International Journal of Healthcare Sciences ISSN 2348-5728 (Online)
Vol. 2, Issue 2, pp: (305-320), Month: October 2014 - March 2015, Available at: www.researchpublish.com

P63 and Cyclin D 1 Expression in Benign Prostatic Hyperplasia versus Prostatic Adenocarcinoma: A Clinicopathologic, Radiologic, and Immunohistochemical Study

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Abstract: **Histopathological diagnosis of benign prostatic hyperplasia (BPH) and prostatic carcinoma (PC) may be problematic. P63, is confined to basal cells/myoepithelial cells in prostate. Cyclin D1 is expressed in the G1 phase of cell cycle and play important role in regulating the cell cycle and cancer progression and Its over-expression is believed to play a role in tumorigenesis including prostatic carcinoma.**
Objectives: This study is to assess the expression of P63 and cyclin D1 in BPH and PC, to examine the correlation between results of expression P63 and cyclin D in such lesions, determine the relation between the immunostaining and histologic grade, stage of PC as well as clinical and radiologic findings.
Material and methods: 50 cases of BPH and 50 cases of PC were obtained by TURP (62 cases) and radical prostatectomy (38 cases). For each case, clinical data and radiographic findings were obtained. All immunohistochemical (IHC) analysis was performed on routinely processed, formalin-fixed, paraffin embedded tissue. Tissue sections were cut at 4 µ and mounted on poly-L-lysine-coated slides. Percentage of positive cells was calculated and positive staining scored as: 1+ (weak)= less than 10%, 2+ (moderate) = 11 to 50% and 3+ (strong) = more than 50% tumor cells stained positive.
Results: For P63; 98% of BPH showed positivity and 96% of PC cases showed negativity. For Cyclin D1; 84% of BPH showed negativity while 90% revealed positivity. Degree of reactivity was increased with high Gleason grade but this correlation is not significant.
Conclusion: p63 and Cyclin D1 were highly expressed in BPH and PC respectively, so they may be a valuable tool in differential diagnosis of BPH versus PC lesions.

Keywords: **P63, Cyclin D1, Prostate, BPH, PC.**

1. INTRODUCTION

Benign prostatic hyperplasia (BPH) is an extremely common condition in elderly men and is a major cause of outflow obstruction. By the age of 60, 50% of men have BPH, and by 90 years of age the prevalence has increased to 90%. As such it is often thought of essentially as a 'normal' part of ageing [1]. Prostatic cancer (PC) is the worldwide leading cause of cancer and the second cause of cancer-related death in men after lung cancer [2].

The diagnosis of PC on routine biopsies can be challenging when pathologists are faced with certain problems such as limited tissue sample, small foci of carcinoma, or benign mimics of prostate cancer like atrophy and atypical adenomatous hyperplasia. It has been well documented that that benign prostatic glands retain their basal cells while infiltrating adenocarcinomas do not [3-5]. Therefore histologically, absence of a basal cell layer provides supportive evidence for prostatic carcinoma (PC) [6].

Differentiation of prostatic adenocarcinoma (PC) from benign prostatic lesion and hyperplasia sometimes cannot be done on the sole basis of morphologic findings. In these cases, the diagnosis can be made according to the presence or absence of the basal cell layer, considering the fact that in the PC there is no basal cell layer whereas benign lesion is encirclement by this layer. Therefore, using basal cell markers should be useful in distinguishing these two important categories of prostatic lesions [7-14].

The discovery of p63 as basal cell markers makes it a useful stain in difficult cases to distinguish some benign lesions as benign prostatic hyperplasia (BPH) from prostatic carcinoma (PC) especially in association with cyclin D 1 [15,16].

p63, a p53-homologue nuclear transcription factor that is located on 3q27-29 and encodes six different isoforms, which harbor either trans-activating or negative dominant effects on p53 reporter genes[17,18]. p63 protein (p63) is a nuclear protein, a transcription factor plays a critical role in the growth and development of many epithelial organs. p63 is confined to basal cells of squamous epithelia (including epidermis and hair follicles) and urothelium, as well as basal cells/myoepithelial cells in breast, sweat glands, salivary glands, and prostate[19,20].

Cyclin D1 is expressed in the G1 phase of the cell cycle and that has an important role in regulating the cell cycle and cancer progression. Its over-expression is believed to play an important role in both the tumorigenesis and grading of many cancers, including prostatic carcinoma, if its expression is deregulated, mainly overexpressed [21]. in spite of overexpression of cyclin D1 it does not increase proliferation[15] In prostatic cancer, cyclin D1 acts as a critical regulator of androgen-dependent transcription and cell cycle progression [22]. Expression of cyclin D1 has been shown to be upregulated by a complex mechanisms involving RB and P53 and downregulation caused by oncogenic proteins of transforming DNA viruses, including SV40 large T antigen and E6, E7 proteins of human papilloma virus [23]. Chen et al [24,25] reported that overexpression of cyclin D1 increases cell growth and tumorigenicity in human prostate cancer.

Cyclin D1 overexpression secondary to its gene amplification has been identified in variety of tumors, including adenoma, B cell lymphoma, and carcinoma of breast, liver, oesophageus, urinary bladder, lung and prostate [25].

It has been shown that some PC show basal cell layer a few benign prostatic hyperplasia (BPH) do not express basal cell markers [8].

The objectives of our work is to investigate the expression of P63 and cyclin D1 in prostatic hyperplasia and prostatic carcinoma, to examine the sensitivity and specificity of both as immunomarker in distinguishing some confusing foci of some benign lesions as BPH from PC, to examine the correlation between results of expression P63 and cyclin D in such lesions and also determine the the relation between the immunostaining and histologic grade, stage of PC as well as radiologic findings.

2. MATERIAL AND METHODS

This study was performed on 100 prostatic specimens in the pathology department, of King Fahd hospital in Al-Baha province, KSA. These specimens were collected between 2011-2013. Out of the 100 cases; 50 cases were BPH (cancer mimickers), and 50 cases were prostatic adenocarcinoma of different Gleason's grade. Sampling procedures were different including transuretheral resection prostatectomy (TURP) (62 cases) and radical prostatectomy (38 cases).

For each case, clinical data including radiographic findings were obtained from patient's file as well as from reference sheet. The clinical data include age and clinical presentation. A pre-operative blood sample was collected to PSA assay.

Histopathological examination:

Tissue samples were routinely fixed in 10% formalin, embedded in paraffin, cut into 4 µm thick sections and stained with hematoxylin and eosin stain. Slides were reviewed for lesions of BPH and PC as well as presence or absence of PIN. For cases of PC, each case was graded according to the Gleason grading system and cases were distributed according to their Gleason score into three groups (score ≤ 5) or (score 6 and 7) and (score >7). Stage of the tumor, was applied on 38 cases that were obtained by radical prost-atectomy specimens. Staging was applied according to modified Whitmore-Jewett staging system. PC cases were distributed according to their pathological stage into two groups; organ confined (<T2) or extension outside capsule (>T2).

Immunohistochemical staining:

All IHC analyses was performed on routinely processed, formalin-fixed, paraffin embedded tissue. Tissue sections were cut at 4 µ and mounted on poly-L-lysine-coated slides.

For P63 immunostaining, Immunostaining was performed in all tissue specimens and paraffin-embedded cell lines using the 4A4 anti-p63 antibody [6], which recognizes all six p63 isotypes. The antibody was diluted 1/50. For p63 immunostaining, 5-µm sections were deparaffinized, rehydrated, and subjected to microwaving in 10 mmol/L citrate buffer, pH 6.0 in a 750 W oven for 15 minutes. Slides were allowed to cool at room temperature for 30 minutes. The diluted antibody was applied at room temperature for 2 hours in an automated stainer (Optimax Plus 2.0 bc; BioGenex, San Ramon, CA). Detection steps were performed by the instrument using the MultiLink-HRP kit (BioGenex). Peroxidase activity was localized using 3,3-diaminobenzidine or 3,3-diaminobenzidine-nickel chloride. Standardized development time periods allowed accurate comparison of all samples.

