Immunohistochemical Detection of P21 and P27 Expression in Uterine Tumors

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Abstract

Background: Several studies assessed gene and protein expression of p21 in endometrial carcinoma(EC), and mention that P21 represent an important participant in EC Cell invasion and metastasis ,while some researchers indicat that there were no apparent differences in immunostaining for p21.Other studies found that p27 expression significantly reduced in the endometrial carcinoma and inactivation ofP27 proteins is a specific feature in the progression of this cancer. **Methodology:** This study has used Immunohistochemistry for detection the gene expression of p21 and p27 in tissue specimens from 70 hysterectomized patients diagnosed with malignant uterine tumors (30 cases), non-malignant uterine tumors (25 cases),and 15 cases as control tissues groups.

Results: The results of moleculare detection of P21 revealed12 (40%) in malignant uterine tumor 8(32%) non-malignant uterine tumor and 5(33.3%) in control groups and low expression of P27 revealed in all groups :5 (16.7%) in malignant uterine tumor 6(24%) non-malignant uterine tumor and 2(13.3%) in control groups.

Conclusions: Decrease in expression of P21 was found mostly in malignant endometrial uterine tumors and this expressions occur could have correlated to the an early events of their tumorgenesis while low expression of P27 in hysterectomized patients mostly appear in non-malignant uterine tumors at the endometrial sites.

Keywords: P21, P27, Uterine Tumors.

الخلاصة

عدة دراسات قيمت التعبير الجيني ل p21 في سرطان بطانة الرحم واشارت الى ان p21 يمثل مساهم مهم في اجتياح و انتقال سرطان بطانة الرحم, في حين اثبت بعض الباحثين عدم ظهور اختلاف في التصبيغ المناعي لل p21.وجدت دراسات اخرى اختزال ملحوظ في تعبير p27 وان التثبيط في فعالية بروتين ال p27 صفة مهمة في تقدم هذا النوع من السرطان .**طريقة العمل:** اعتمدت هذه الدراسة تقنية Immunohistochemistry للكشف عن التعبير الجيني ل p21,p27 في عينات الأنسجة الماخوذة من 70 مريضة اجريت لها عملية استئصال الرحم منها: (30 حالة) شخصت ضمن الأورام الخبيثة للرحم و(25حالة) ضمن أورام الرحم غير الخبيثة و(15 حالة) كمجموعة انسجة السيطرة.**النتائج:** اظهرت الدراسة بان التعبير الجيني ل p21 كان (40 %) 12 من مجموعة أورام الرحم الخبيثة و(32٪) في ثمان حالات في المصابات بأورام الرحم غير الخبيثة. اما في أنسجة مجاميع السيطرة، تم الكشف عن خمس حالات فقط (33.3٪). اما التعبير الجيني ل p27 كان (16.7%)5 من مجموعة أورام الرحم الخبيثة و(24٪) في ست حالات في المصابات بأورام الرحم غير الخبيثة. وفي حالتين (13.3%) من أنسجة مجاميع السيطرة. **الاستنتاج:** اغلب الانخفاض في التعبير الجيني ل p21 كان في مجاميع سرطانات الرحم الخبيث وممكن ان يكون له علاقة بالمراحل الاولى للسرطان,في حين p27 اظهر انخفاض في التعبير الجيني اغلبه كان ضمن مجاميع سرطانات الرحم غير الخبيث في بطانة الرحم.

الكلمات المفتاحية: بي21، بي27، ورم الرحم.

Inroduction

Cyclin dependent kinase inhibitors (Cdks) belong to 2 families(1)The inhibitors of Cdk4 (INK4) family ($P16^{2NK49}$, $P15^{2NK4b}$, $P18^{2NK4C}$, and $P19^{2NK4d}$), which inhibit CdK4 and Cdk6,and (2)Cip/Kip family ($P21^{WAF/Cip1}$, $P27^{Hip1}$, and $P57^{Kip2}$), which exhibit a boarder range of inhibition (Sherr & Roberts, 1999).

P21 protein play important roles in a wide range of cellular process, including cell morphogenesis mortality, survival, cell cycle progression, angiogenesis, cell invasiveness and transformation. Also regulation of cytoskeleton activate C-jan

NH2terminal Kinase and extra cellular signal-regulated Kinase, thus influences nuclear signaling**(** Balasenthil *et al.,* 2004; Siu *et al.,* 2010)

P21 can be regulated via many pathways, include:The tumor suppressor P53, activities P21 expression by binding to its promoter,oncogene MYC,and E-box-binding proteins(Abukhdeir & Park 2009**).**

The encoding gene ($P27^{Kip1}$) , play role in regulating the progression from G1 to the S-phase.The P27 gene has a DNA sequence similar to other member of the "Cip/Kip" family and similar functional characteristic of being able to bind several different classes of cyclin A ,CDK2 ,and cyclin D-CDK4 complexes (Denicourt & Dowdy, 2004). Authors investigated the clinical significance of p21 expression and its functional roles in Ec (Lu *et al.,* 2013) by promote epithelial hyperplasia throught phosphorylation and transactivation of estrogen receptor α-(ER- α) that involved in the pathogenesis of Ec (Di Cristofano & Ellenson, 2007) Also several studies indicated that P21 expression was associated with the progression of Ec and involved in rapid proliferation of cancer cells through the Nf-Kp dependent pathway (Saegusa *et al.,* 2012).

