dl. Cases were statistically distributed according to their preoperative serum PSA level into two groups (≤20ng/dl) or (>20ng/dl), cases with serum PSA level (≤ 20ng/dl) constitutes (55.9%) of all cases while (44.1%) of all cases >20ng/dl. The forty cases revealed the conventional type of prostatic adenocarcinoma, with one case with Gleason score 7 showing focal hypernephroid patterns.

The presence of PIN was estimated and was found adjacent to invasive cancer in 37 out of 40 cases. All foci of PIN found were high grade PIN (HGPIN). Distribution of PIN in relation to the Gleason score of adjacent cancer was estimated as shown in Table 1. The highest frequency of cases with PIN (55%) occurs in Gleason score 7.

The Gleason grade was estimated in all cases taking the primary grade (most prevalent) into consideration which ranged from Gleason grade 3 up to Gleason grade 5. The most prevalent grade in the studied cases was Gleason grade 3 (67.5%). The Gleason score ranged from 6 up to 9 and cases were grouped according to their Gleason score. The most prevalent score in the studied cases was Gleason score 7 which constitutes (60%). Gleason score 7 (3+4) constituted (54.2%), while (45.8%) of the studied cases were Gleason score (4+3) as shown in Table 2.

According to the Gleason score, cases were distributed into two groups, most of the studied cases (67.5 %) had Gleason score more than 6 (poorly differentiated). The most prevalent stage in the studied cases was stage T2cN0 (32.5%). Lymph nodes were excised bilaterally in 32 cases with positive metastases in two cases having lymph node stage N2. Both cases show Gleason score (4+3 = 7) and tumor stage T3b in one case and T4a in the other case. According to pathological stage, cases were distributed into two groups, half of the cases (50%) were organ confined (≤T2), while the other half were extended through the capsule (>T2).

Estimation of Cyclin D1 Expression: All cases (100%) revealed foci (>10 % of cancer cells) with positive nuclear staining for Cyclin D1 with different grades of intensity ranging from moderate (grade 2) to strong (grade 3). Twenty three (57%) cases were grade 3 cyclin D1 intensity as shown in Table 3.

Normal prostatic tissue found adjacent to cancer in few cases revealed absent Cyclin D1 expression. Cyclin D1 expression was noticed only in the nuclei of ganglia present adjacent to the capsule. Estimation of Cyclin D1 expression in foci of PIN found in 37 (92.5%) out of 40 cases, 30 cases (75%) which showed positive Cyclin D1 expression with grades of intensity ranging from weak (Grade 1) up to strong (Grade 3), while 7 cases (18.9%) did not take the stain Most of the foci of PIN (29.7%) had G1 intensity of Cyclin D1 as shown in Table 4.

Grade 3 cyclin D1 intensity constitutes (59.1%) of cases (≤65 years), while (55.9 %) of grade 3 intensity occurs in age group above 65 years. According to results of Chi square test, there was no significant relation between Cyclin D1 and age (P value = 0.822). Statistical

Table 1: Distribution of cases with PIN in relation to Gleason score of adjacent cancer.

PIN		Number of cases	Percentage (%)
Absent	3	7.5	
Present	S6	13	32.5
	S7	22	55.0
	S8	0	0.0
	S9	2	5.0
Total	40	100.0	

Table 2: Distribution of cases with Gleason score 7.

Gleason score 7	Number of cases	Percentage (%)
3 + 4	13	54.2
4 + 3	11	45.8
Total	24	100

Table 3: Distribution of intensity of Cyclin D1 expression among studied cases.

Intensity of Cyclin D1	Number of cases	Percentage (%)
Grade 2	17	42.5
Grade 3	23	57.5
Total	40	100.0

Table 4: Cyclin D1 expression in foci of PIN.

	Cyclin D1 expression									
	G0		G1		G2		G3		Total	
PIN	No.	%	No.	%	No.	%	No.	%	No.	%
	7	18.9	11	29.7	14	37.8	5	13.6	37	100

Table 5: Relation between intensity of Cyclin D1 expression and Gleason score.