For cyclin D1; Immunohistochemical staining was performed with monoclonal anti-cyclin D1 antibody (Novocastra/Vector, Burlingame, CA), at dilution of 1:20, using a standard avidin/biotin complex (ABC)method as implemented on a Techmate 1000 (BioTek) automated immunostainer. The staining procedure consisted of a 45-min incubation in the primary antibody, followed by brief buffer washes, and then incubation in a cocktail of biotinylated anti-mouse IgG/IgM (BioTek) for 30 min. The slides were then washed, incubated in avidin/biotin complex (BioTek) for 30 min, washed, and then reacted with diaminobenzidine and hydrogen peroxide to visualize the end product. The sections were counterstained with hematoxylin. A breast cancer known to express cyclin D1 served as positive control for cyclin D1. For negative control, nonimmune serum was substituted for primary antibody.

Evaluation of Immunostaining:

Positive results were considered as brown stain of the nuclus of basal cell layer with negative stain of the stroma and the secretory epithelium of prostatic acini. Immunostained sections were evaluated by estimating the percentage of tumor cells stained with monoclonal anti-cyclin D1 antibody. Only a distinct brown nuclear staining of tumor cells was considered as positive. The nuclear staining in the normal prostate tissue surrounding the tumor was used as an internal negative control for cyclin D1. The percentage of positive cells was then calculated and staining categories was as follow scored as: 1+ (weak)= less than 10%, 2+ (moderate) = 11 to 50% and 3+ (strong) = more than 50% tumor cells stained positive [66]. PSA assay was carried out using human PSA total ELIZA kit (RABO331 Sigma).

Statistical Analysis:

Chi-square test and Fisher's exact tests were used to compare the P63 and cyclin D1 percentage and staining intensity data. The degree of agreement between P63 and cyclin D1 expression was measured by the Kappa measure of agreement. All p-values were two-sided. P-values less than or equal to 0.05 were considered significant.

The sensitivity, specificity and positive predictive values of p63 and Cyclin D 1 were calculated using the following formula

Sensitivity = True Positive / True Positive + False Negative x 100%

Specificity = True Negative / True Negative + False Positive x 100%

Harvard Graphics was used for drawing figures. Computer software Statistical Package for the social science (SPSS) version 17 was used in the analysis of the presenting study.

3. RESULTS

All selected cases were ranged from age between 50-90 years, age of PBH cases was ranged from 50-90 years with mean ± SD; 72±3.6; of these 18 cases (36%) were in range group between 50-70 with mean± SD; 61 ± 2.1 years while 32 cases(64%) were in range group 70-90 years with mean±SD; 80 ± 1.6. Age of PC cases showed 12 cases (24%) lies in range group between 50-70 years with mean±SD; 60±1.8while 38 case (76%)lies in range group between 70-90 years with mean ±SD; 80±2.

Level of PSA in BPH was ranged from 2-5 ng/mL with mean of 2.5 ± 1 ng/mL while in PC cases ranged from 7-84 with mean 34 ± 4 ng/ mL.

Clinical data ranged from degree of lower urinary tract obstructive symptoms. All cases of BPH were radiologically investigated by TRUS and MRI.

In cases of BPH, 32 cases out of 50 did prostatic U/S on prostate that showed an increase in volume of the prostate with a calculated volume exceeding 30 cc (A x B x C) /2). The central gland is enlarged, and is hypoechoic or of mixed

echogenicity. Calcification was seen both within the hypertrophied gland as well as in the pseudocapsule (representing compressed peripheral zone) with elevated Post-maturation residual volume.

26 out of 50 cases of BPH did Fluoroscopy and IVP that showed that The bladder floor is elevated and the distal ureters lifted medially (J-shaped ureters or Fishhook ureters). 20% of these cases showed detrusor hypertrophy, trabeculation 2 cases showed bladder diverticula.

MRI showed that an enlarged central zone which is heterogenous in signal with an intact low signal pseudocapsule in its periphery.

As regard PC cases, Transrectal ultrasonography (TRUS) was performed in order to detect abnormalities and to guide biopsy, usually following an abnormal PSA level. In our retrospective study TRUS was done in about 42 out of 50 (84%) cases of PC which revealed the presence of a hypoechoic lesion in about 26 (61.9%) cases in the peripheral zone of the gland,10 cases (23.8%) showed hyperechoic and 6 cases(14.2%) showed isoechoic lesions. All lesions were situated in peripheral zone.

MRI was done on 48 (96%) cases out of 50 following after a ultrasound guided prostate biopsy has confirmed cancer in order to determine or evaluate presence of extracapsular extension[25-27, 28-30] to detect and localize cancer especially for those cases with elevated PSA but routine TRUS biopsy is negative.

All cases showed MRI parameters that include the presence of a mass with low T2 signal, restricted diffusion with reduced ADC and increased tissue capillary permeability using dynamic gadolinium contrast enhanced imaging and calculation of the so-called Ktrans (a calculated time constant for permeability). 42 out of 48 cases have no extra-capsular extension and 6 cases with extracapsular extension have been recorded to involve the urethra and seminal vesicles and one case was seen with obliteration of rectoprostatic angle.

Histopathological findings: 50 cases of BPH and 50 cases were of PC. 45 cases out of PC cases were associated with PIN of different grades. Also cases of PC showed different Gleason grades; of these 16 cases (32%)were less than grade 5, 30 cases (60%) of grade 7 and 4 cases (8%) more than grade 7.

Results of P63 immunostaining; 49 cases (98%) out of 50 BPH showed positive immunostaing for P63 while only one case (2%) showed negative staining, sensitivity (98%) and specificity (96%) ; of the positive cases; 36 cases (73%) showed diffuse strong positive staining, 12 cases (24.4%) showed moderate staining and 1 case(2.6%) revealed focal staining.

Cases of PC: 48 cases out of 50 (96%) showed negative immunostaining while 2 cases (4%) showed weak focal staining for P63, no moderate or strong staining were obtained. There is no significant correlation between Gleason grade and the results of P63 immunostaining. Some Foci of PIN (10%) showed interrupted focal P63 immunostaining (Table 1 &2) (Fig: 1-7).

There is no significant statistical differences between PSA and intensity of P63 staining in both BPH and PC cases(P-value=0.621, 0.581 respectively).

Table: 1: Results of P63 immunostaing in BPH and PC in details

Lesion	**P63 reactivity percentages**			**Negative**	**Total**
	Focal(+)	Moderate(++)	Strong(+++)		
BPH	1 (2%)	12 (24%)	36 (72%)	1 (2%)	50
PC	2 (4%)	0 (0%)	0 (0%)	48(96%)	50
Total	3 (3%)	12 (12%)	36 (36%)	49 (49%)	100

Table 2: Summary of the results of P63 immunostaing in BPH and PC

Results of P63 immunostaining	BPH	PC	Total
Positive	49(98%)	2(4%)	51
Negative	1(2%)	48(96%)	49
Total	50	50	100