In addition P21 represent an important participant in Ec Cell invasion and metastasis by reorganization through several reported substrates, such as phosphorylates LIM Kinase (Kichna *et al.,* 2010) Moreover ,P21 anchorage-independent growth and protects Ec cell from apoptosis induced by TNf- α via caspase-3 activation (Lu *et al.,* 2013).

A study found that p27 expression was present in the proliferative ,secretory phases; and In complex hyperplasia with atypia, but significantly reduced in the endometrial carcinoma (Özkara *et al.,* 2004). Other study found that inactivation of PTEN/$P27^{Kip}$ proteins is a specific feature in the progression of endometrial carcinoma (Bansal *et al.,* 2009).

This study amid to Evaluation of the expression functionally tumor suppressor genes (i.e. p21and p27) among patients hysterectomized for cancers in their tissues using immunohistochemistry technique.

Materials and Methods

1-Subjects (Patients Tissue Samples)

This retrospective study has enrolled seventy (70) cases represented by 158 selected formalin fixed paraffin embedded uterine tissues blocks were belonging to patients who had undergo hysterectomy.For each patients we were chose blocks from endometrium, myometrium ,polyp,fibroid as well as cervix and these blocks were collected from the archives of histopathology laboratories at teaching Laboratories in medical city, Al-Yarmok teaching hospital and private laboratories. These samples were related to the period from 2012 to 2014. The study tissues group comprised thirty cases represented by 66 malignant uterine tumor,25 non-malignant uterine tumors represented by (62 samples),and 15 control tissues group represented by 30 samples. Immunohistochemical method was used to demonstrate the product of gene expression of P21 and P27 in those uterine tumors tissue and was done according to the manufactoring company(Abcam/UK,Code No. ab80436). This kit used for detection of :Anti-P21 antibody(ab18209) and Anti-P27antibody (ab54563).

Evaluation of IHC results: Proper use of this IHC detection system will given an eintense brown precipitate in positive cell on tissue sections.IHC was given an intensity grading of the positive signals and scoring of the number of cells contain these signals.

I-P21: for cytoplasmic (P21) expression, the staining intensity was scored in the following manner: 0= negative ,1= weak ,2=moderate ,3= strong .And the staining percentage was scored as: 0=0-5% ,1= 5-25% ,2= 25-75% ,3= 50-75% .And 4= 75-100%.We obtained a composite histoscore by multiplying the value of the 2 parameters percentage epithelium stained x stain intensity: 0-4weak, 5-8moderate, 9-12strong (Lu, 2013) .

II. P27: For P27 labeling analysis, only nuclear staining was defined as positive and visually counting up to 500 nuclei using high power (x 40 at 8-10 fields), the average of immunopositive nuclei of 10 fields were determine .The finding were recorded as the percentage of immunopositive nuclei, and graded as:0(negative),1(<10%),2(10-50%),3(>50%) ,the staining intensity was scored as: 0=negative,1= weak,2=moderate,3= strong (Dellas *et al*., 2009).

Results

1- Detection of IHC staining for P21 in the endometrial tissues among hysterectomized patients:-

The P21 protein staining was captured in endometrial glandular epithelial cell cytoplasm, and the results were as follows:12 cases (40.0%) among malignant uterine tumors,8 cases (32.0%) among non-malignant uterine tumors, and 5 cases (33.3%) among control tissues group. No significant differences (P>0.05) were found among the study groups (Table1).

Table(1):IHC-results of P21 expression in the endometrial lesions .

Endometrial-IHC signal results		Malignant uterine tumors		Non-malignant uterine tumors		Control uterine tissues	
		No	%	No	%	No	%
P21-IHC Signal results	Positive	12	40.0	8	32.0	5	33.3
	Negative	18	60.0	17	68.0	10	66.7
	P compared to NT	0.664		0.066		-	
	P compared to Con	0.539		-		-	

Significant difference between proportions using Pearson Chi-square test at 0.05 level.
P:p-value,
NT:Non-malignant uterine tumors,
Con:Control uterine tumors

High percentage (33.3%) of score 3 was found among malignant uterine tumors with predominated moderate intensity that constituted (20%), while score 2 was predominated among non-malignant tumors (20%) with predominated moderate intensity was predominated which constituted (20%).No significant differences among the study groups were noticed.(Table 2) (Figure1,2) .

Table (2): Distribution of IHC-results of P21according to their signal scoring &intensity in the endometrial lesions tiusse.