	Cyclin D1expression					
Total Gleason score	G2		G3		Total	
	No.	%	No	%	No	%
≤6	6	46.2	7	53.8	13	100
>6	11	40.7	16	59.3	27	100
Total	17	42.5	23	57.5	40	100

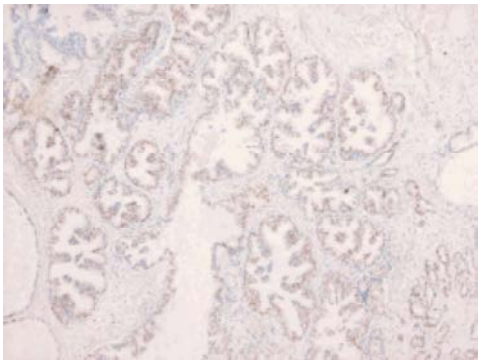

Fig. 1: Prostatic adenocarcinoma pattern 3 & adjacent PIN, both showing moderate nuclear Cyclin D1 staining, grade 2. (Cyclin D1 x 100)

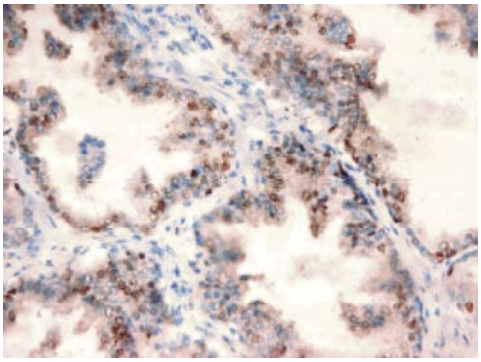

Fig. 2: PIN showing strong nuclear Cyclin D1 staining, grade 3. (Cyclin D1 x 400)

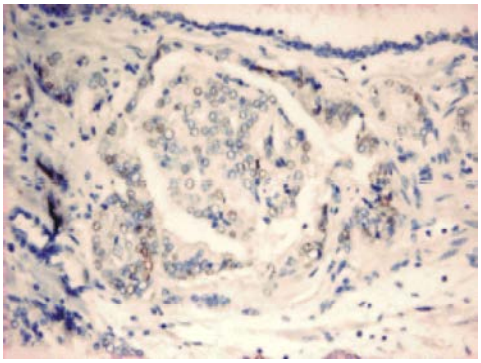

Fig. 3: Prostatic adenocarcinoma pattern 4 with glomeruloid feature showing moderate nuclear Cyclin D1 staining, grade 2. (Cyclin D1 x 400)

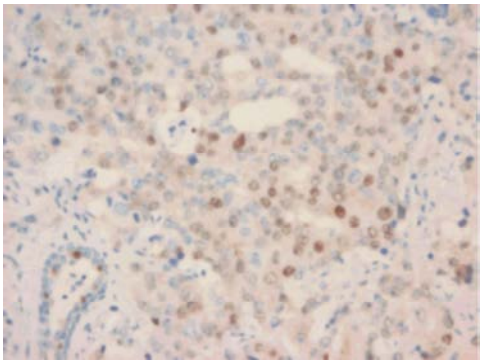

Fig. 4: Prostatic adenocarcinoma pattern 4 with fused acini showing moderate nuclear Cyclin D1 staining, grade 2. (Cyclin D1 x 400)

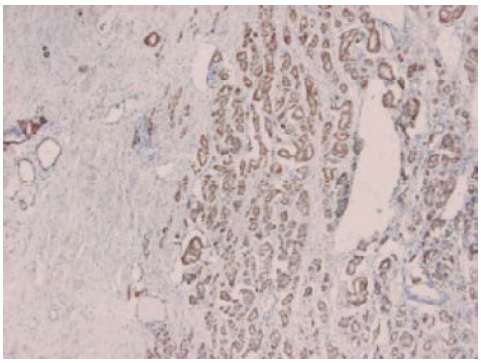

Fig. 5: Prostatic adenocarcinoma pattern 3 with rounded and angulated glands showing strong nuclear Cyclin D1 staining, grade 3. (Cyclin D1 x 100)