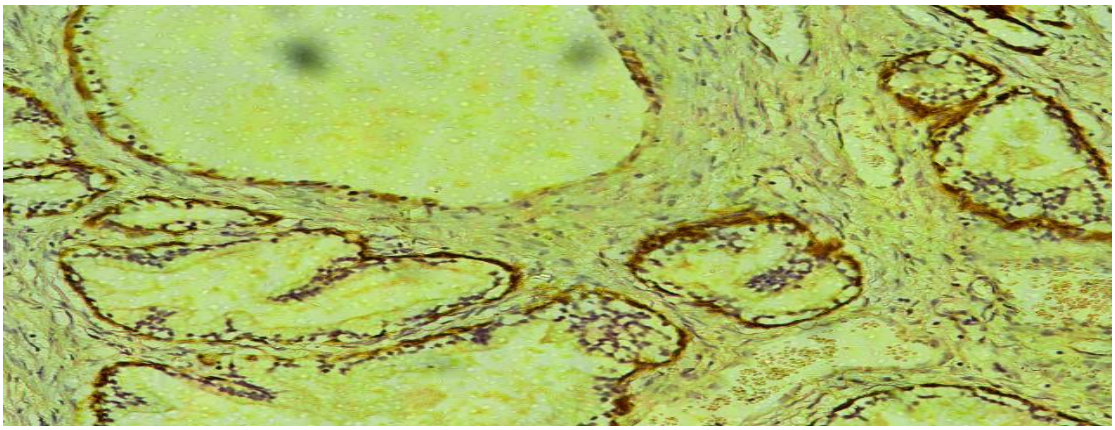

Fig 1: A case of BPH showing strong positivity for p63 within the basal cells of prostatic acini. (x 200)

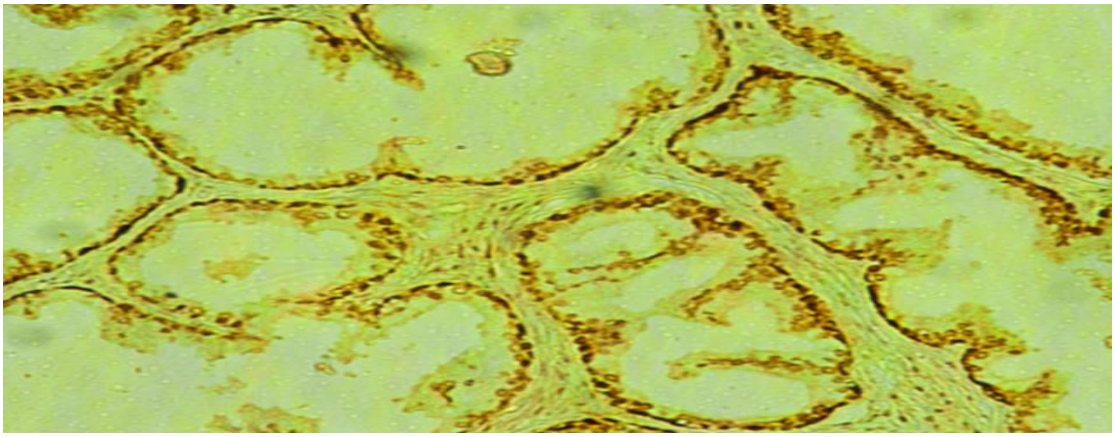

Fig 2: A case of BPH showing strong positivity for p63 within the basal cells of prostatic acini. (x 400)

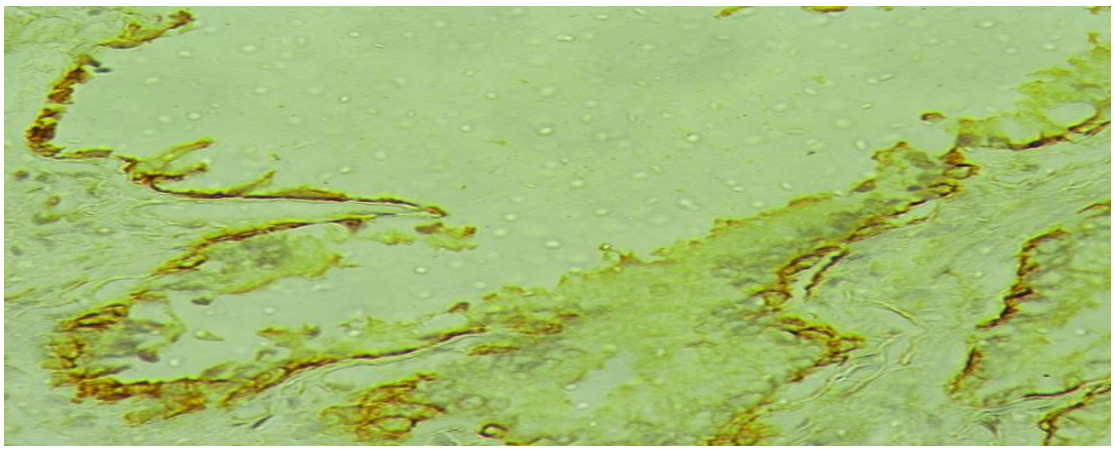

Fig 3: A case of BPH showing strong positivity for p63 within the basal cells of prostatic acini. (x 400)

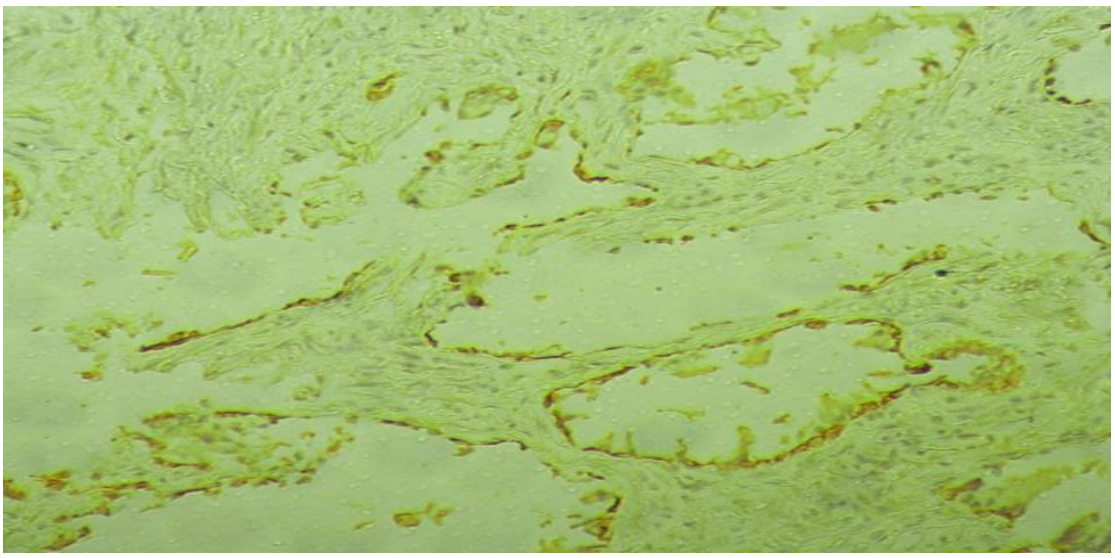

Fig 4: A case of BPH showing moderate positivity for p63 within the basal cells of prostatic acini. (x 200)

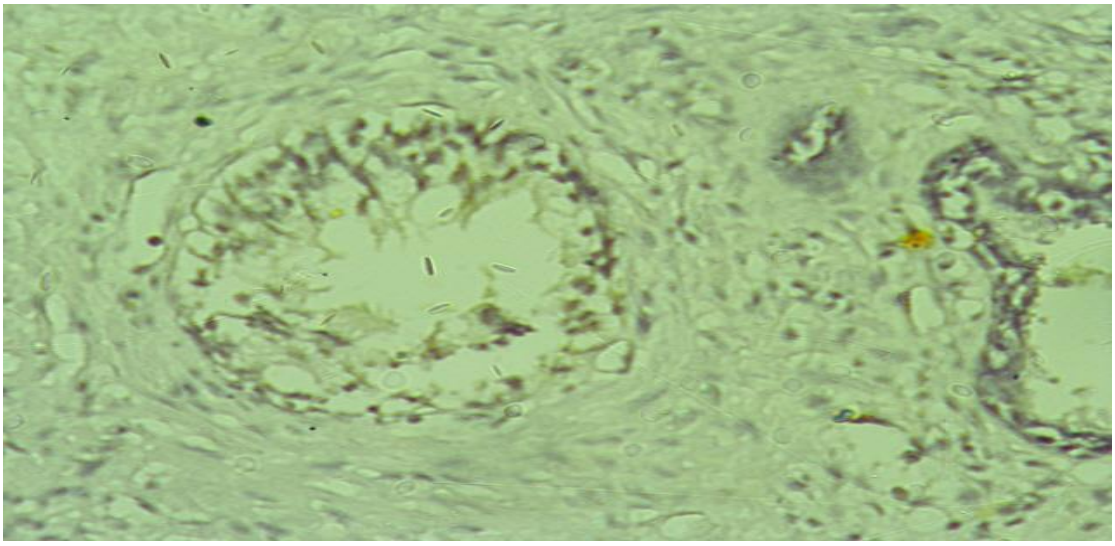

Fig 5: A case of PIN showing negativity for p63 within the basal cells of prostatic acini. (x 200)