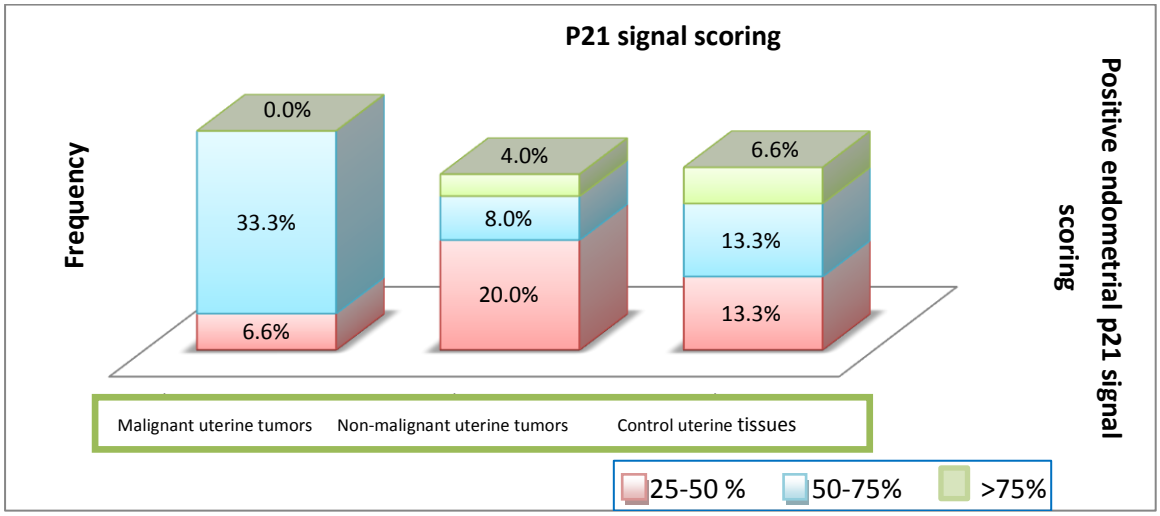
Pathological Type	IHC Negative results	IHC Positive results	Signal score*				Signal Intensity**			P value x Control uterine tissue	P value x non malignant uterine tissue
			Score1	Score2	Score3	Score4	L	M	H		
Malignant uterin tumors (30)	18 60%	12 40%	0	2 6.6%	10 33.3%	0	1 3.3%	6 20%	5 16.6%	0.664	0.539
Non-malignant uterin tumors (25)	17 68%	8 32%	0	5 20%	2 8%	1 4%	2 8%	5 20%	1 4%	0.931	
Control uterine tissues (15)	10 66.66%	5 33.3%	0	2 13.33 %	2 13.33 %	1 6.6%	0	2 13.3 %	3 20%		

Significant difference between proportions using Pearson Chi-square test at 0.05 level.

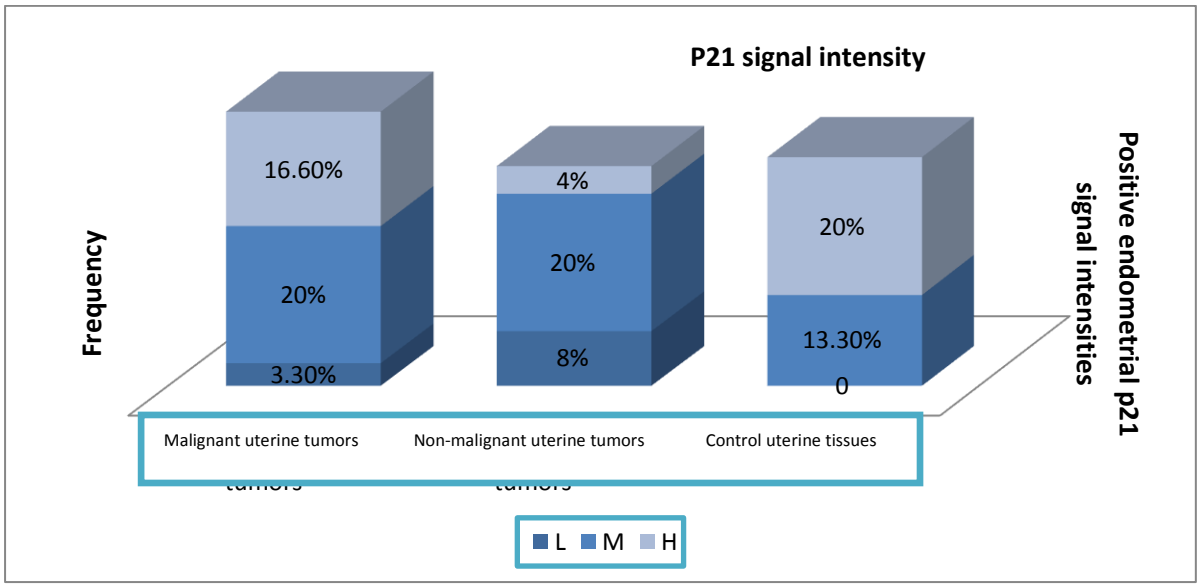
*Score 1(<25%),Score2(25-50%),Score3(50-75%),Score4(>75%)

