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ORIGINAL ARTICLE

Differential expression of cyclin D1 in human pituitary tumors: relation to MIB-1 and p27/Kip1 labeling indices

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Abstract *Background:* Pituitary tumors are a common form of endocrine neoplasia. However few studies assessed the expression of the principal cyclin regulating checkpoint exit, cyclin D1. Cyclin D1 expression in pituitary tumors and its possible relation to MIB-1 and p27/Kip1 labeling indices (LIs) was explored.

Design: We studied a total of 199 pituitaries, including normal pituitaries ($n = 7$), pituitary adenomas ($n = 187$), and pituitary carcinoma ($n = 5$). All tissues were tested as cores of archived tissue microarrays that were immunostained for cyclin D1, MIB-1 and p27 using a standard technique. Tissue cores were subjected to automated analysis to evaluate the staining LIs.

Results: No cyclin D1 positive cells in the normal anterior pituitary gland was found. Sparse nuclear staining was noted in pituitary tumors. Higher expression of cyclin D1 was noted in pituitary carcinomas compared to adenomas ($p < 0.001$), in non-functioning adenomas compared to functioning ones ($p < 0.001$) in macroadenomas versus microadenomas ($p = 0.017$) and in recurrent non recurrent adenomas ($p < 0.001$). Cyclin D1 LI and MIB-1 LI were related among adenomas ($p < 0.001$) and carcinomas ($p = 0.041$). p27 LI was neither related to pituitary adenoma recurrence nor invasion.

Conclusions: Expression of cyclin D1 in pituitary tumors is related to cell proliferation, recurrence, and metastatic potential. Nuclear cyclin D1 expression is a good marker of aggressive behavior in pituitary tumors.

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Introduction

Pituitary neoplasms are relatively common tumors that exhibit a wide range of hormonal activities and proliferative behaviors [1,2]. These tumors are responsible for an estimated 10% of all intra-cranial tumors [3]. Their classification is a dynamic area requiring continuous critical review due to advances in molecular genetic techniques and new immunohistochemical

stains providing a clearer understanding of cell lineages and pathogenetic mechanisms in adenohypophysial tumors [2].

The best predictive markers remain those that subclassify adenomas accurately based on hormone content and cell structure. A silent corticotroph adenoma will recur more often and more aggressively than a silent gonadotroph adenoma. However, the diagnosis of primary pituitary carcinoma remains based solely on the identification of distant metastasis. While this facilitates the diagnostic approach to these tumors, it seems inappropriate [2].

Pituitary tumors that can cause significant morbidity due to hormone hypersecretion, often invade the brain, causing blindness and nerve palsies, require radiotherapy, and ultimately cause death in some patients. The wide spectrum of biological behavior that they display and the linkages or lack thereof between differentiated activity and cell proliferation makes pituitary adenomas an ideal model for the study of mechanisms of tumorigenesis [2].

The deregulation of genes involved in the control of the cell cycle is one of the most common alterations in tumor growth; cells with such a defect often have a growth advantage over their neighbors. Progression through G1 to the S phase of the cycle is mediated by the interplay between proteins controlling pRb phosphorylation. Under mitogenic stimulation, the cyclin D1 protein binds to cyclin-dependent kinase 4 (CDK-4), which is subsequently activated and phosphorylates pRb. In a hyperphosphorylated form, pRb is inactivated, and the cells are released from G1 arrest. In contrast, under growth-inhibitory conditions, kinase inhibitors bind competitively to CDK 4, effectively keeping the cell in G1 and thus preventing cell division [4–6]. It has been demonstrated that cyclin D1 may play a role in sequestering p27 in the cell cytoplasm. This suggested that prevention of the functional nuclear localization of p27 protein could also potentially play a role in cell cycle dysregulation [7].

The cyclin D1 gene (CCND1), located at 11q13, is one of the most frequently amplified genes observed in human tumors, with amplification of CCND1 frequently leading to cyclin D1 protein over expression [4–6]. In head and neck cancers [8,9], cyclin D1 amplification and over expression are associated with a poor prognosis, whereas in other cancers such as breast [10], bladder [11], and non-small cell lung cancers [12], over expression of cyclin D1 correlates with good prognosis.

Although cyclin D1 has been studied extensively in a number of human malignancies, including breast, lung, and bladder cancers, and lymphomas, there are currently only limited data on cyclin D expression in pituitary tumors [13,14].

The aim of the current study is to investigate: 1 – the possible role of cyclin D1 over-expression in pituitary tumors compared to normal pituitary gland, 2 – the possibility of categorizing different pituitary tumors according to their cyclin D1 expression in addition to immunophenotype and clinical behavior, and 3 – the relationship between cyclin D1 and both MIB-1 or p27 labeling indices.