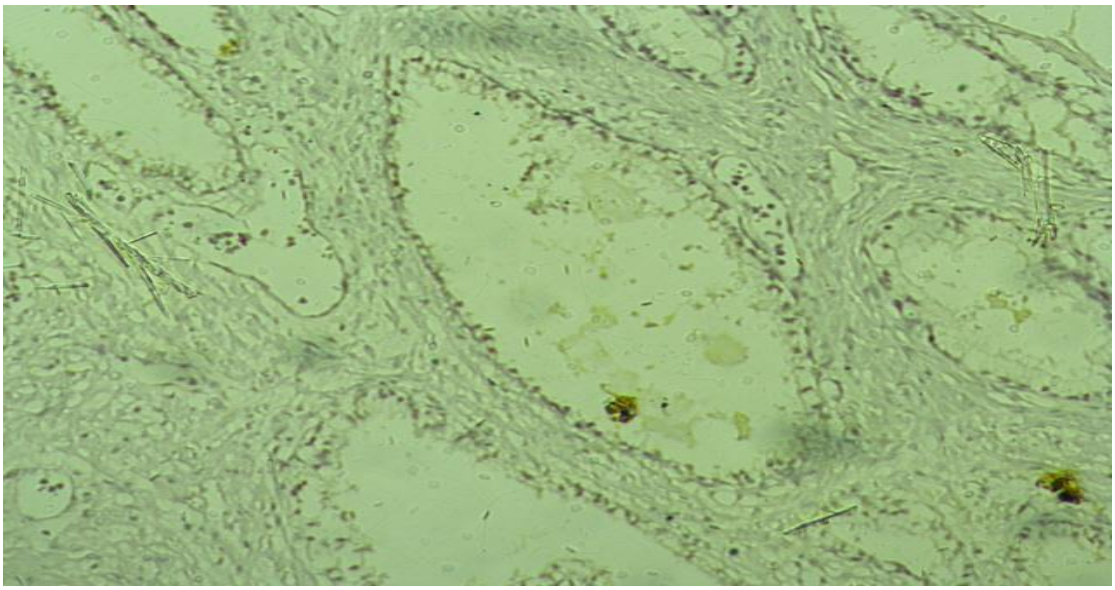

Fig 6: A case of BPH showing negative staining for P63 (x200).

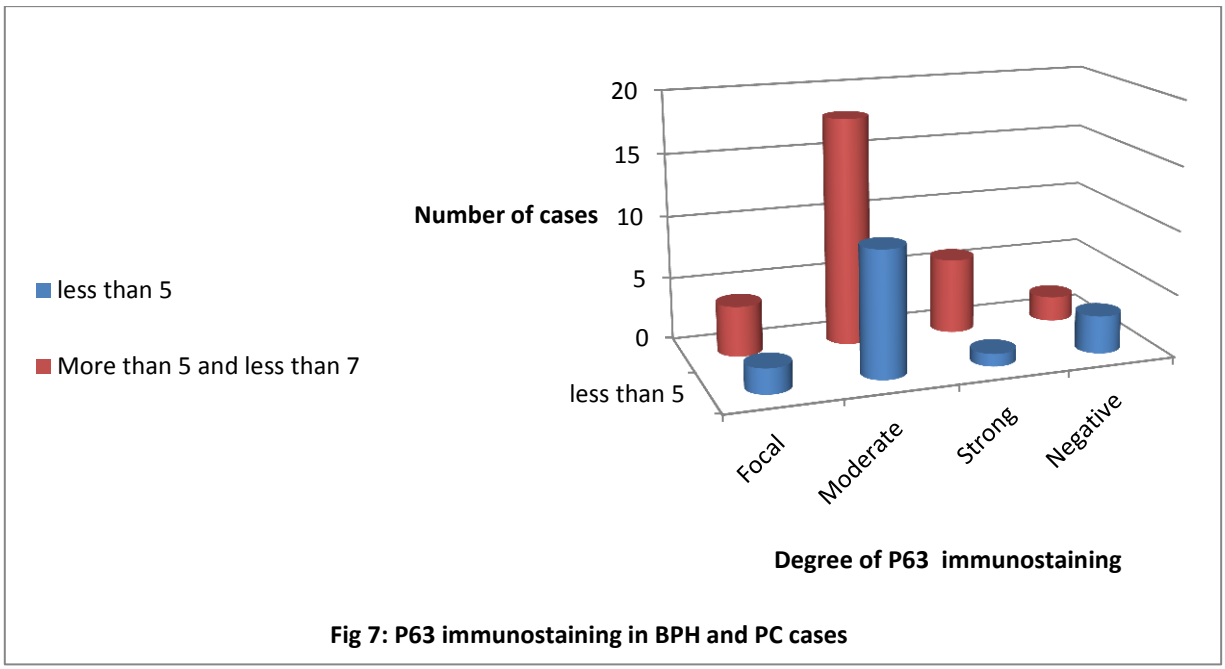

Fig 7: P63 immunostaining in BPH and PC cases

Results of Cyclin D 1 immunostaining: As regard Cyclin D1 immunostaining, our presenting study revealed that; 42 out of 50 BPH cases (84%) showed negative immunostaining for cyclin D1 while 8 cases (16 %) showed focal positivity. 45 cases of PC out of 50 (90%) yield positive reaction for cyclin D1 while 5 cases (10%) showed negative immunostaining (fig 7-15). Sensitivity and specificity of Cyclin D 1 for PC cases were 90% and 84% respectively. Of the 45 PC positive cases; 7 cases showed focal positivity, 29 cases showed moderate activity and 9 cases showed strong positivity for cyclin D1 (table 3 &4). 90% of PIN foci associated with PC cases showed positive immunostaining for cyclin D 1. The degree of reactivity was increased with the Gleason grade according to results of Chi square test but this correlation was not significant (P-value=0.586) (fig 16, 17), also, there was no significant correlation between Cyclin D1 expression and PSA either in BPH or PC cases with P-value = 0.534 and 0.434 respectively, . level of PSA in positive cyclin cases was 25.8±2.6 ng/ml, while in negative cases it was 17.2± 1.6 ng/ml, no significant correlation between PSA and stage of tumor(P- value= 0.189) (table 5).

Correlation between results of P63 and Cyclin D 1 immunostaining in both BPH and PC cases showed highly significant difference with P- value of 0.001 (table 6 &7).

Table: 3: Results of Cyclin D 1 immunostaing in BPH and PC in details

Lesion	Cyclin D1 reactivity percentage			Negative	Total
	Focal	Moderate	Strong		
BPH	8 (16%)	0 (0%)	0 (0%)	42 (84%)	50
PC	7 (14%)	29 (58%)	9 (18%)	5 (10%)	50
Total	15(15%)	29 (29%)	9(9%)	47(47%)	100

Table 4: Summary of the results of Cyclin D 1 immunostaing in BPH and PC

Results of Cyclin D1 immunostaining	BPH	PC	Total
Positive	8	45	53
Negative	42	5	47
Total	50	50	100

Table 5: Relation between the results of Cyclin D 1 immunostaing and Gleason score in PC cases.