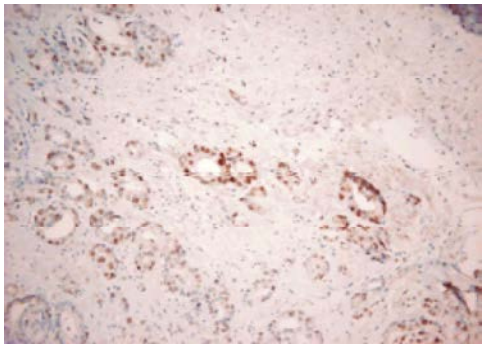

Fig. 6: Prostatic adenocarcinoma pattern 3 with rounded and angulated glands showing strong nuclear Cyclin D1 staining, grade 3. (Cyclin D1 x 200)

analysis using Spearman's rho test revealed that there was no correlation between intensity of Cyclin D1 expression and age (P value =0.89). According to the results of T test, there was a significant statistical difference between G2 (mean 23.7) and G3 (mean 18.4) according to serum PSA level. Cases with Gleason score >6 were (59.3%) of grade 3 cyclin D1 intensity. According to results of Chi square test, there was no significant relation between intensity of Cyclin D1 and Gleason Score (P value =0.746) as shown in Table 5.

As regard to the relation between the intensity of Cyclin D1 expression and pathological stage, forty five percent (45%) of the studied cases with grade 2 Cyclin D1 intensity were confined to the organ, while (60%) of cases

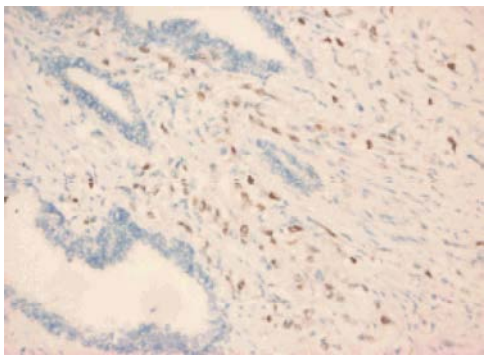

Fig. 7: Strong nuclear Cyclin D1 staining (grade 3) in pattern 4 (signet cell ring feature) + negative nuclear staining (grade 0) in adjacent PIN. (Cyclin D1 x 400).

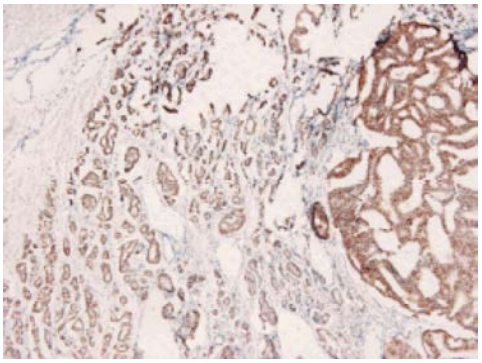

Fig. 8: Strong nuclear Cyclin D1 staining (grade 3) in both pattern 4 (cribriform pattern) & adjacent pattern 3 (rounded and angulated glands) prostatic adenocarcinoma. (Cyclin D1 x 100).

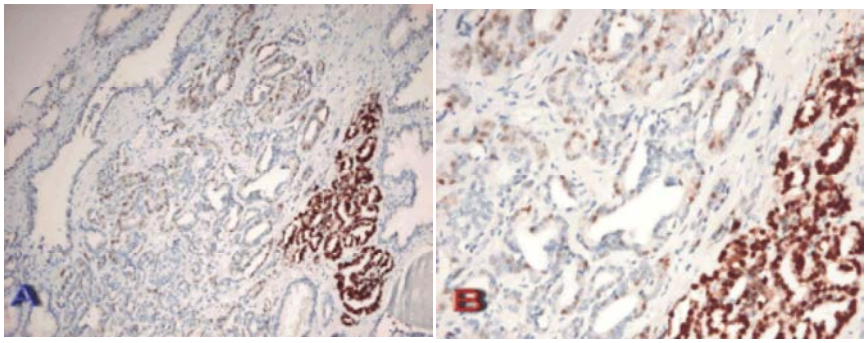

Fig. 9: Prostatic adenocarcinoma pattern 4 with fused acini showing strong nuclear Cyclin D1 staining (grade 3) + adjacent pattern 3 with rounded and angulated glands showing moderate nuclear Cyclin D1 staining (grade 2). A) (Cyclin D1 x 200) & B) (Cyclin D1 x 400)

with grade 3 Cyclin D1 intensity were extended through the capsule. According to of Chi square test, there was no significant relation between intensity of Cyclin D1 and Pathological stage (P value = 0.749).