**L = Low intensity, M = Moderate intensity,H = High Intensity

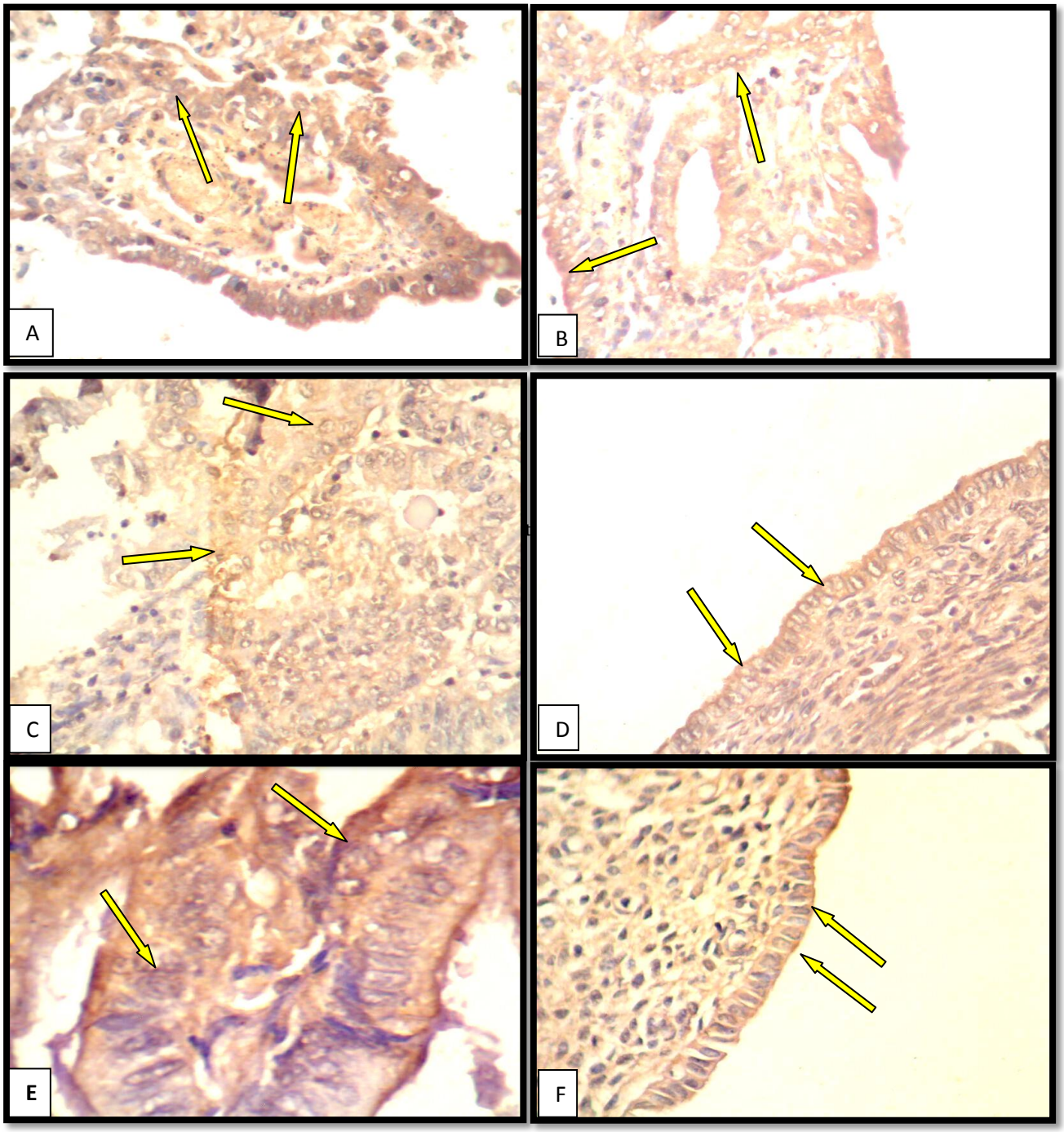
A-



B-



Figure(1):A-Frequency distribution of IHC-results for P21 according to signal score in the endometrial lesions ,B- Frequency distribution of IHC-results for p21 according to signal intensity in the endometrial lesions.



Figure(2):Microphotographs of IHC positive staining for p21 in cell cytoplasm(yellow arrow) of glandular tissue in (A,B)endometrial carcinoma show score 3 with high intensity (400x) (C) endometrial carcinoma show score 2 with low intensity (1000x) (D)non-secretory endometrial gland associated with polyp show score3 with high intensity(400x)(E) non secretory endometrial gland show score 2 and Moderate intensity (1000x) (F)secretory endometrial gland show score 2 and low intensity(400x).

2-The association between P21 Protein expression, TNM/FIGO staging system and grading of endometrial carcinoma .

Eight (38.1%) cases revealed positive expression of p21 in T1/IB stage lesions,4(44%) cases revealed in T2/IIB stage ,and 10 (37.0%) of cases had well differentiation grade (figure:3&4)

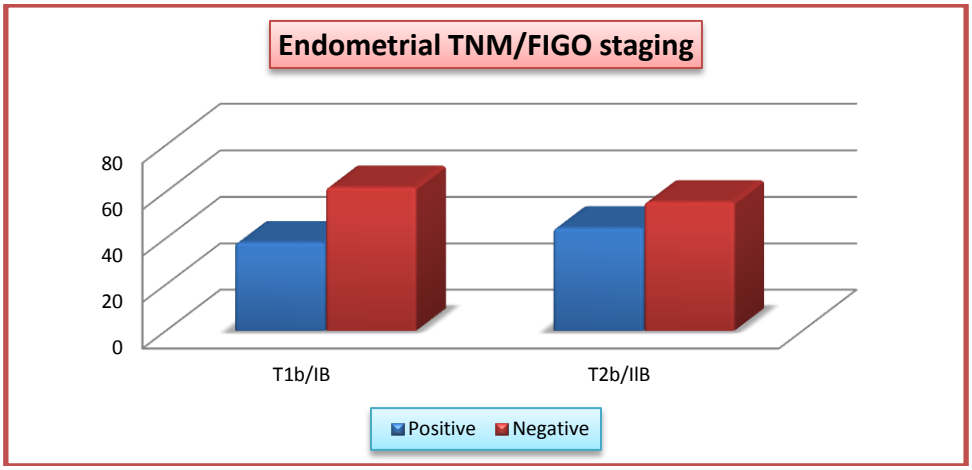


Figure (3): Distribution of p21 positive expression according to TNM/FIGO staging of the endometrial cancers.