PC Gleason grade	Cyclin D1 immunostaining in PC cases				P value
	-	+	++	+++	
2-4	3	2	10	1	**0.586**
5-7	2	4	18	6	
8-10	-	1	1	2	
Total	**5 (10%)**	**7 (14%)**	**29 (58%)**	**9 (18%)**	

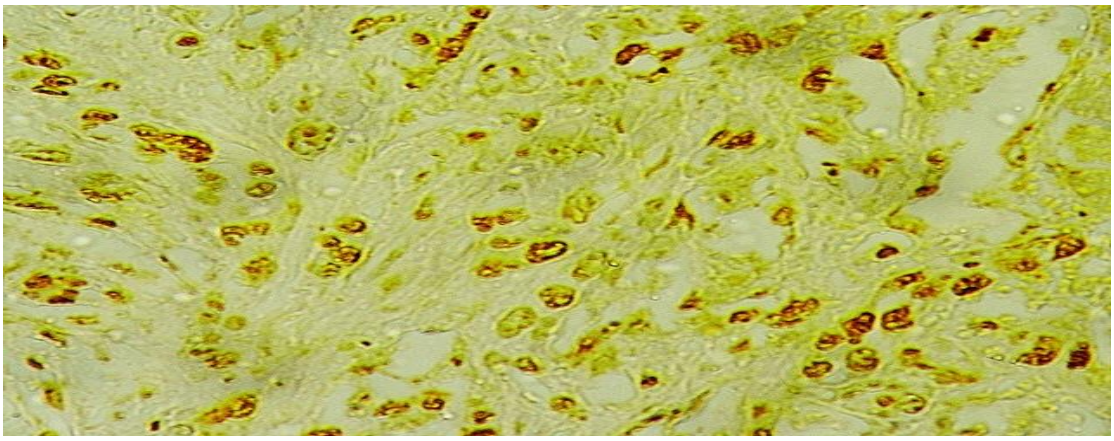

Fig 7: Prostate carcinoma (PC) pattern 4 reveal fused acini showing strong positivity for cyclin D1 within carcinomatous glands. (x 400)

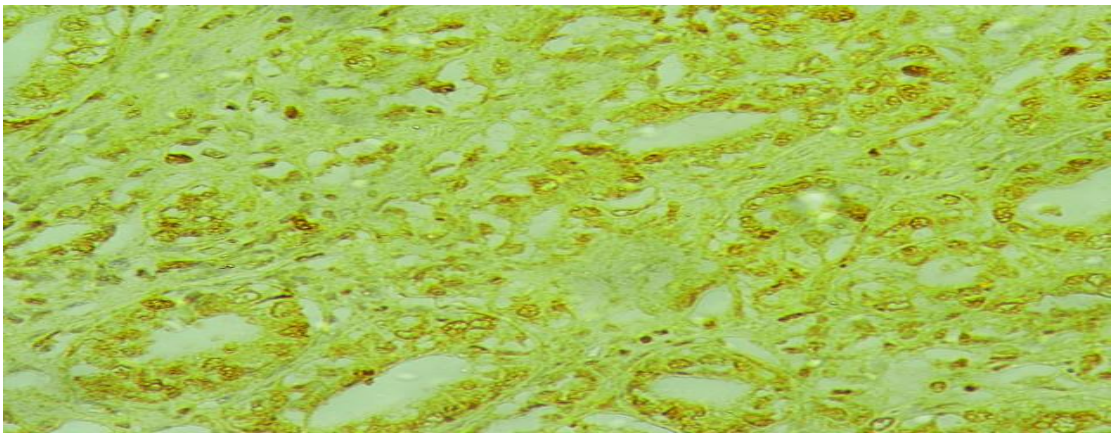

Fig 8: Prostate carcinoma (PC) pattern 3 reveal rounded acini showing strong positivity for cyclin D1 within carcinomatous glands. (x 400)

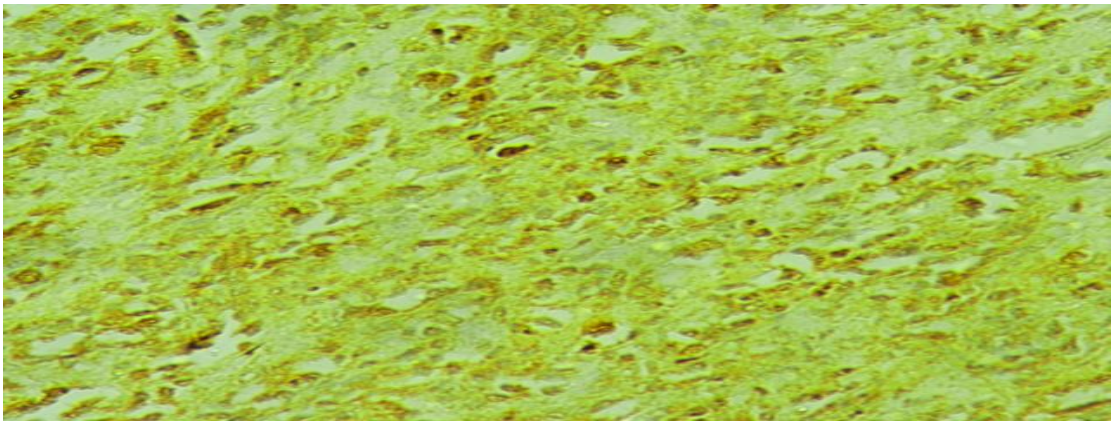

Fig 9: Prostate carcinoma (PC) showing moderate positivity for cyclin D1 within carcinomatous glands. (x 400)

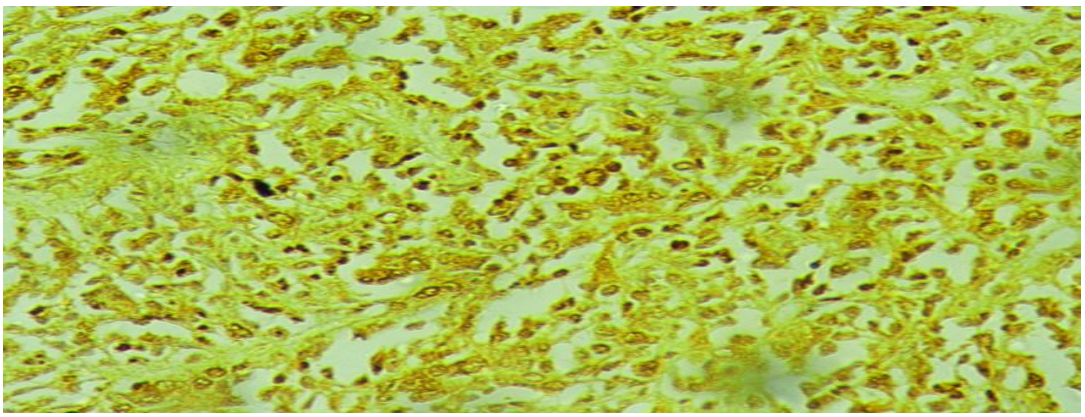

Fig 10: Prostate carcinoma (PC) showing very intense positivity for cyclin D1 within carcinomatous glands. (x 400)