Material and methods

Tissue samples

This study included formalin-fixed paraffin-embedded tissues from 192 pituitary tumors, in addition to 7 normal anterior pituitary tissues. All cases were operated upon at Saint Mary's Hospital; one of the Mayo hospitals in Rochester–MN–USA; between 1982 and 2002 through transsphenoidal approach.

The study was approved by both the Biospecimens Subcommittee of the Mayo Rochester Research Committee and the Institutional Review Board (IRB).

(I) *Pituitary adenomas* studied (Total $n = 187$) included 34 prolactin (PRL) producing adenomas, 29 growth hormone (GH) producing adenomas, 20 adrenocorticotropic hormone (ACTH) producing adenomas, 2 thyroid stimulating hormone (TSH) producing adenomas, 44 gonadotroph (LH/FSH) adenomas, and 58 null cell adenomas. All had previously been characterized by immunostaining with pituitary antibodies. The GH adenomas group included plurihormonal GH adenomas and adenomas producing both GH and PRL but not acidophil stem cell adenomas.

Tumors producing GH and PRL were all endocrinologically functioning at presentation, while eight ACTH producing tumors were non-functional "silent". The remaining functioning ACTH producing tumors presented with either Cushing disease ($n = 10$) or Nelson's syndrome ($n = 2$).

The designation of the adenomas as microadenomas vs. macroadenomas and non-invasive vs. invasive was assigned upon revision of both image and surgery reports for each single case. All the adenomas studied were retrieved as primary excisions. Their post-operative two years follow-up revealed recurrence of only 16 cases, which we designate as recurrent adenomas.

(II) *Pituitary carcinomas* (Total $n = 5$) were previously characterized by immunostaining and included three PRL, one ACTH, and one LH/FSH carcinomas. Carcinomas were very rare and defined by the presence of metastatic disease.

(III) *Non-tumorous anterior pituitary tissue samples* ($n = 7$), surgically removed in conjunction with small microadenomas through transsphenoidal approach, were also studied as a control group. The number of the control group was few as the normal anterior pituitary tissue was difficult to be obtained.

For the present immunohistochemical study, we used tissue microarrays (TMA) that were formerly created as described by Kononen et al. [15] consisting of three cores of each specimen interspersed with normal liver tissues.

Immunohistochemistry

The original tumors – both adenomas ($n = 187$) and carcinomas ($n = 5$) used for construction of the tissue microarrays have been previously subjected to the seven pituitary battery antibodies (Table 1). Antigen unmasking procedure with protease II was performed only for β-FSH staining while no pretreatment was required for the remaining of the pituitary battery hormones. Staining was then carried out by Ventana ES (320) automated immunohistochemical stainer using AEC-detection kit (Ventana Medical Systems Inc. "VMS"–Tucson–AZ–USA).

The resultant TMA slides have been presented to immunostaining applying antibodies for cyclin D1, p27 and Ki-67 (Table 1). Antigen unmasking procedure was carried out using citrate buffer pH6 for cyclin D1 and 1 mM EDTA pH 8 for both p27 and Ki-67 staining. Staining was performed by DAKO EnVision Autostainer (DakoCytomation–Carpenteria–CA–USA) with diaminobenzidine (DAB) for color development.

Table 1 Technical characteristics of immunohistochemical methodology.

Protein	Origin	Clone	Dilution
PRL	Vector Labs, Burlingame, CA, USA	INN-hPRL-3	1: 75
GH	Vector Labs, Burlingame, CA, USA	Polyclonal	1: 250
ACTH	Vector Labs, Burlingame, CA, USA	Clone 56	1: 300
β-FSH	Vector Labs, Burlingame CA, USA	INN-h FSH-60	1: 2500
TSH	Vector Labs, Burlingame, CA, USA	QB2/6	1: 100
β-LH	DakoCytomation, Carpenteria, CA, USA	C93	1:75
∝-SU	Chemical Reagent Lab, Mayo Foundation, MN, USA	Polyclonal	1: 50,000
Cyclin D1	Thermo Fisher Scientific, Fremont, CA, USA	Polyclonal	Ready-to-use
P27	DakoCytomation, Carpenteria, CA, USA	SX53G8	1: 1000
Ki67(MIB-1)	DakoCytomation, Carpenteria, CA, USA	Ki-S5	1:100

Controls included appropriate positive tissues (normal human pituitary for pituitary battery antibodies, breast carcinoma for cyclin D1, and tonsillar tissue for both p27 and Ki-67). Slides with 'no primary antibody' served as negative control.

Quantitation

The TMA slides were scanned using the BLISS System–Slide scanner (Bacus Laboratories Inc.–Lombard–IL–USA).

