INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES

(p-ISSN: 2348-5213: e-ISSN: 2348-5221)

www.ijcrcps.com

Coden: IJCROO(USA) Volume 3, Issue 4 - 2016

Research Article

SOI: http://s-o-i.org/1.15/ijcrcps-2016-3-4-1

Immunohistochemical Targeting of p21WAF1 and p27Kip1 Gene Expression in the Cervical Cancers Tissue Infected with High Oncogenic Risk HPV

Dr.Saad Hasan Mohammed Ali[1], Dr. Basim Shehab Ahmed[2], Dr. Isra'a M. Al-Sudani[3] and Sura Dhafer AL-Aziz[4]

[1]Communicable Diseases Research Unit, Baghdad Medical College, Baghdad, Iraq.

[2,3]Department of Pathology, College of Medicine, AL-Mustansyria University, Baghdad, Iraq.

[4]Department of Microbiology, College of Medicine, AL-Mustansyria University, Baghdad, Iraq .

*Corresponding Author: dr.alkarkhi@gmail.com

Abstract

Background: Several studies assessed the role of gene and protein expression of p21and p27 in cervical lesions, and mention that there is an important changing in the levels of these Cyclin -dependent Kinase(CdK) Inhibitors protiens during the progression from normal epithelium through precancerous lesions to invasive cervical cancer,while some researchers indicat that there were no apparent differences in immunostaining for p21 and p27. Also, It is known that during HPV infection, E7 also activates cyclins (E and A) and blocks the cellular proliferation- inhibiting activities of cyclin-dependent kinase (CDK) inhibitors such as p21 and p27. **Methodology:** This study has used *in situ* hybridization to localized HR- HPV16/18 and used Immunohistochemistry for detection the gene expression of p21 and p27 in tissue specimens from 70 hysterectomized patients diagnosed with malignant endometrial tumors (30 cases), non-malignant endometrial tumors (25 cases),and 15 cases as control tissues groups.These 70 cases have enrolled 48 accompanying cervical lesions involving: chronic cervicitis (40 cases) and cervical cancers that were invasive from endometrium cancers sites(8 cases) as well as (22 cases) with normal cervical tissues. **Results:** The results of HR-HPV16/18 CISH signals&results in different cervical lesions was:Eight cases (26.7%) in malignant endometrial tumor group contain HPV16/18 DNA,where as 10 cases(40%) in non- malignant endometrial tumors group, and 4 cases (26.7%)in control endometrial tissues were containing this DNA in their tissues. No significant correlations (P>0.05) of HR-HPV16/18 infections among cervical lesions were reported. Moleculare detection of P21 revealed 4 cases (13.3%) of cervical lesions in the malignant uterine tumor group,11 cases (44%) of cervical lesions in non-malignant uterine tumor group and 5(33.3%) in control groups and there were a significant difference in the p21 expression in cervical tissue of malignant groups compared to the control group. low expression of P27 revealed in all groups :7(23.3%) in cervical lesions of malignant uterine tumor group, 8(32%) in cervical lesions of non- malignant uterine tumor group. **Conclusions:** No significant correlations (P>0.05) of HR-HPV16/18 infections among cervical lesions were reported while a significant deference in expression of P21 was reveal mostly in cervical lesions of malignant uterine tumor group and this expressions occur could have correlated to the an early events of their tumorgenesis but low expression of P27 in hysterectomized patients mostly appear in cervical lesions of all study groups . There was an association between p21 expression and viral infection among malignant endometrial tumors group in the cervical lesions but no significant association was observed between p21 expression and HR-HPV16/18 infection among other groups. Also,a Significant association was revealed between P27 expression and HR- HPV16/18 infection in the cervical site of malignant endometrial tumor group while no significant association was revealed between p27 expression and HR-HPV16/18 infection in non-malignant endometrial groups.

Keywords: Cervical Cancers, p21WAF1, HPV.