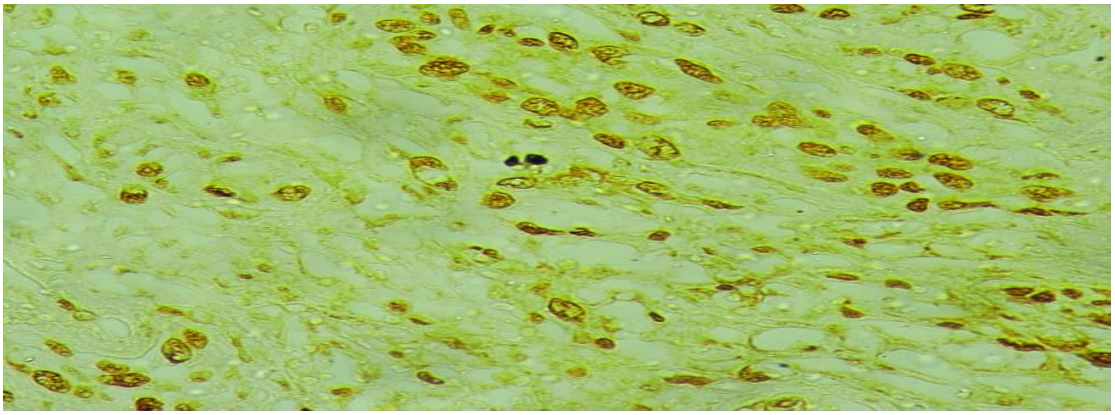

Fig 11: Prostate carcinoma (PC) showing strong positivity for cyclin D1 within carcinomatous glands (x 400)

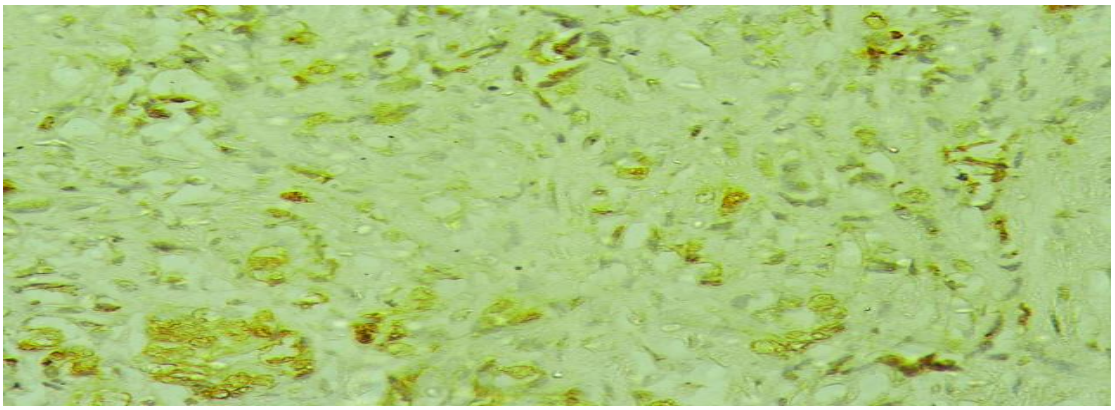

Fig 12: Prostate carcinoma (PC) showing moderate positivity for cyclin D1 within carcinomatous glands. (x 400)

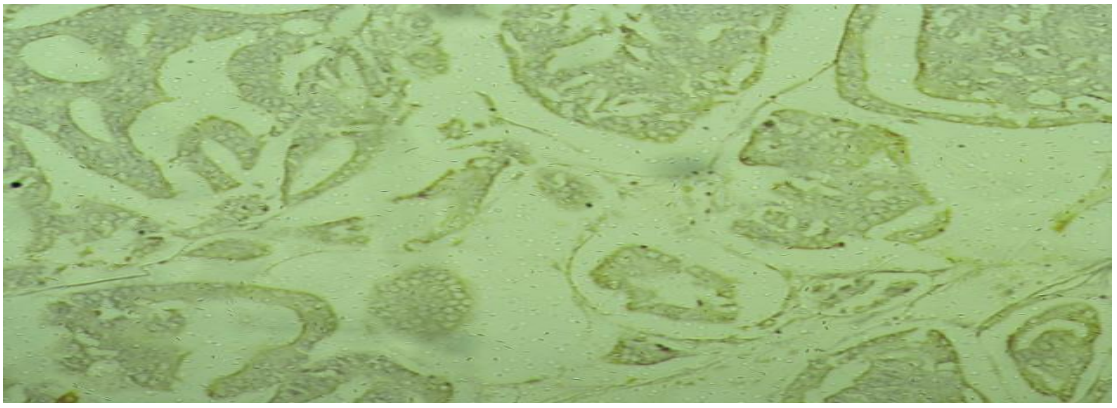

Fig 13: Prostate carcinoma (PC) showing negative staining for cyclin D1 within carcinomatous glands. (x 400)

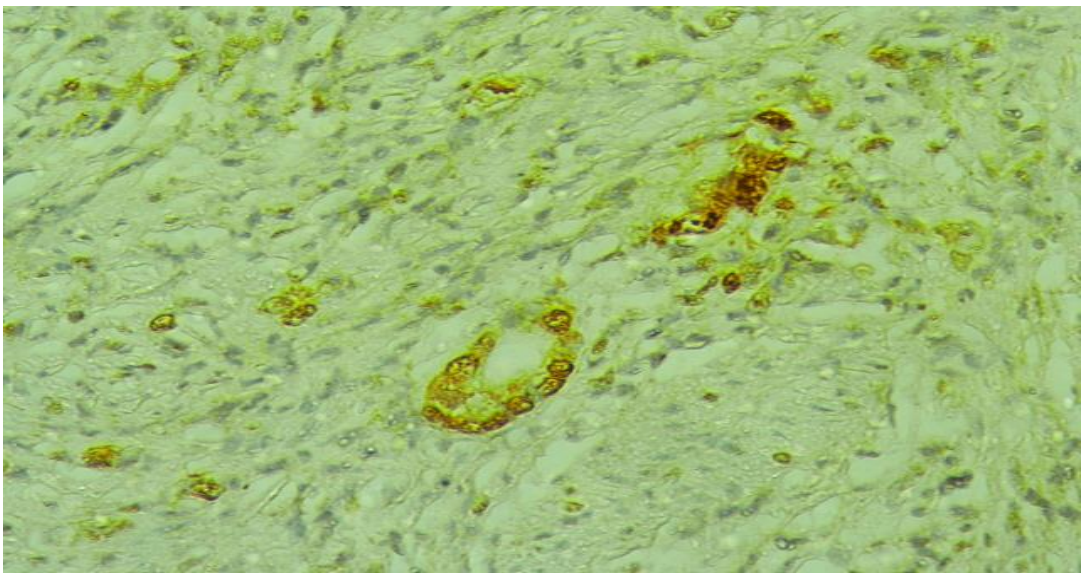

Fig 14: Photomicrograph of foci of malignant acini showing diffuse positivity for cyclin D1 in carcinomatous acini (left) and foci of PIN with focal week positivity (lower right) (x 200)

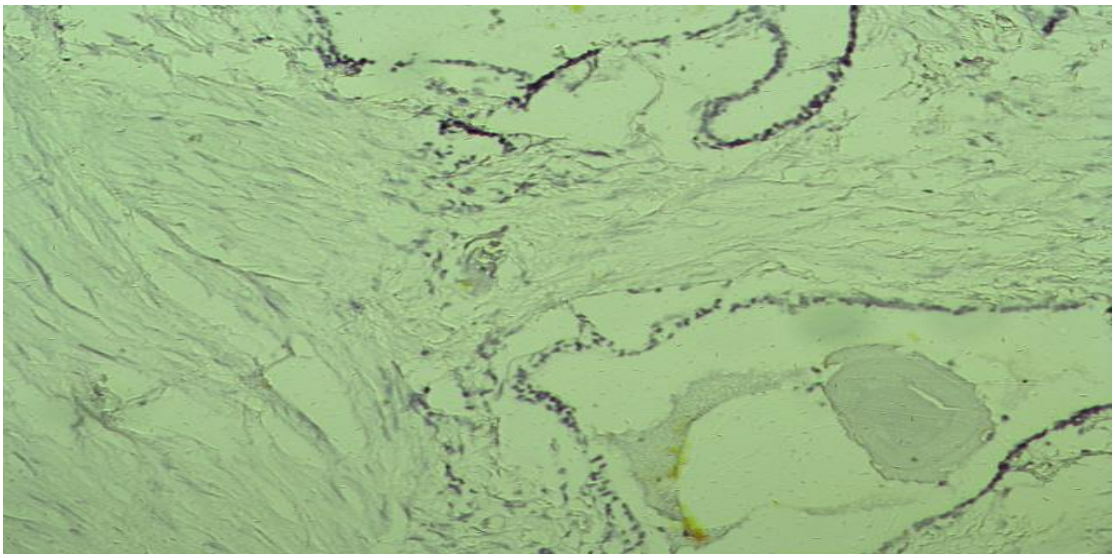

Fig 15 : A case of BPH showing negative staining for cyclin D1 (x200)