Images were digitally captured at 20× magnification using a 12-tile matrix of images per core for a final resolution of 1920×2256 pixels per TMA core. Quantification of the labeled cells was performed using computer assisted TMA score software (Bacus Laboratories Inc.). Assessment of the cyclin D1, MIB-1, and p27 labeling indices was subsequently calculated as the percentage of cells showing definite positive nuclear staining with examination of at least 1000 cells in the three different cores per tumor specimen according to Turner et al. [14].

Table 2 Clinical characteristics by pituitary tumor type.

Tumor type	Clinical characteristics						
	n	Sex (% male)	Age (mean)	Size		Invasion n (% +)	Recurrence n (% +)
				Macro-n (%)	Micro-n (%)		
Pituitary adenomas							
All Adenomas	187	63.1	49.1	165 (88.2)	22 (11.8)	115 (61.5)	16 (8.6)
PRL	34	52.9	40.1	25 (73.5)	9 (26.5)	22 (64.7)	3
GH	29	65.5	42.2	26 (89.7)	3(10.3)	15 (51.7)	1
ACTH							
Cushing	10	30	50.9	2 (20)	8 (80)	2 (20)	1(10)
ACTH Nelson	2	0	52	2 (100)	0 (0)	2 (100)	0 (0)
Silent ACTH	8	50	46.2	7 (87.5)	1 (12.5)	4 (50)	1 (12.5)
TSH	2	100	46.5	2 (100)	0 (0)	1 (50)	0 (0)
LH/FSH	44	75	60.5	43(97.7)	1(2.3)	28 (63.6)	0 (0)
Null	58	67.2	54.7	58 (100)	0 (0)	41 (70.7)	10 (17.2)
Pituitary carcinomas							
Carcinomas	5	20	54.5	5 (100)	0 (0)	5 (100)	–

(% +): percent of invasive or recurrent cases.

Table 3 Cyclin D1 expression in different pituitary tumor types.

Tumor type	Protein expression n of cases (%)				
	0	1+ [LI 0.4–<0.6]	2+ [LI 0.6–<1.5]	3+ [1.5–<1.8]	4+ [LI 1.8–2.5]
All adenomas	103 (55.1)	21 (11.2)	27 (14.4)	24 (12.8)	12 (6.4)
PRL	20 (58.8)	4 (11.8)	8 (23.5)	1(2.9)	1 (2.9)
GH	15 (51.7)	5 (17.2)	9 (31)	0 (0)	0 (0)
ACTH	11 (55)	4 (20)	5 (25)	0 (0)	0 (0)
TSH	1 (50)	0 (0)	1 (50)	0 (0)	0 (0)
LH/FSH	32 (72.2)	8 (18.2)	4 (9.1)	0 (0)	0 (0)
Null	24 (41.1)	0 (0)	0 (0)	23 (39.7)	11 (19)
Carcinomas	0 (0)	0 (0)	0 (0)	1 (20)	4 (80)

Statistical analysis

Predictive Analytics Software (PASW) statistical software package (V. 18.0, IBM Corp., USA, 2010) was used for data analysis. Data were categorized according to their distribution and expressed as number (*n*) and percentage (%) for each group or subgroup. All tests were two-sided and *p*-values less than 0.05 were considered statistically significant. Any comparison of two discrete variables was made with chi-square test. Ranked Sperman correlation test was used to study the possible association between two variables among each group for non-parametric data. Diagnostic validity tests were performed and Receiver Operating Characteristic (ROC) curves were constructed to obtain the most sensitive and specific cut-off for cyclin D1, MIB-1 and p27 LIs in discriminating between pituitary adenomas and carcinomas. For statistical purpose, cyclin D1 LIs in pituitary tumors were classified into five groups; 0 (LI 0%), 1+ (LI 0.4–<0.6), 2+ (LI 0.6–<1.5%), 3+ (1.5–<1.8%), and 4+ (LI 1.8–2.5%).

Results

The clinical characteristics for the studied pituitary tumors are summarized in Table 2.

Cyclin D1 expression

All the studied tissue samples showed no evidence of cytoplasmic staining for cyclin D1. Overall, positive nuclear expression was seen in 84 of 187 (44.9%) pituitary adenomas, were graded either 2+ in 27 cases (14.4%) or 3+ in 24 cases (12.4%). All the five studied pituitary carcinoma cases revealed positive nuclear staining for cyclin D1 and four (80%) of them were graded as 4+. Cyclin D1 LI for all the positive cases of pituitary tumors ranged between 0.4% and 2.5%. In contrast, in the normal anterior pituitary gland, there were no cyclin D1 positive cells (Table 3 and Fig. 1). On comparing adenomas to carcinomas, a highly significant difference was revealed (*p* < 0.001), while no statistical significant difference was obtained on comparing adenomas to normal pituitaries (*p* = 0.236).