DISCUSSION

Prostatic carcinoma is a common and growing health problem. It is the third most common male cancer after malignancies of lung and stomach [11, 12]. It represents about 50% of male genital tract malignancies in Egypt [13]. Recognition of prognostic factors that can separate potentially life threatening tumors from less aggressive ones would allow for more disease specific selection and timing of therapeutic options [9]. Cyclins are involved in the regulation of cell cycle progression. Previous studies revealed that overexpression of Cyclin D1 is known to shorten the G1-S transition and thus, it promotes cell progression and differentiation [7]. Chen *et al.* [14] reported that overexpression of Cyclin D1 increases cell growth and tumorgenecity in human prostate cancer LNCaP cells. It has been previously shown through *in vitro* studies that Cyclin D1 can influence androgen dependant prostate cancer cell proliferation through its dual ability to modulate both CDK4 and androgen receptor activity [15, 16].

Few studies have addressed Cyclin D1 expression or localization in primary prostatic adenocarcinomas and the criteria used to establish positive Cyclin D1 staining have been divergent [4]. Chang *et al.* [17] proved that Cyclin D1 has a prognostic significance in early glottic cancer. This is in agreement with the findings of Bilalovic *et al.* [18], which proved the role of Cyclin D1 in invasive breast cancer and Yang *et al.* [19], who proved the role of Cyclin D1 in prognosis of urothelial carcinoma. The prognostic role of Cyclin D1 in prostate cancer has been studied in few researches and its prognostic role in this kind of tumors has to be more investigated. In the present study we examined whether the expression of Cyclin D1 correlated with the prognosis of prostatic carcinoma

through studying the correlation and significance of association between the intensity of Cyclin D1 expression and different prognostic clinicopathological factors as age, preoperative serum PSA, Gleason grade, Gleason score and pathological stage of the disease.

In the present work, 40 cases of prostatic adenocarcinoma removed by radical prostatectomy procedure; Cyclin D1 cytoplasmic expression either in cancer cells or few foci of adjacent normal prostatic tissue was very few and thus ignored and only nuclear staining was taken into consideration. This finding goes with Kallakury *et al.* [9], Ozbek *et al.* [7] and Drobnjak *et al.* [5] who considered only nuclear Cyclin D1 staining, but it was in contrast with Comstock *et al.* [4], who reported differential cytoplasmic and nuclear staining. This might be explained by the use of different antibodies and techniques of antigen retrieval used in this study. There was no nuclear staining for Cyclin D1 in the epithelial cells of the few foci of benign prostatic tissue seen adjacent to invasive cancer or PIN, which was used as a control, nuclear staining was observed in ganglia of adjacent capsular tissue in one case. These results were very close to Ozbek *et al.* [7] who noticed absent nuclear staining in adjacent normal prostatic tissue. In the present study, all cases (100%) revealed foci (>10 % of cancer cells) with positive nuclear staining for Cyclin D1 with different grades of intensity ranging from moderate (Grade 2) to strong (Grade 3).These results were in compatible with Ozbek *et al.* [7], who reported positive nuclear Cyclin D1 expression in all the studied cases (100%), but they were in contrast with Kallakury *et al.* [9] who noticed positive nuclear Cyclin D1 expression in 22% of the studied cases and Drobnjak *et al.* [5] who noticed Cyclin D1 expression in 11% of the studied cases. More advanced techniques and methods used for detection of Cyclin D1 in this study might explain a better chance of detecting Cyclin D1 positive cells.