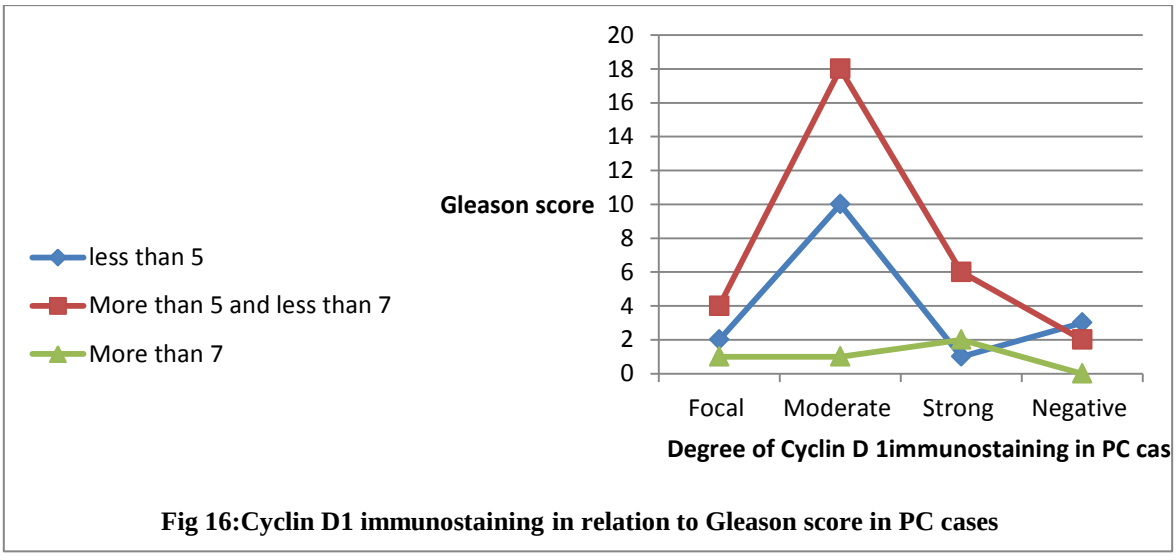

Fig 16:Cyclin D1 immunostaining in relation to Gleason score in PC cases

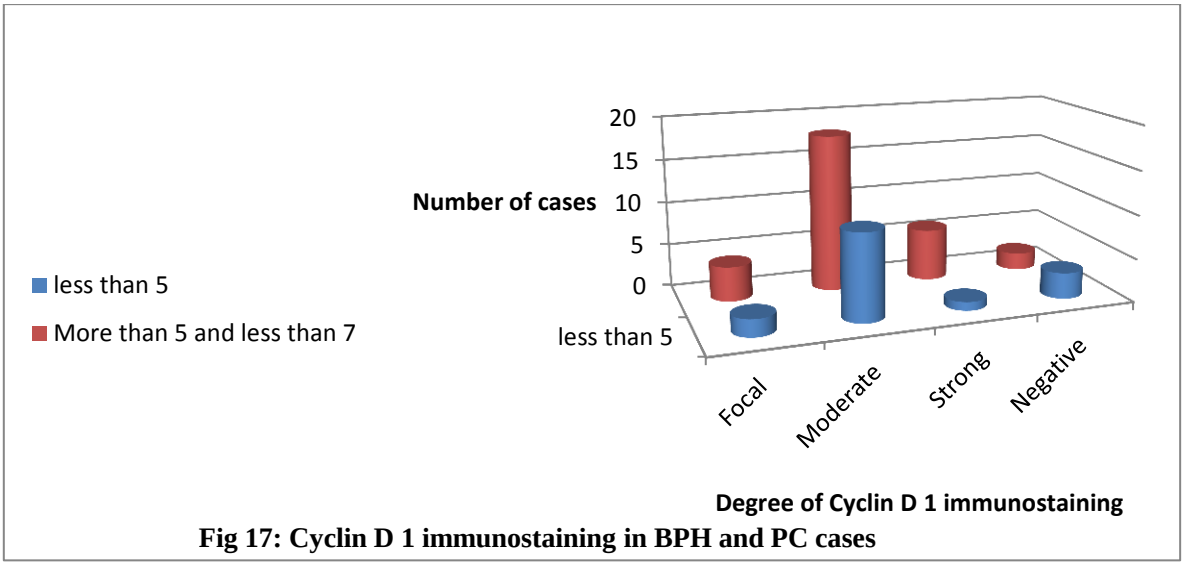

Fig 17: Cyclin D 1 immunostaining in BPH and PC cases

Table 6: Correlation between results of P63 and Cyclin D 1 in BPH cases

IHC	Results of P63 and Cyclin D 1 in BPH				Total	P-value
	Focal	Moderate	Strong	Negative		
P63	1	12	36	1	50	0.001
Cyclin D 1	8	0	0	42	50	
Total	9	12	36	43	100	

Table 7: Correlation between results of P63 and Cyclin D 1 in BPH cases

IHC	Results of P63 and Cyclin D 1 in PC				Total	P-value
	Focal	Moderate	Strong	Negative		
P63	8	-	-	42	50	0.001
Cyclin D 1	7	29	9	5	50	
Total	15	29	9	47	100	

4. DISCUSSION

It has been reported that all basal cells express P63, therefore it can be used in distinguishing benign lesion from prostatic adenocarcinoma [26,27].

As regard results obtained for P63 immunostaining, these results are consistent with those obtained by Signoretti et al, [14] they reported that all BPH cases showed universal p63 immunostaining of basal cell nuclei, whereas secretory cells were consistently negative, (97%) invasive prostate cancers were negative for p63, whereas in four cases, <1% of cells were positive for p63. They reported that p63 expression is necessary for the normal development of the mouse prostate, suggesting that p63-positive basal cells may represent/include prostate stem cells. Also our results coincide with that obtained by Parsons et al [28], they reported that basal epithelial cells in normal, BPH, high grade PIN stained intensely for P63 polypeptide but the vast majority of PC (94%) did not, therefore P63 immunohistochemistry represents a potential novel adjuvant method for facilitating the pathologic diagnosis of PC

Weinstein et al [20], reported that p63-positive basal cells were seen in every one of these benign foci, some of which showed significant cautery artifact. No clusters of architecturally and cytologically benign glands without p63-positive basal cells were seen, although scattered single p63-negative benign glands could be found. In one block, a focus of adenocarcinoma was present. It was negative for p63. Also they concluded that p63 staining is sensitive in identifying basal cells in benign lesions and will not lead to false-positive diagnoses of malignancy in needle biopsies of the prostate. Moreover, staining of cells other than basal cells was not observed, indicating that use of this stain would not lead to false-negative diagnoses. Also they concluded that P63 is at least as sensitive and specific for the identification of basal cells in diagnostic prostatic specimens.

Leong et al [39] found that Of 134 PBH samples stained, 128 cases showed positive staining and 113 malignant samples stained 106 did not stain for p63. Shiran et al [6] found that out of 43 cases of BPH stained for PBH ; 38 showed positive staining and all of the malignant glands showed total absence of p63 staining leading to sensitivity 83.37% a specificity of 100% for p63 and the positive predictive value was 100% for p63.