Relationship of cyclin D1 LI to tumor type and clinical characteristics

The carcinoma group clearly demonstrated higher cyclin D1 expression compared to adenoma group (Pearson chi-square, *p* < 0.001). Among the adenoma group, null cell adenomas showed significantly higher cyclin D1 expression compared to all the other adenoma subgroups; PRL (*p* < 0.001), GH (*p* < 0.001), ACTH (*p* < 0.001), TSH (*p* < 0.001), and LH/FSH (*p* < 0.001). On comparing adenomas which showed recurrence on follow up to those who did not have an evidence of recurrence, a significant higher cyclin D1 expression was noted in the former subgroup (*p* < 0.001). Also, non functioning adenomas [LH/FSH and Null cell adenomas] showed significantly higher cyclin D1 expression upon comparison with the endocrinologically functioning ones [PRL, GH, Cushing and Nelson ACTH cases] (*p* < 0.001). On comparing silent ACTH adenomas to the functioning ones
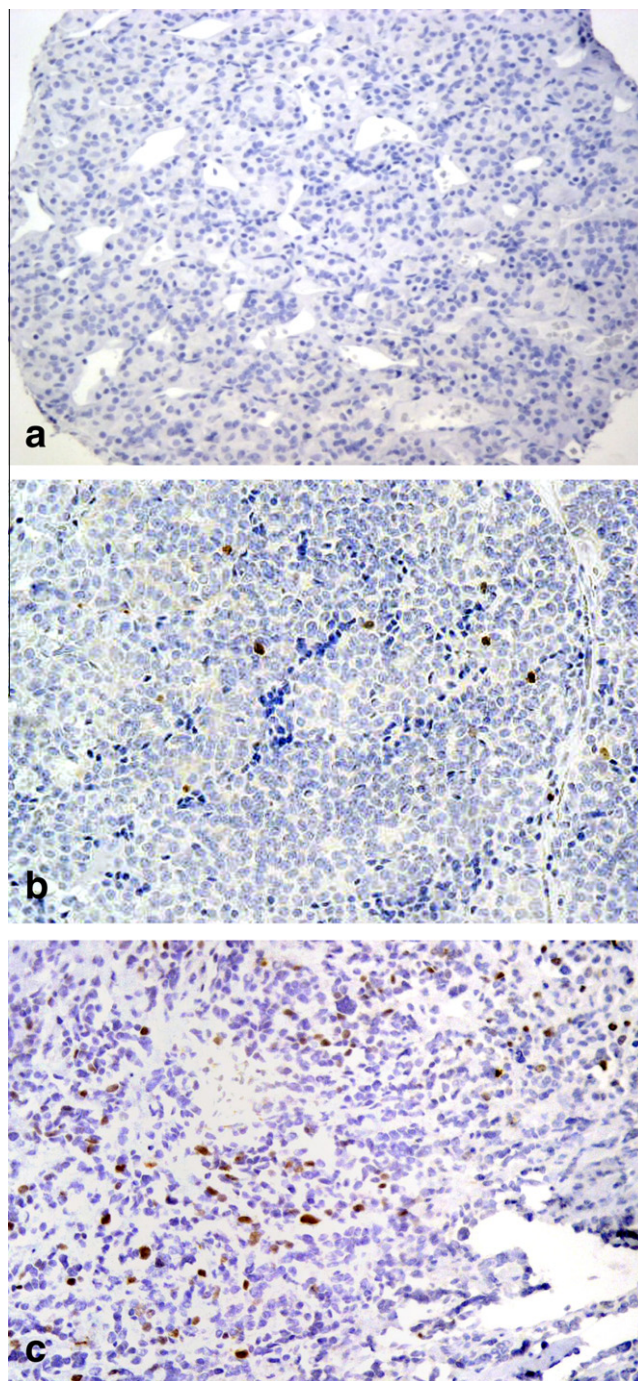

Figure 1 Cyclin D1 expression in human pituitary tissues. (a) Normal anterior pituitary tissue with negative staining for cyclin D1 [X 200]. (b) Null cell adenoma showing scattered cells expressing cyclin D1 [X 400]. (c) PRL cell carcinoma with 4+ cyclin D1 expression [X 400].

(Cushing and Nelson adenomas), no significant difference was observed regarding cyclin D1 expression (*p* = 0.893). There is a significant relationship was noted between Cyclin D1 expression and adenoma size (*p* = 0.017), while no significant difference between Cyclin D1 expression and adenoma invasion (*p* = 0.067) (Table 4).

Table 4 Cyclin D1 expression by pituitary adenomas clinical characteristics.