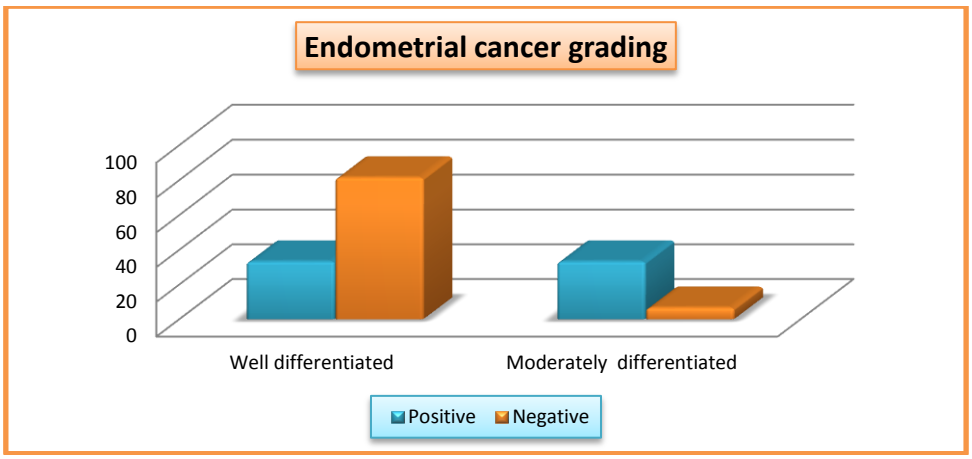


Figure (4): Distribution of p21expression according to the grade of endometrial cancer.

2- Detection of IHC staining for P27 in the endometrial tissues among hysterectomized patients:-

The P27 protein staining was captured cellular nuclei of the endometrial glandular epithelium tissues.Five cases(16.7%) of malignant uterine tumors showed p27 expression while Six cases (24.0%) of non-malignant uterine tumors and two cases (13.3%) of control groups showed such p27 expression.

No significant differences (P>0.05) were found among study groups (Table 3).

Table(3):IHC-results of P27 expression in the endomterial lesions .

Endometrial IHC results		Malignant uterine tumors		Non-malignant uterine tumors		Control uterine tissues	
		No	%	No	%	No	%
P27- IHC signal results	Positive	5	16.7	6	24.0	2	13.3
	Negative	25	83.3	19	76.0	13	86.7
	P compared to NT	0.771		0.624		-	
	P compared to CON	0.498		-		-	

Significant difference between proportions using Pearson Chi-square test at 0.05 level.
P:p-value .
NT:non-malignant uterine tumors .
CON:Control

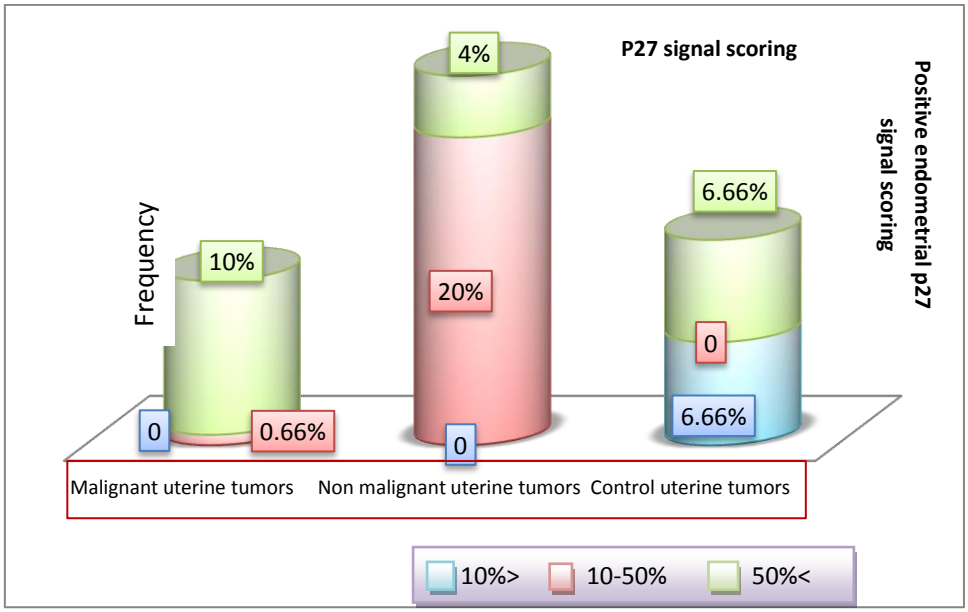
In malignant uterine tumor the highest percentage observed (10%) of score 3 with intensity predominated constituted (10%), and in non-malignant tumor (20%) of score 2 with moderate intensity was predominated constituted (20%) .There were no differences (P>0.05) according to score and intensity between the study groups (Table 4)(Figure 4,5).