الخلاصة:-
عدة دراسات قيمت دور التعبير الجيني والبروتيني ل p21 و p27 في آفات عنق الرحم وقد اشارت الى ان هنالك تغيرات مهمة في معدلات هذه البروتينات المثبطة خلال مراحل التطور من النسيج الطلائي الطبيعي مرورا بالافات ما قبل حدوث السرطان الى انتشار سرطان عنق الرحم, في حين بعض الباحثين اثبتوا عدم ظهور اختلافات في التصبيغ المناعي ل p21 و p27 وكذلك لوحظ بان اثناء الإصابة بال HPV فان CYCLIN E&A يؤثر على P21,P27

طريقة العمل:
اعتمدت هذه الدراسة تقنية التهجين الموضعي للكشف عن الفايروس الحليمي البشري و Immunohistochemistry للكشف عن التعبير الجيني ل p21,p27 في عينات انسجة عنق الرحم والماخوذة من 70 مريضة اجريت لها عملية لاستئصال الرحم منها 30 (حالة) شخصت ضمن الأورام الخبيثة للرحم و 25 (حالة)ضمن أورام الرحم غير الخبيثة و 15 (حالة)كمجموعة
انسجة السيطرة .هذه ل 70 حالة تضمنت 48 حالة من آفات عنق الرحم وتشمل 40 حالة لالتهاب عنق الرحم المزمن و 8 حالات من سرطان عنق الرحم الممتد من بطانة الرحم, بالإضافة الى 22 حالة لديها نسيج عنق رحم طبيعي. **النتائج:** كانت نتائج الإصابة بفايروس الحليمي كالاتي:ثمانية عينات في مجموعة المصابات باورام الرحم الخبيثة و 10 في مجموعة المصابات باورام الرحم الحميدة وأربعة عينات ضمن مجاميع السيطرة اظهر الكشف الجزيئي بان التعبير الجيني ل p21 كان 4) % 13.3 (في آفات عنق الرحم من مجموعة أورام الرحم الخبيثة و 44) 11 ٪)في آفات عنق الرحم من مجموعة المصابات بأورام الرحم غير الخبيثة. اما في أنسجة مجاميع السيطرة، تم الكشف عن خمس حالات فقط 33.3 (٪.)ظهر انخفاض للتعبير الجيني ل p27 في كافة المجاميع و كان 7 %(23.3 (في آفات عنق الرحم من مجاميع اورام الرحم الخبيثة و)% 32) 8 في
آفات عنق الرحم من مجموعة أورام الرحم غير الخبيثة

الاستنتاج:
لا توجد إصابة معنوية للفايروس الحليمي البشري ضمن مجاميع الدراسة وظهر اختلاف معنوي للتعبير الجيني ل p21 في آفات عنق الرحم لمجموعة سرطان الرحم الخبيث وقد يكون له علاقة بالمراحل السرطانية الأولية في حين ظهر انخفاض في التعبير
الجيني ل p27 في آفات عنق الرحم لكافة المجاميع الدراسية. هنالك علاقة توافقية بين نسبة الإصابة بال فايرس الحليمي البشري والتعبير الجيني للكل P21 ,P27 في انسجة عنق الرحم لمجاميع اورام الرحم الخبيثة عند المقارنة بالمجاميع الأخرى.

Introduction

Several studies have recognized HR-HPV16/18 as the sexually transmitted etiological agents of cervical neoplastic lesions and subsequently cervical carcinoma (Hammouda *et al.,* 2004) where the HR-HPV 16/18 are the most commonly detected HR-HPV types in these cervical lesions (Bahnassy *et al.,* 2006). Cervical cancer is the most important outcome of HPV infection with over 500.000 new cases and 275.000 attributable deaths world-wide in 2008 (Defilippis *et al*., 2003).

1. Cyclin Dependent Kinase (Cdk) Inhibitors:-

In mammals, Cyclin-dependent Kinases (Cdk) and their binding partners, the cyclins, together drive progression through cycle towards S phase and later to initiate mitosis. Cdk activity is regulated at multiple levels including phosphorylation, cyclin expression levels, and binding of Cdk inhibitors (Cdk) that tightly regulate function of these regulatory molecules(cyclins and Cdk) by binding and inhibiting activity of cyclin Cdk complexs (Denicourt & Dowdy, 2004).Cdk inhibitors (Cks) 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 broader range of inhibition (Sherr & Roberts, 1999).

1.2. P21

Xiong *et al.,* first Identified P21 in 1992 as partner of Cyclin D1 and D3by using immunoprecipitation test, and they noted that P21 associated with another protein (Proliferating cell nuclear antigen -PCNA). El-Deity and his worker in 1993 found that P21 downstream target of P53 that was up regulated following P53 activation, and mapped gene to the chromosomal region 6P21.2 and named this gene wild-type P53 activate fragment1 (WAF1) (Abukhdeir & park, 2009).