Clinical characteristics	Protein expression n of cases (%)					Statistical analysis* (significance)
	0	1+ [LI 0.4–<0.6]	2+ [LI 0.6–<1.5]	3+ [1.5–<1.8]	4+ [LI 1.8–2.5]	
Recurrence						
Recurrent	4 (25)	2 (12.5)	0 (0)	6 (37.5)	4 (25)	$p < 0.001$
Non-recurrent	99 (57.9)	19 (11.1)	27 (15.8)	18 (10.5)	8 (4.7)	(H.S.)
Functional status						
Functioning	42 (56)	11 (14.7)	20 (26.7)	1 (1.3)	1 (1.3)	$p < 0.001$
Non-functioning	56 (54.9)	8 (7.8)	4 (3.9)	23 (22.5)	11 (10.8)	(H.S.)
Size						
Macroadenomas	88 (53.3)	20 (12.1)	21 (12.7)	24 (14.5)	12 (7.3)	$p = 0.017$
Microadenomas	15 (68.2)	1 (4.5)	6 (27.3)	0 (0)	0 (0)	(S.)
Invasion						
Invasive	56 (48.7)	12 (10.4)	19 (16.5)	17 (14.8)	11 (9.6)	$p = 0.067$
Non-invasive	47 (65.3)	9 (12.5)	8 (11.1)	7 (9.7)	1 (1.4)	(N.S.)

* Pearson chi-square test. H.S.: highly significant; S.: significant; N.S.: non-significant.

MIB-1 and p27 LIs

The normal pituitaries studied showed nil to trivial MIB-1 expression with LI ranged between 0% and 0.04%. The median MIB-1 LI among studied adenomas was 0.37% and ranged between 0% and 9.76%, while the five studied carcinomas showed median MIB-1 LI of 2.47% and ranged between 0.6% and 7.5%. Regarding p27 expression, a wide range of LI was noted among normal pituitaries (median 0.77; range 0.02–21. 39), adenomas (median 2.95; range 0.01–82.97), and carcinomas (median 1.76; range 0.54–3.39) (Figs. 2 and 3).

Relationship between cyclin D1 and MIB-1 LIs

A significant positive relationship was noted between cyclin D1 and MIB-1 labeling indices among adenomas ($r = 0.619$, $p < 0.001$) and carcinomas ($r = 0.894$, $p = 0.041$). This positive relationship between cyclin D1 and MIB-1 LIS was consistently noted among all the studied adenoma subgroups divided according to immunophenotyping [PRL ($r = 0.879$, $p < 0.001$), GH ($r = 0.849$, $p < 0.001$), ACTH ($r = 0.854$, $p < 0.001$), LH/FSH ($r = 0.781$, $p < 0.001$), and Null ($r = 0.895$, $p < 0.001$)]. Also, this significant relation was observed upon dividing adenomas according to size [macroadenomas ($r = 0.663$, $p < 0.001$), and micro-adenomas ($r = 0.612$, $p = 0.002$)], functional status [functioning ($r = 0.675$, $p < 0.001$), and non functioning ($r = 0.79$, $p < 0.001$)], Invasive status [invasive ($r = 0.629$, $p < 0.001$) and non invasive ($r = 0.601$, $p < 0.001$)], and finally according to recurrence [recurrent ($r = 0.625$, $p = 0.01$) and non recurrent ($r = 0.615$, $p < 0.001$)].

Relationship between cyclin D1 and p27 LIs

No significant relationship was noted between cyclin D1 and p27 LIs among either adenomas ($r = 0.059$, $p = 0.424$) or carcinomas ($r = -0.112$, $p = 0.858$).

Discriminating cut-off value for cyclin D1, and MIB-1 labeling indices between adenomas and carcinomas

Although the number of studied pituitary carcinoma cases is only five owing to the extreme rarity of the documented cases, an attempt to mark out cut-off values to discriminate carcinomas from adenomas with reasonable specificity and sensitivity was conducted for the studied markers namely cyclin D1. The ROC curves created for this purpose are shown in Fig. 4. The best cut-off cyclin D1 LI detected was 1.7% at which the sensitivity (Sn) was = 80; the specificity (Sp) was = 93.6; the negative predictive value (P−) was = 99.4, the positive predictive value (P+) was = 25 and the efficacy was = 93.23. When MIB-1 LI was investigated, a cut-off value of 0.59 was advocated but with overall efficacy of only 70.31 (Sp = 69.52, Sn = 100, P− = 100, P+ = 8.06). p27 LI was totally unreliable in this tedious attempt to segregate carcinomas from adenomas. Multi-ROC curve analysis reveal that combined best cut-off for cyclin D1 and MIB-1 LIS at 1.7 and 0.55 respectively offered slightly higher accuracy compared to cyclin D1 only (Sn = 100, Sp = 94.7, P− = 100, P+ = 33.3, and efficacy = 94.8).