Regarding foci of PIN detected in 37 (92.5%) out of 40 cases, 30 cases (75%) showed positive Cyclin D1 expression in the nuclei of PIN cells, with grades of intensity ranging from weak (Grade 1) up to strong (Grade 3), while 7 cases (18.9%) did not take the stain. No previous studies had investigated the expression of Cyclin D1 in foci of PIN, that is why in this study we assumed that the chance of finding different grades of intensity in the nuclei of PIN including weak, or even its complete absence in some foci, might indicate that Cyclin D1 is likely to be expressed more in the foci of invasive cancer. This might be explained by the fact that Cyclin D1 might act as an activated oncogene and thus playing a role in the pathogenesis of cancer development, but not in very early stages of cancer. In this study, no significant association or correlation was found between the intensity of Cyclin D1 expression and other clinicopathological factors as age of the patients ($P = 0.822$). Although more intense Cyclin D1 positivity "G3" was observed with high Gleason grade, poorly differentiated carcinoma with score >6 and advanced pathological stage as in tumors extending to the capsule, yet Cyclin D1 positivity did not show statistical significance and did not correlate with the previous prognostic variables. These results are in agreement with those obtained by Comstock *et al.* [4], Drobnjak *et al.* [5] Kallakury *et al.* [9] and Shiraishi *et al.* [20],but they were in contrast with Ozbek *et al.* [7] who observed a positive correlation between Gleason grade and staining intensity of Cyclin D1. Larger sample size, variable grades, scores and pathological stages of cancer used in this study as well as different methods of immunohistochemical assessment might explain the contradictory results. The results of our study are in harmony with comparative studies which investigated the relationship between Cyclin intensity of expression and clinicopathological features done on other tissues as gastric carcinoma [16], endometrial carcinoma [17], breast cancer and laryngeal squamous cell carcinoma [18] who all noticed absence of significant correlation between Cyclin D1 and other clinicopathological factors. Our results are also very close to those observed by Yang *et al.* [19], who found no correlation between Cyclin D1 and grade of urothelial carcinoma.

In some other tumors like laryngeal squamous cell carcinoma [21, 22], lung and colorectal cancer [23], Cyclin D1 expression correlated with a worse outcome and a positive correlation with proliferative markers was found. The contradiction between results of study of immunohistochemical evaluation of Cyclin D1 and its correlation with different clinico-pathological variables done on different types of tissue as well as different types of human cancers might indicate that Cyclin D1 activities might not be only diverse but also tissue specific [19]. In our study, cases which were found to have positive lymph node metastases were associated with strong grade of intensity of Cyclin D1 (G3), as a matter of fact the correlation between Cyclin D1 expression and the presence of lymph node metastases was studied in details in other researches done on other tissues as laryngeal squamous cell carcinoma [20, 21] and papillary thyroid carcinoma [10] where Cyclin D1 expression was correlated with the presence of lymph node metastases and

advanced tumor stage and thus researchers assumed that Cyclin D1 overexpression predicts lymph node metastases. Contradictory results noticed by researchers investigating this point whether on the same tissue as laryngeal carcinomas [18] or different tissues as gastric carcinoma [16] was found. Correlation between Cyclin D1 expression and the presence of lymph node metastases in prostatic carcinoma is a point that had not been studied in details before and yet to be investigated. Drobnjak *et al.* [5] found a strong association between Cyclin D1 positive phenotype in prostatic carcinoma and metastatic bone disease, thus they hypothesized that Cyclin D1 overexpression act as an activated oncogenic event and that it might be related to the development of bone metastases in prostatic carcinoma and to the evolution of androgen independent disease. On the other hand there was a significant statistical difference between cases showing moderate expression of cyclin D1 (mean 18.4) and cases showing strong expression of cyclin D1 (mean 23.7) according to preoperative serum PSA level (P = 0.029). Drobnjak *et al.* [5] reported significant correlation between intensity of expression of Cyclin D1 and preoperative serum PSA level (P = 0.029), while, Comstock *et al.* [4] and Kallakury *et al.* [9] noticed that Cyclin D1 positive tumors were more likely to be derived from patients with lower preoperative PSA value. These contradictory results about the relation between preoperative serum PSA level and Cyclin D1 intensity and whether any of these factors influences one another is a point of question that needs to be more investigated.

We concluded that lack of correlation between Cyclin D1immunoreactivity and prognostic markers of aggressive disease, support the accumulating evidence that Cyclin D1 plays an important role in the pathogenesis and evolution of prostate cancer rather than the prognosis, thus Cyclin D 1 is not a reliable prognostic factor in cancer prostate. The use of targeted therapy which targets Cyclin D1 might be of interest in prevention of tumor progression in prostatic carcinoma. We recommended that more and more studies on the molecular study of Cyclin D1 in cancer prostate specimens as well as its relationship with androgen receptors, thus establishing the appropriate mean of cancer therapy by targeting this marker.

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