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 ; Siu *et al*., 2009).

P21 protein play important roles in a wide range of cellular process, including promoting cell cycle arrest in response to various stimuli. Additionally, acts as a master effector of multiple tumour suppressor pathways for promoting anti- proliferative activities that are independent of the classical p53 tumour suppressor pathway (Roninson, 2002).
Recent study suggest that, under certain conditions, p21 can promote cellular proliferation and oncogenicity.

Consequently, p21 is often misregulated in human cancers, but its expression, depending on the cellular context and circumstances, suggests that it can act as a tumour suppressor or as an oncogene (Abbas & Dutta ,2009).

Mutations in the p21 gene were very rarely detected in cervical carcinoma. Authors found increased p21 expression in invasive carcinomas during the progression from normal epitheliaum through precancerous lesions to invasive cervical cancer(Jo &Kim , 2005). Others detected expression of p21 in micro-invasive and invasive cervical cancer compared to normal cervical epithelium and in SCC compared to normal epithelium (Kim &Zhao, 2005).

1.3. P27

Investigators were analyzing a protein with similar size and function to P21. The group led by Joan Mass ague in 1994 , identified the gene for P27 and found that P27 inhibited cyclin CDK complexes from phosphorylating histone H1,and overexpression of P27 was found to prevent CDK activation and entry into the S- phase of the cell cycle . Studies have identified that P27 degradation is initiated by different ubiquitin ligases. Among these, the KPC1 complex ubiquitinaes free unphosphorylated P27 and S-phase Kinase interacting protein-2 (SKP2) ((Kamura *et al*., 2004; Lahav-Baratz *et al*., 2009).

The encoding gene ($P27^{Kip1}$) is located on chromosome 12P13, 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 and Dowdy, 2004). It's level is high in quiescent cells at G0/G1, but following mitogenic stimuli, it is rapidly degraded by ubiquitin system, allowing the CDK-cyclin complexs to drive the cell into S-phase (Ciechanover, 2003)**.**

A study showed that a decrease in p27 expression and a stepwise increase in cervical epithelium as it progressed from normal to a neoplastic .Authors can not find a proper explanation for this complexity in the result for expression of p27,assume that the mechanism might represent an alternative pathway for p27 inactivation in SCC of the uterine cervix through binding and sequestration by cdk2 and cyclin E which render it inactive.Also it might be explained simply as a direct consequence of the increased cell proliferation, in addition to ,geographic, racial, or methodological differences may be contributing to these differences in reported levels of expression(Kim& Zhao, 2005;Bahnassy *et al* ., 2006).

The Study aimed to:

Investigation of the HR-HPV16/18 infection in cervical lesions obtained from patients with hysterectomy using in situ hybridization technique. **Also** , assessment of the expression functionally tumor suppressor genes (i.e. p21and p27) in cervical lesion tissues amog patients hysterectomized for cancers using immunohistochemistry technique.

Materials and Methods

1-Subjects (Patients Tissue Samples)

This research was designed as retrospective study has envolved 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 cervix and these blocks were collected from the archives of histopathology laboratories at teaching Laboratories in medical city, Al-Yarmook teaching hospital and private laboratories. These samples were related to the period from 2012 to 2014. The study tissues group comprised 30 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. These 70 cases have also enrolled 48 accompanying cervical lesions involving: chronic cervicitis (40 cases) and cervical cancers that were invasive from endometrium cancers sites(8 cases) as well as (22 cases) with normal cervical tissues .

2-Laboratory methods

Thick-tissue sections (4 mm) were prepared and stuck onto positively charged slides. An *in situ* hybridization (ISH) detection system (Zytovision/Germany) was used to target DNA sequences in tissue specimens using a biotinylated long DNA probe for HPV genotypes 16, 18 in tissue specimens. The procedure of the (CISH) assay adopted by this study was carried out in accordance with the manufacturer company leaflet (zytovision/Germany) in the Research Laboratories at Communicable Disease Research Unit/ Baghdad Medical College . Positive reactions were performed by replacing the probe with a biotinylated house keeping gene probe. For the negative control, all reagents were added except the diluted probe. Proper use of this ISH detection system gives an intense blue signal at specific sites of the hybridization probe in positive test tissues, The enzymatic reaction of NBT/BCIP leads to the formation of strong blue violet signals that can be visualized by light microscopy at (10-20x) dry lens. CISH signals were determined for at least 10 high power fields. Nuclear staining was considered as a positive result for HPV-DNA . Positive CISH signal patterns were classified as follows:

1. Diffuse (D), when nuclei were completely stained.

2. Punctuated (P), when distinct dot-like intra nuclear signals were noted .
3. Mixed, diffuse and punctuated (D/P), when both patterns are noted[9] .