Discussion

This study provides further evidence that deregulation of genes involved in the G1-S phase transition is important in pituitary tumorigenesis. Cyclin D1 protein was frequently over expressed in human pituitary tumors compared to the normal pituitary, where no staining was observed in the latter.

The majority of pituitary tumors showed either negative immunostaining for cyclin D1 or its presence in less than 10% of the cells. It should also be noted that all positive pituitary tumors showed staining confined to the nucleus. This is in agreement with previous studies [16,17]. However, this disagrees with another study group [18] who found cytoplasmic staining for cyclin D1 in the U-2-OS pituitary cell line. They mentioned a differential solubility of cyclin D1 protein to ex-
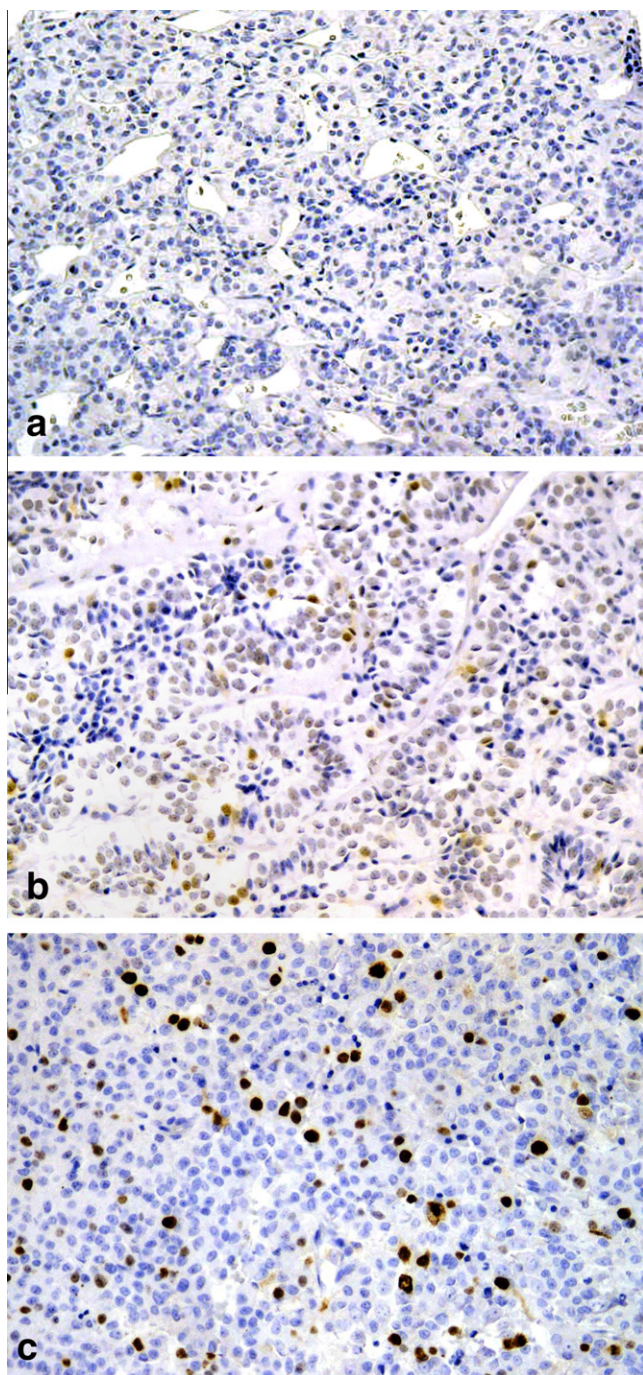
Figure 2 MIB-1 expression in normal anterior pituitary tissue (a), TSH adenoma (b), and PRL cell carcinoma (c) [All X 400].

Figure 3 p27 Expression in LH/FSH adenoma (a), and LH/FSH carcinoma (b) [All X 400].

plain the apparent different subcellular localization. The importance of cytoplasmic cyclin D1 expression in tumors showing this phenomenon is not understood.