Table (4): Frequency Distribution of IHC-Test for P27 according to Signal Score & Intensity in the endometrial lesions.

Pathological types	Negative signaling	Positive signaling	Signal score*			Signal intensity**			Pvalue x Control uterine tissue	P value x non-malignant uterine tumor
			Score 1	Score2	Score3	L	M	H		
Malignant uterine tumors (30)	25 83.3%	5 16.6%	0	2 0.66%	3 10%	1 3.3 %	1 3.3%	3 10%	0.771	0.498
Non-malignant uterine tumors (25)	19 76%	6 24%	0	5 20%	1 4%	0	5 20%	1 4%	0.414	
Control uterine tumors 15	13 86.6%	2 13.33%	1 6.66%	0	1 6.66%	0	2 13.3%	0		

Significant difference between proportions using Pearson Chi-square test at 0.05 level.
*Score 1(<10%), score 2 (10-50%) , score 3(>50%)
**L = Low intensity, M = Moderate intensity,H = High Intensity

A-



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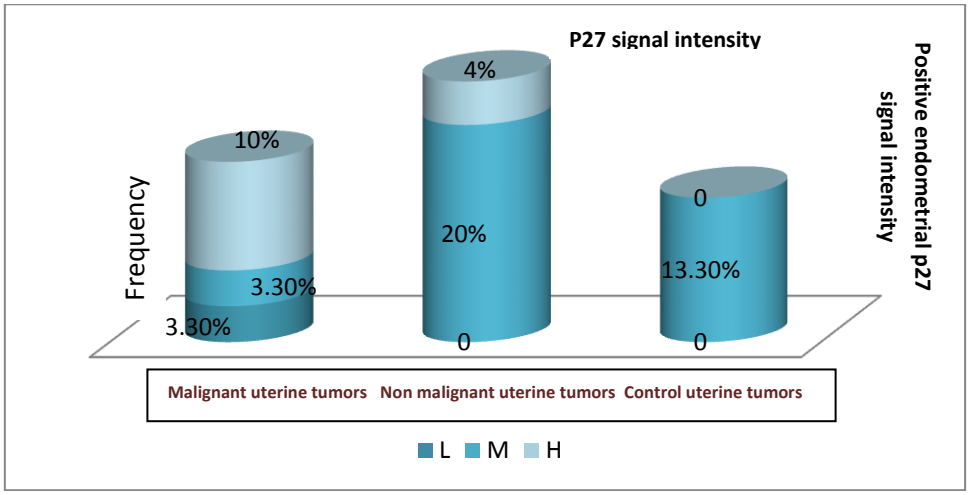
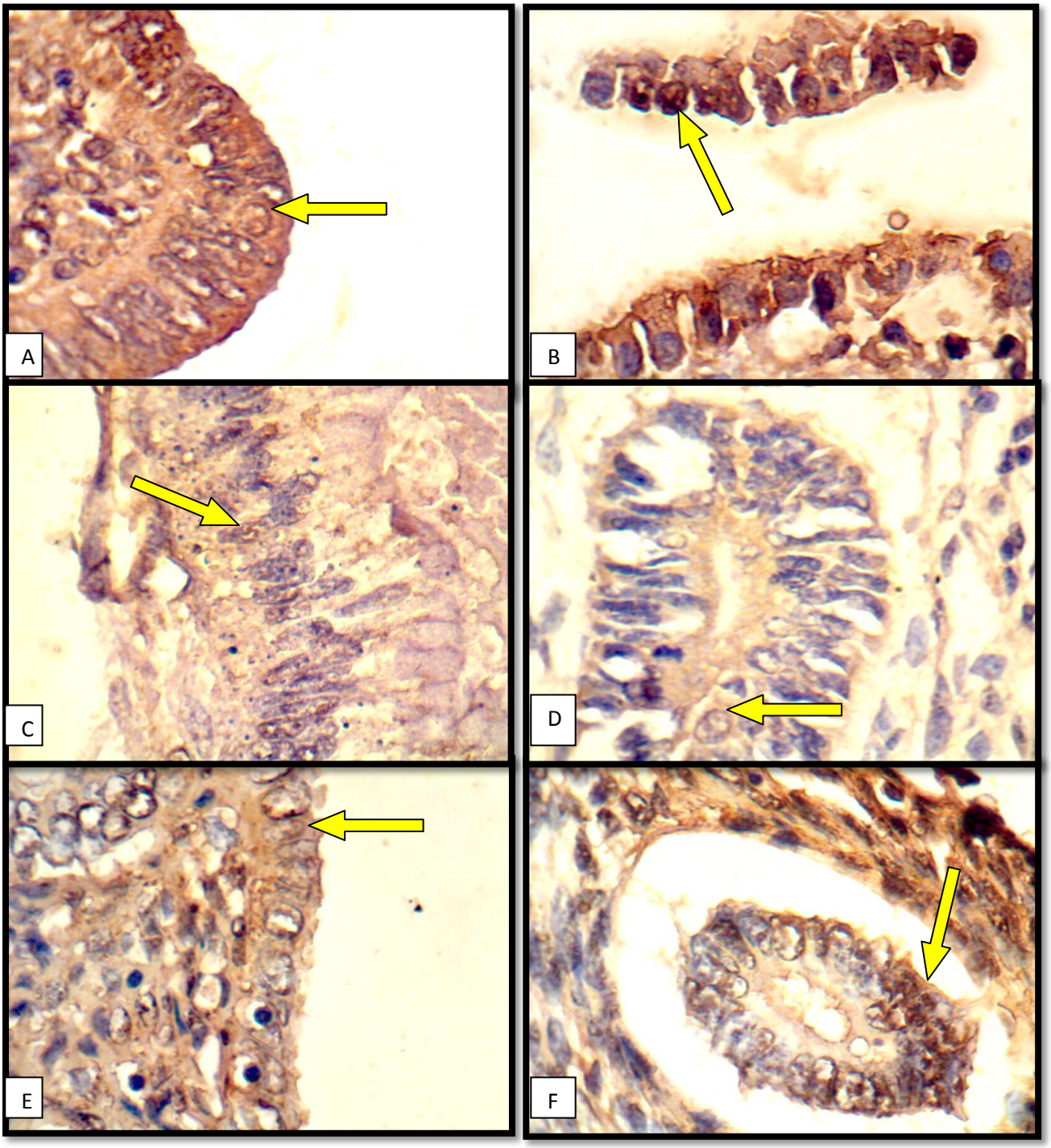