Immunohistochemical method was used to demonstrate the product of gene expression of P21 and P27 in those cervical lesions 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).

3-Statistical Evaluation:

Chi-square exact test was used to find out the effect of different patients criteria on the reading of each marker of in situ hybridiazation and immunohistochemical techniques.

Results

1-Molecular detection of HR-HPV16/18 in cervical lesions among the study groups:

(Table 1 and 2) Shows the results of HR-HPV16/18 CISH signals&results in different cervical lesions : Eight cases (26.7%) in malignant endometrial tumor group contain HPV16/18 DNA,where as 10 cases(40%) in non-malignant endometrial tumors group, and 4 cases (26.7%)in control endometrial tissues were containing this DNA in their tissues. No significant correlations (P>0.05) of HR-HPV16/18 infections among cervical lesions were reported(Table 1).

Table(1): Distribution of HR-HPV16/18 CISH-results among cervical tissues from different leions.

Cervical CISH signaling results		Malignant endometrial tumors		Non- malignant endometrial tumors		Control endometrial tissues	
		No	%	No	%	No	%
HPV16/18	Positive	8	26.7	10	40.0	4	26.7
CISH Results	Negative	22	73.3	15	60.0	11	
P compared to NT	-	0.692*					
P compared to NT	0.294*						

*No Significant difference between proportions using Pearson Chi-square test at 0.05 level. P:p-value .NT:Non-malignant endometrial tumors. Con: Control endometrial tissues.

The DNA patterns of HR-HPV were punctate form in: (62.5%) of cervical lesions among malignant endometrial tumor cases, (90%) in cervical lesion among non- malignant endometrial uterine tumor and (100%) in cervical lesion among control group(Table 2)(Figure 1: a,b,c,d) ,while mixed form appear in (37.5%) of cervical lesion among malignant endometrial uterine tumor (Figure1: c,d) and (10%) in cervical lesion among control group.

Table(2): Distribution of HR-HPV16/18 CISH-signal among cervical tissues from different leions .

Cervical CISH signal results		Malignant endometrial tumors		Non- malignant endometrial tumors		Control endometrial tissue	
		No	%	No	%	No	%
HPV16/18	Punctate alone	5	62.5	9	90.0	4	100.0
CISH signal	Diffuse alone	-	-	-	-	-	-
patterns	Mixed [punctate &diffuse]	3	37.5	1	10.0	-	-