We were able to demonstrate that pituitary tumors as a group had a large number of samples with significant cyclin D1 positivity compared with normal pituitary. Overall, positive nuclear expression was seen in 84 of 187 (44.9%) pituitary adenomas and LI for all the positive cases of pituitary tumors were mostly graded either 2+ or 3+. The cyclin D1 positive expression occurred with sub-group analysis and principally
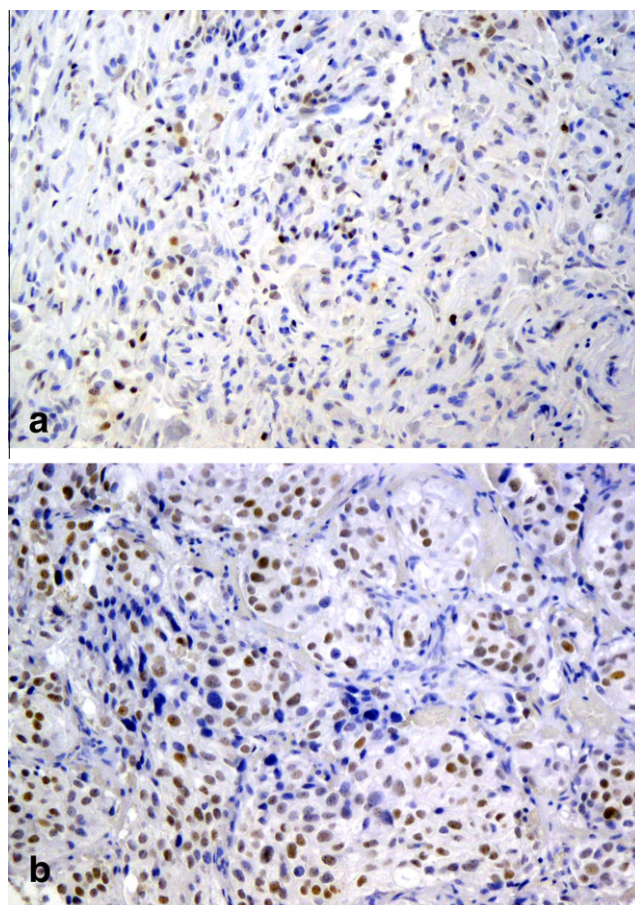
due to the non-functioning tumors mainly null cell adenomas; indicating that the inappropriate expression of cyclin D1 may play a more important role in nonfunctional pituitary tumors than in functioning tumors. This is in agreement with other studies which demonstrated that the expression of different genes or proteins is altered during tumorigenesis of different pituitary subtypes [16,19,20] with some evidence of cyclin D1 allelic imbalance in one fourth of the tumor samples analyzed by Hibberts and colleagues [13].

Non-functioning pituitary tumors are generally larger than other types [21,16], the current study could establish a significant relationship between cyclin D1 expression and adenoma size. Therefore the noted difference between null cell adenomas and other adenoma types is likely to be related to the immunophenotype as well as the adenoma size. These results are in accordance with that of Turner et al. [14].

Amplification of cyclin D1 gene (CCND1) is more common in invasive than in noninvasive pituitary adenomas [22]. In our study, the expression of cyclin D1 was noted in 51.3% of invasive adenomas compared to only 34.7% of non invasive ones but this difference did not reach statistical significance. Pituitary carcinomas showed a significantly higher expression of cyclin D1 compared to adenomas. However, Jordan and his group in 2000 [16], found that pituitary carcinomas did not appear to differ from the more benign tumors. They found that combining the groups according to tumor grade (adenoma,
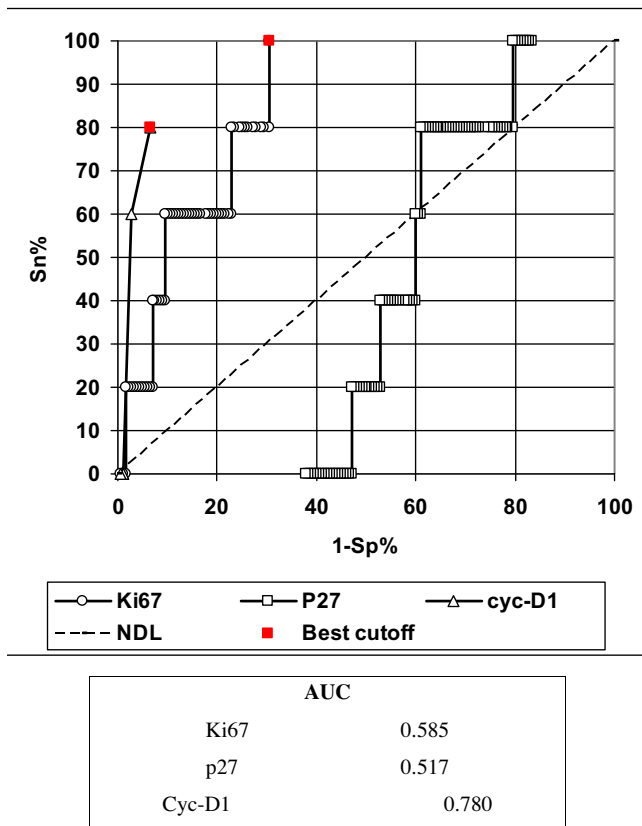
AUC	
Ki67	0.585
p27	0.517
Cyc-D1	0.780

Figure 4 ROC curve analysis showing the diagnostic performance of Ki67, p27 and cyc-D1 for discriminating patients with carcinoma from those with adenoma.

aggressive adenoma and carcinoma) showed a significant group effect.