Romics et al [33] studied the expression of p21(waf1/cip1), p27(kip1), p63 and androgen receptor proteins in relation to serum PSA levels in normal prostate and PC of low and high Gleason grade to find differentially expressed markers of malignant progression. They found that P63 and p21(waf1/cip1) proteins detected in normal basal cell nuclei were lost in all but one studied tumors respectively.

We reported a case of PC showing focal P63 positivity ; this coincides with that obtained by Osunkoya et al [34] who reported that rarely, prostate cancer can aberrantly express diffuse p63 staining in a non-basal cell distribution leading to the erroneous diagnosis of atrophy or atypical basal cell proliferation.

Also our results coincides with that obtained by Sirnivasan and Parwani [35], they found that P63 was positive in 119 out of 132 of urothelial carcinoma and BPH and negative in all cases of PC. Also our results coincides with that obtained by UdDin et al [36], they studied expression of P63 in both urothelial carcinoma(UC) and PC and found 44 of 50 UC (88%) was positive while None of the prostatic adenocarcinomas expressed p63 and Concluded that p63 can be used as a reliable marker to distinguish prostatic adenocarcinomas from urothelial carcinomas in difficult cases in conjunction with other markers like PSA.

In the presenting study, one case of BPH showed negative staining for P63; this means absence of basal cell layer (fig 6), this observation coincides with that obtained by Shiran et al [6], they identified rare benign glands showing lack of basal cell staining in nine cases. This could be explained as, It is common for some benign glands to show absence of basal cell staining due to the effects of prolonged formalin fixation, as extended formalin fixation decreases the P63 antigenicity [37,38]. Shah et al [39] reported that absence of basal cell staining in more than two benign glands occurred in 9% of needle biopsies stained with p63 , and also be attributed to the true absence of basal cells, or diminished or absent gene expression of basal cell markers. Technical variabilities, including those resulting from surgical procedures and antigen retrieval methods could be another important source of negative basal cell IHC reactions. Prostatic glands in the transition zone are especially susceptible to such variability. Also it coincides with that observation obtained by Weinstein et al., [12], they found absence of basal cells in some TURP specimens in benign lesions, especially in areas with cautery artifact.

As regard Cyclin D1 immunostaining, we selected only on nuclear staining and exclude cytoplasmin staining of cyclin D1. This coincides with Kallakury et al [40], Ozbek et al [41], Drobnjak et al [42], Anis et al [43], and in contrast to Comstock et al [44] and Gupta et al [45].

Our results for cyclinD1 are consistent with study done by Ozbek et al [41], they reported that positive nuclear Cyclin D1 expression in all cases of PC (100%) subjected to study and in contrary to results of Kallakary et al [41], they noticed positive nuclear staining only in about 22% and also to study done by Droinjack et al[42], they reported only 11% of cases of PC with positive cyclin D1 immunostaing.

We found that cyclin D1 positivity in PC cases showed increased intensity of stain with high Gleason grade with insignificant P –value. This coincides with Kallakary et al [40] as they stated that there was marked nuclear staining of cyclin D1 with high Gleason but with insignificant results in spite of low percent of positive cyclin D1 expression in PC cases of Kallakary' study.

Han et al., [46]studied the Cyclin D1 expression in human prostate carcinoma cell lines and primary tumors on 50 primary prostate cancer samples. They found that cyclin D1 protein was expressed at relatively high levels in all of the six human prostate cancer cell lines examined and 24% cases of PC revealed regions of moderate to strongly positive staining for cyclin D1, but was not detected in the cultures of normal human prostate cells and recommended a further studies on the expression of this gene for understanding the pathogenesis of prostate cancer.

Drobnjak et al [42]found a correlation between cyclin D 1expression and presence of metastatic bone disease and concluded that cyclin D1 expression along with the proliferative index are associated with the clinicopathological parameter of poor clinical outcome. However, no correlation was observed between cyclin D1 overexpression and either Gleason's score, neo-adjuvant hormone treatment, or PSA relapse.

Also our results go in accordance with that of Ueda et al [47], who found that 53.8% of cases of BPH and 84.6% of cases of prostatic carcinoma showed cyclin D1 expression. They indicated that cyclin D1 expression tends to increase in malignant prostate tissue.

We found a part of our PIN and PC results in the presenting study goes with results obtained by Fleischmann et al [48] as they found that all cases of PIN and PC while in cases of BPH. Our results are out of concordance to Fleishman' study who reported that no expression of cyclin D1 in 27.7% of BPH cases in contrast to 84% of our study, this may be due to technical varieties. Fleischmann et al., [48] reported that high nuclear cyclin D1 expression was significantly correlated with poor tumor differentiation and large nodal tumor burden. Also our BPH results are in contrast with the results of Gupta et al., [45] who found that of the BPH cases, 13 out of 18 showed cyclin D1 expression, of which 8 cases showed only nuclear positivity and 5 cases showed both nuclear and cytoplasmic positivity PC results coincides with these of Gupta et al., [45] who found that all cases showed both positivity for Cyclin D1 , of these, 24 out of 30 nuclear and cytoplasmic positivity for Cyclin D1 , whereas 2 cases showed cytoplasmic and 4 cases showed nuclear positivity only. But finally we coincides with them in his observation as regard BPH as they observed that focal and weak staining may be seen in benign cases but that it never reaches a significant proportion as seen in carcinoma of the prostate.

Our findings coincide with study done by Anis et al [43], who found that all cases of PC (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). They found also that normal prostatic tissue found adjacent to cancer in few cases revealed absent Cyclin D1 expression.

Our findings revealed no significant correlation between cyclin D1 expression with Gleason score. This coincides with Gupta et al., [45] , Ueda et al., [47] and Shiraishi [49] but in contrast with others as Ozbek et al [41] and Comstock [44]. This differences may be attributed to that most of studies including ours were focused largely on nuclear cyclin D1, and exclude cytoplasmic study.

In our study, there is no significant correlation between PSA level and cyclin D1 expression. Level of PSA in positive cases was 25.8±2.6 ng/ml, while in negative cases it was 17.2± 1.6 ng/ml. This is in contrary to that obtained by Comstock et al [53], they found that the mean PSA value for the cyclin D1 in positive group (13.5±4.03ng/ml) was significantly lower than cyclin D1 in negative group(27.7±7.43ng/ml).

Additionally, we found a case of BPH showed a strong positivity for P63 and negativity for cyclin D1 with PSA borderline level of 5 ng/ml. The could be explained according to the fact that false positive PSA may be due to several factors such as digital rectal examination [50], presence of bacterial prostatitis [51], acute retention of urine, prostatic biopsy, ejaculation and BPH[52-54].

Romics et al., [33] studied the expression of p21, p27, p63 and androgen receptor proteins in relation to serum PSA levels in normal prostate and PC of low and high Gleason grade to find differentially expressed markers of malignant progression. They found that all cases except one in each group were androgen receptor positive. P63 and p21 proteins detected in normal basal cell nuclei were lost in all but one studied tumors respectively. P27(kip1) protein, however, was detected in all low Gleason score prostate cancers, but it was found in only 7/13 high score cases. Prostate specific antigen levels, either pre- or post-treatment, did not show strict correlation with the p27(kip1) results. The low to high grade dedifferentiation of prostate adenocarcinoma is accompanied with the down-regulation of p27(kip1) protein, which may be an important molecular sign of the lost cell cycle control.

5. CONCLUSION

p63 and cyclin D1 immunohistochemistry has a meangiful valuable tool in the differential diagnosis of BPH *versus* PC to great extent, especially when some conflicts in diagnosis are presented as poor tissue sample, or presence of atypical lesion. P63 has high sensitivity to identify basal cells and so its presence is a track for diagnosis of BPH and vice versa. Sensitivity of cyclin D1 for diagnosis of PC is high and so it can be used alone or in conjugation with P63 for confirmation of diagnosis of PC.

Conflict of interest:

We have no conflict of interest to declare.

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