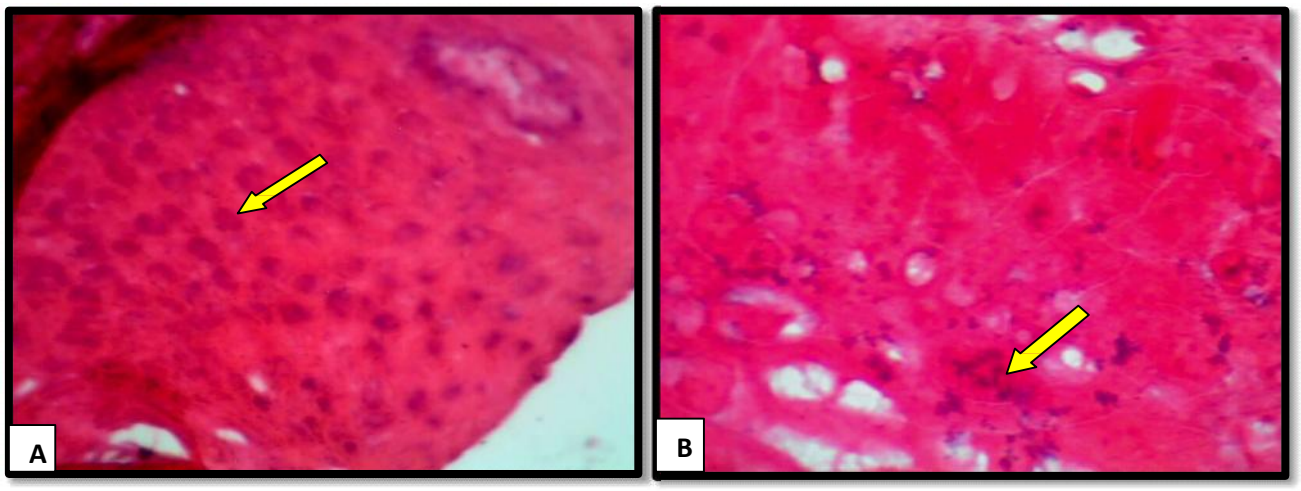
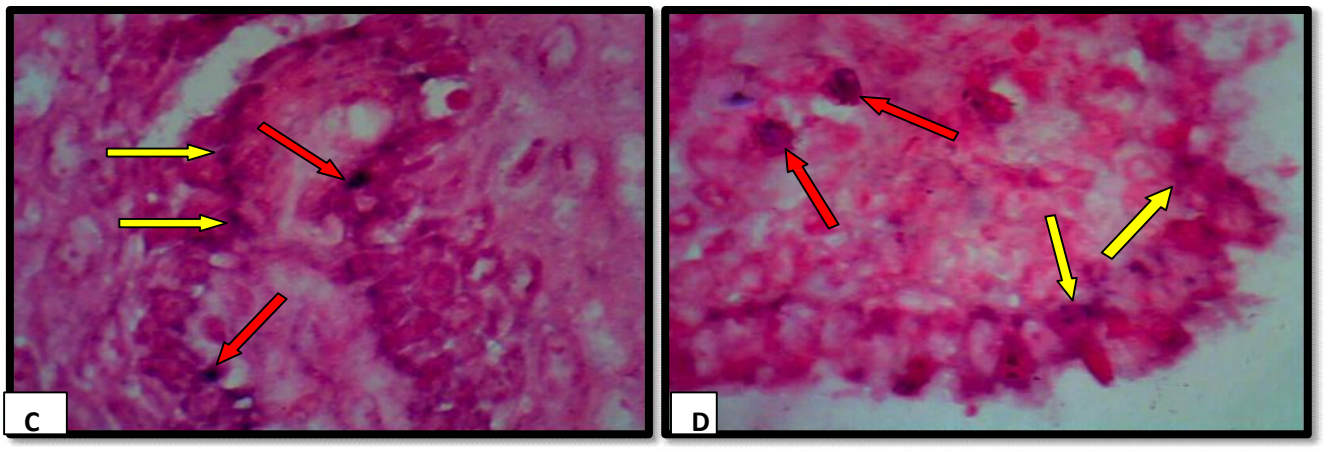


Figure (1): Microphotographs of the HR-HPV 16/18 DNA CISH positive signals in the cell nuclei of ecto-endocervical tissues :A&B-Chronic cystic cervisitis. Punctate patterns (dot_like intranuclear Blue signals) are detected at complementarity sequence sites(yellow arrows)(x1000).



C&D-Chronic cystic cervisitis.mixed signal patterns (yellow arrow;punctate form,red arrow:diffused form) Blue signals are detected at complementarity sequence sites(yellow arrows)(x1000).

2- Detection of IHC staining for P21 in the cervical lesions among the study groups:-