Previous studies have also shown that in colorectal [23], breast [24] and lung tumors [25], cyclinD1 expression is an early event in tumorigenesis and is maintained in more advanced stages of the disease. Two studies suggested that over expression of cyclin D1 is an early event in tumorigenesis responsible for initial clonal expansion of the tumor, whilst subsequent loss of pRb expression results in complete disruption of this G1 check point, thereby giving an additional growth advantage to tumor cells [26,27].

Previous studies, especially on the cell cycle regulation, have shown that deregulation of the cyclin D/cyclin-dependent kinases (CDK)/CDK inhibitor /pRb pathway in the G1/S phase may represent an obligatory step in pituitary tumorigenesis [28,29]. However in addition to amplification, inappropriate cyclin D1 expression may be due to another principal mechanism which is cytogenic inversion as described in parathyroid tumors [30]. This is further highlighted in the few reports on the cyclin D1 overexpression and its genetic alteration in pituitary adenomas suggesting that the overexpression of cyclin D1 occurs early and late in pituitary tumorigenesis and this is not necessarily associated with cyclin D1 gene allelic imbalance [13,16,31].

In our study, a significant positive correlation was noted between cyclin D1 and tumor recurrence, which was previously described in squamous cell carcinoma of the head and neck [9]. Conversely, cyclin D1 over expression was associated with a lower rate and/or interval to recurrence in breast, bladder and non-small lung cancer. The conflicting data regarding the correlation between cyclin D1 over expression and tumor recurrence support that the influence of gene polymorphism is perhaps dependent on tumor type [17].

Theoretically, proliferation markers should help in differentiating aggressive or rapidly growing tumors from those with slower growth, as cellular atypia is not helpful for identifying aggressive adenomas of the pituitary [32]. In our study; a significant positive correlation was noted between cyclin D1 and MIB-1 LIs among adenomas and carcinomas. This positive correlation was consistently noted among all the studied adenoma subgroups. This positive correlation suggests that a constant ratio of cells that have entered the cell cycle continue to proliferate. Therefore the signal to cyclin D expression is strong enough to override the G1/S checkpoint where cells then become 'committed' to the cell cycle. This confirms the hypothesis that cyclin over-expression is secondary to an increased number of cells entering the cell cycle and therefore increased proliferation rather than cell cycle deregulation [16]. These results are in agreement with studies showing a correlation between cyclins expression, cell proliferation and tumor progression [14,33,34].

Both cyclin D1 and MIB-1 but not p27 LIs were reliable predictors of carcinoma. We displayed that combined cyclin D1 LI higher than 1.7% and MIB-1 LI higher than 0.55% were capable of predicting carcinoma among studied pituitary tumors with overall efficacy of 94.8% though with a low (33%) predictive value of positive test. This means that only one third of the cases that have combined cyclin D1 and MIB-1 LIs above 1.7% and 0.55%; respectively are truly carcinomas. These cut off levels were excellent in excluding a given tumor from the carcinoma group with zero false negative prediction. In other words, a tumor having combined cyclin D1 and MIB-1 LIs less than 1.7% and 0.55% respectively are in this series exclusively adenomas.

Some groups have investigated p27 in pituitary tumors [35–37]. In the current study, we failed to demonstrate a significant inverse correlation between cyclin D1 and p27 LIs. No available studies, to date, have investigated the parallel expression of both markers in pituitary tumors, while a previous study demonstrated that over expression of cyclin D1 and loss of p27Kip1 was associated with carcinomas of thyroid gland [35,38].

Given the relevance of cell cycle regulators in the correct function of stem cells [39–41]; it is attempting to speculate on the relevance of the cell cycle in pituitary stem cell self-renewal and its implication in pituitary tumors. The observed deregulation of the cell cycle in pituitary has important consequences in the treatment of these tumors. Several small molecular CDK inhibitors are now being evaluated for cancer therapy in many different tumor types [42,43]. Newer techniques including DNA and microRNA microarrays will undoubtedly provide new candidates important to the development and progression of pituitary tumors. In this context, our results recommend cyclin D1 as a novel marker that deserve more studies among pituitary tumors to elucidate its possible rule and perhaps its further therapeutic implication.

Conclusion

Cyclin D1 is promising in predicting the clinical behavior of pituitary tumors as regards adenoma recurrence and perhaps carcinoma development. Whether cyclin D1 expression is a primary event in pituitary tumor initiation and progression or is secondary to other tumorigenic factors is unclear and requires further investigation.

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