Figure (4): A- Frequency Distribution of IHC-results for P27 according to Signal Score in the endometrial lesions, B- Frequency Distribution of IHC-results for P27 according to Signal intensityin the endometrial lesions.



Figure(5):Microphotographs of IHC positive staining for p27 in cell nuclei(yellow arrow) of the glandular tissues in (A,B)Endometrial carcinoma shows score 3 with high intensity (C)Endometrial carcinoma shows score 2 with low intensity(D) Non secretory endometrial gland associated with fiboid shows score3 with low intensity (E)Non secretory endometrial gland show score 3 and moderate intensity (F)Secretory endometrial gland show score 2 and hight intensity (1000x).

2- The association between P27 protein expression and TNM/FIGO system and grading of endometrial carcinoma :

Three cases (14.3%) with T1/BI revealed P27 gene expression and two Cases (22.2%) with T2/BII while four cases(14.3%) of endometrial carcinoma that had well differentiation expressed such P27 protein (Figure:6, 7).

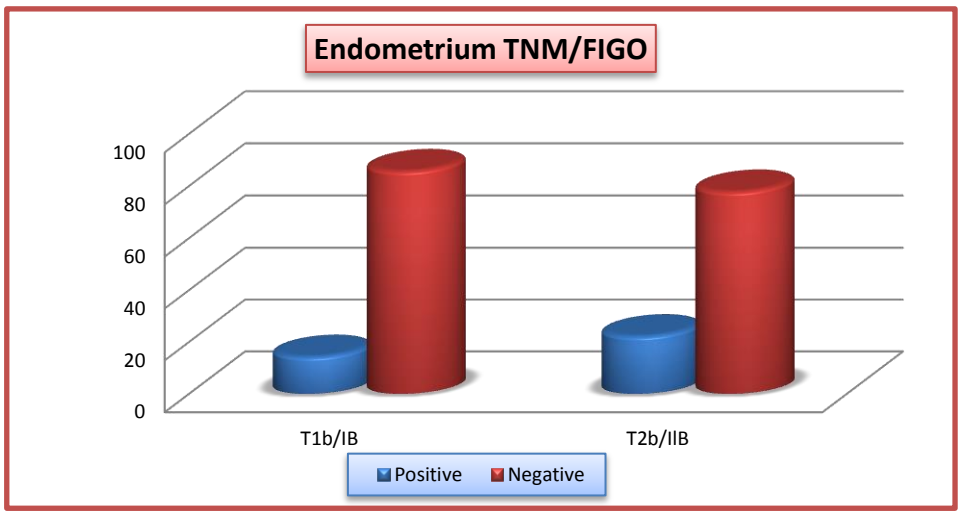


Figure (6): Distribution of p27 positive expression according to TNM/FIGO staging of the endometrial carcinoma.

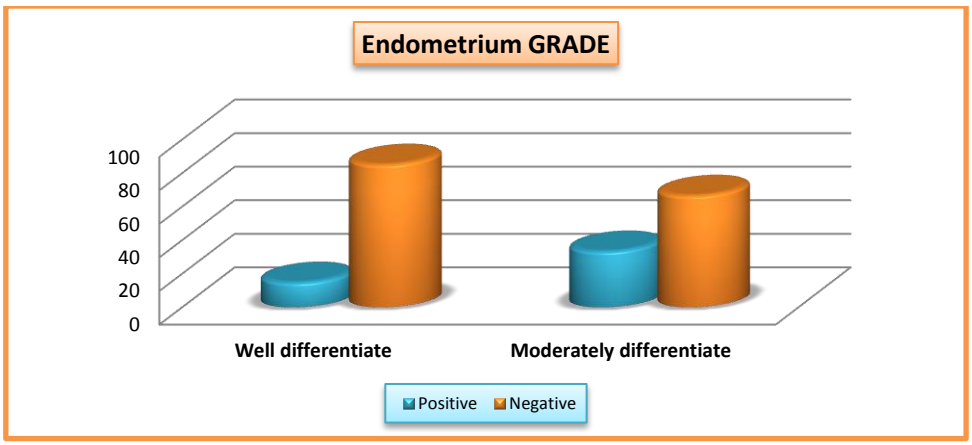


Figure (7): Distribution of p27 expression according to the grade of endometrial cancer.