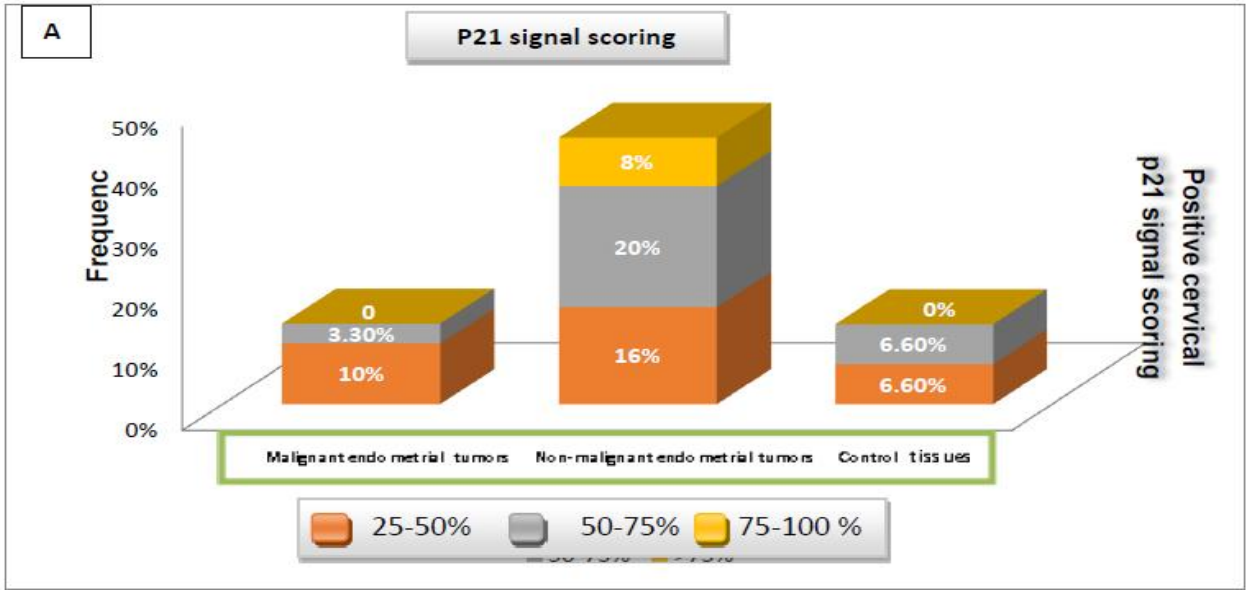
The P21 protein staining was captured in cervical squamous celluler cytoplasm,where the results were found as follows:4 cases (13.3%) of cervical lesions in malignant endometrial tumors ,11 cases (44.0%) of cervical lesions in the non-malignant endometrial tumors and 2 cases (13.3%) in the control tissue groups. A Significant difference in the p21 expression in cervical lesions among malignant endometrial tumor was observed compared to the control group (P<0.05) but no significant difference when compared to the non malignant endometrial tumors (Table 3).

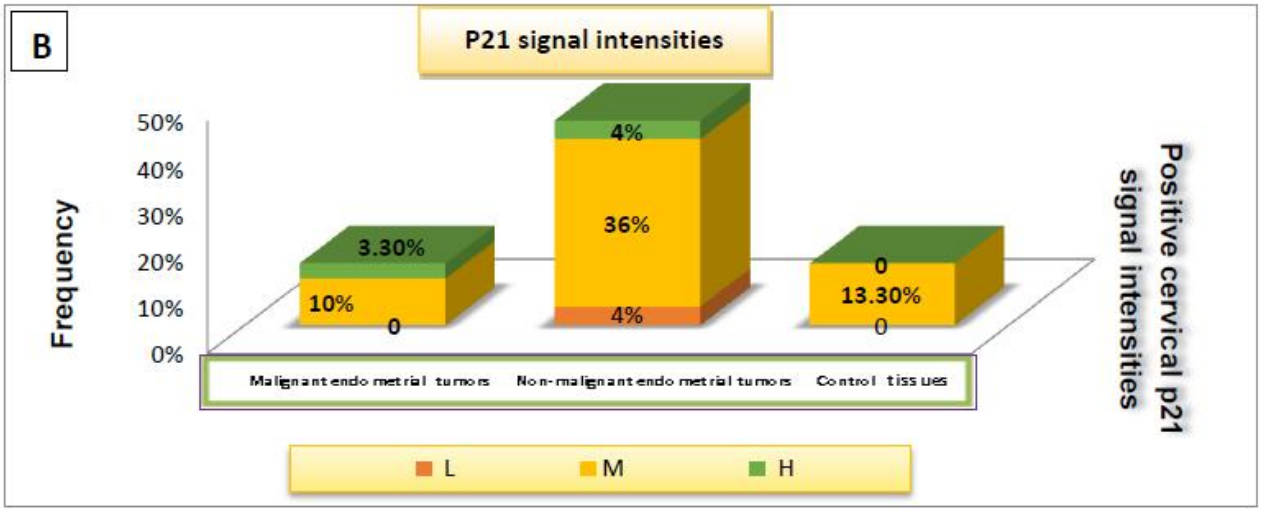
Table(3): Immunohistochemical results of P21 expression in the cervical lesions.