Discussion

I- Detection of P21 expression in endometrial lesions (Table 1&2)(Figure 1&2)

In the present study ,a decreased expression of P21 was detected by IHC technique in cytoplasm of epithelial cells and mostly has been revealed in the malignant uterine tumors as compared to the other two groups with no significant associations among the study groups. Our findings are in agreement with the results of(Palazzo *et al.*, 1997; Palazzo *et al.*, 2000; Semczu *et al.*, 2003 ; Ignatov , 2008; Felix *et al.*, 2015) . They have revealed that a decreased expression of P21 has no independent influence on the endometrial carcinoma and they also reported that P21 have consistent relationship with tumor characteristics among women with endometrial carcinoma where they are supported also by the studies done in china by Lu w *et al.*, 2013 and in Egypt by Abdelmonem & Abdelmajed, 2008 were they concluded that P21 has involved in activation cell growth of endometrial carcinoma and could be useful marker for prognosis.

The expression pattern and function of P21 in endometrial carcinoma remain unknown. Several studies indicated that inactivation of inhibitory factors (CDK inhibitors or pRb) have potential to disturpt the cell cycle that lead to an intial uncontrolled cell proliferation (Fleix *et al.*, 2015). Also aberrant expression of the CDK inhibitors has been frequently characterized in endometrial carcinoma cases (Milde-Langosch & Riethdorf, 2003) and decreased expression of P21 is associated with promotion of tumor formation and poor prognosis in many types of cancer (Abukhdeir & Park, 2009). Elbendary *etal*, 1996 and Migaldi *etal*, 2000 , noted a correlation between P53 mutation and absent or reduce P21 expression consistent with the hypothesis that P53 is an important regulator of P21 expression.Others mention that failure of mutant P53 protein to transactivate P21 may lead to uncontrolled proliferation scince P21 molecule was identified as a mediator of P53-dependent growth suppression and wild type P53 induces the expression of P21, which blocks the progression of cell cycle at G1/S transition by inhibitory (CDKs) (Scian *et al.*, 2004) Other study mention that E7 oncoprotein of HPV can contarget the P21 for degradation during carcinogenesis (Tagle *et al.*, 2014).

Also the present study has revealed a low percentage of expressied of P21 in the early FIGO stage (T2/IIB) (44.4%) and (T1/IB) (38.1%) and in well differentiation grade (33.3%) (Figures 3&4) make a suggestion that low expression of P21 could indicated for a initial tumor event but not progression of the carcinogenesis and has no association with clinicopathological parameters (type, stage and grade) and as supposed by (Fleix *et al.*, 2015).

II- Detection of P27 expression in endometrial sites among hysterectomized patients(Table 3,4)(Figure 5,6):

The results in the present study revealed low expression of P27 in the tissues in all study groups and these results are in agreement with several studies that found a loss or absence in the expression of p27 an important step in promoting the tumor growth(Watanabe *et al.*, 2002, Özkara *et al.*, 2004) .

Our study has revealed a low expression of P27 in the endometrial carcinoma tissue group which was already observed in well differentiated malignant cases (13.33%) and in those with T1b/1B stage (10%)(Figure7& 8) .These findings have suggested that decreased the expression of P27 may be an early event in the initiation of endometrial carcinoma , but not associated with prognostic factor (stage,grade and types) and we in agreement with (Nycum *et al.*, 2001) study who suggested that expression of P27 was not associated with prognostic factor such as FIGO stage.

Several studies suggested that one of the mechanisms that leads to low or absent of P27 expression is up-regulation or disordered of SKP2 (S-phase kinase interacting protein-2) the P27-ligase, which leads to degradation by ubiquitin-proteasome pathway(Watanabe *et al.*, 2002) . Other possible mechanism of the abnormality in P27 expression could be gene mutation ,excessive amount of the complex cyclin E/CDK,or consumption of P27 may be a trapped by other factors such as cyclin D1 and D3(Watanabe *et al., 2*002).

The Current study has observed that P27 expression in the nuclei of glandular cells in some cases of non-malignant tumors and in control tissues that are represented by secretory and late secretory endometrial glands.This findings are in agreement with suggestion of several other studies (Lahav-Barat *et al.*, 2004, Shiozawa *et al.*, 2004).Expression of P27 could probably a result of cellular response to the hormones and the regulation of P27 expression may be induced by the progesterone suggested that a markedly p27 expression induced by progesterone in the secretory phase might develop cell growth arrest by inhibiting the cyclin E/cdk2 complex and it was a result of a persistent accumulation of p27 due to a prolonged half-life by progesterone-mediated impaired proteolytic activity(Watanabe *et al.*,2002).

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