Cervical- IHC signl reults	Malignant endometrial tumors		Non-malignant endometrial tumors		Control endometrial tissues	
	No	%	No	%	No	%
P21-IHC signal results Positive	4	13.3	11	44.0	2	13.3
Negative	26	86.7	14	56.0	13	86.7
P compared to NT	-		0.070		-	
P compared to CT	0.011*		-		-	

Significant difference between proportions using Pearson Chi-square test at 0.05 level.
P:p-value, NT:non-malignant endometrial tumor, CT:Control endometrial tissues.

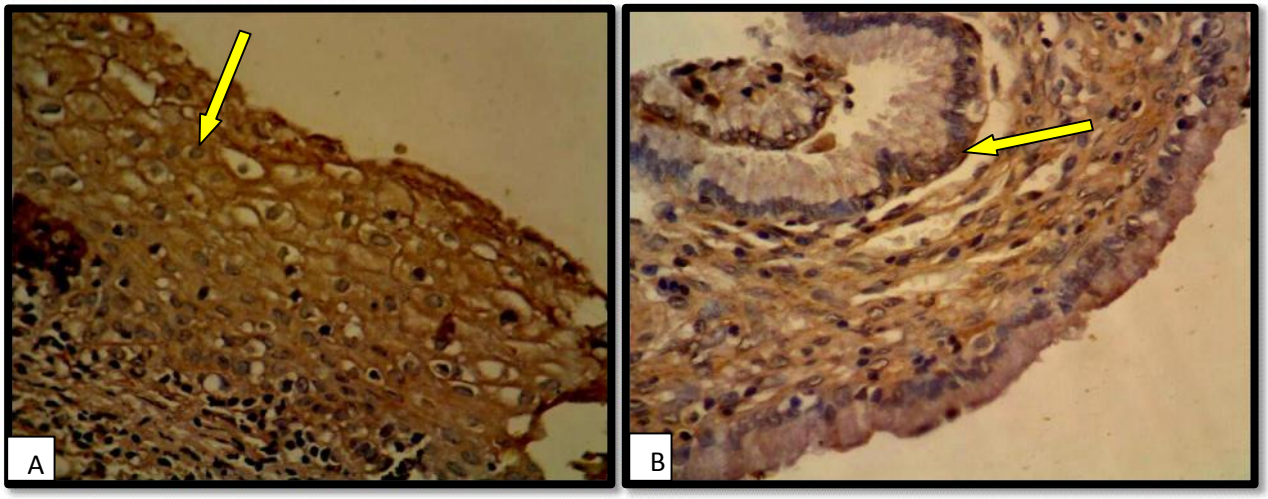
In the cervical lesions of malignant endometrial tumors group the highest percentage of p21 expression has score 2 and also (10%) as highest expression showed moderate intensity. while in the cervical lesions of non-malignant endometrial tumor the highest percentage of p21 expression has score 3 (20%) with moderate intensity was predominated constituted (36 %) .In cervical lesions of the results of cervical lesions of control groups the expression of p21 was reveald in score 2&3 (6.6%) with moderate intensity.Significant difference revealed when compared malignant endometrial tumor to non-malignant endometrial tumor and when compared non-malignant endometrial tumor to control group (Figure2,3,4) .





Figure(2):A-Frequency distribution of IHC-results for P21 according to signal score in the cervical lesions among hysterectomised patients,B- Frequency distribution of IHC-results for p21 according to signal intensity in the cervical lesions among hysterectomised patients.

Figure(3):Microphotograph of IHC positive staining for P21 in cell cytoplasm(yellow arrow) of ecto-endocervical epithelial tissue in : A-Normal cervix tissue show score 2 and moderate intensity (400x).B-Chronic cystic cervicitis show score 2 with high intensity(100X).



Figure(4):Microphotograph of IHC positive staining for P21 in cell cytoplasm(yellow arrow) of ecto-endocervical epithelial tissues in : A-Normal cervix tissue show score 2 and moderate intensity (400x).B-Chronic cystic cervicitis show score 2 with low intensity(400x).

3- P27 expression in different cervical lesions :-

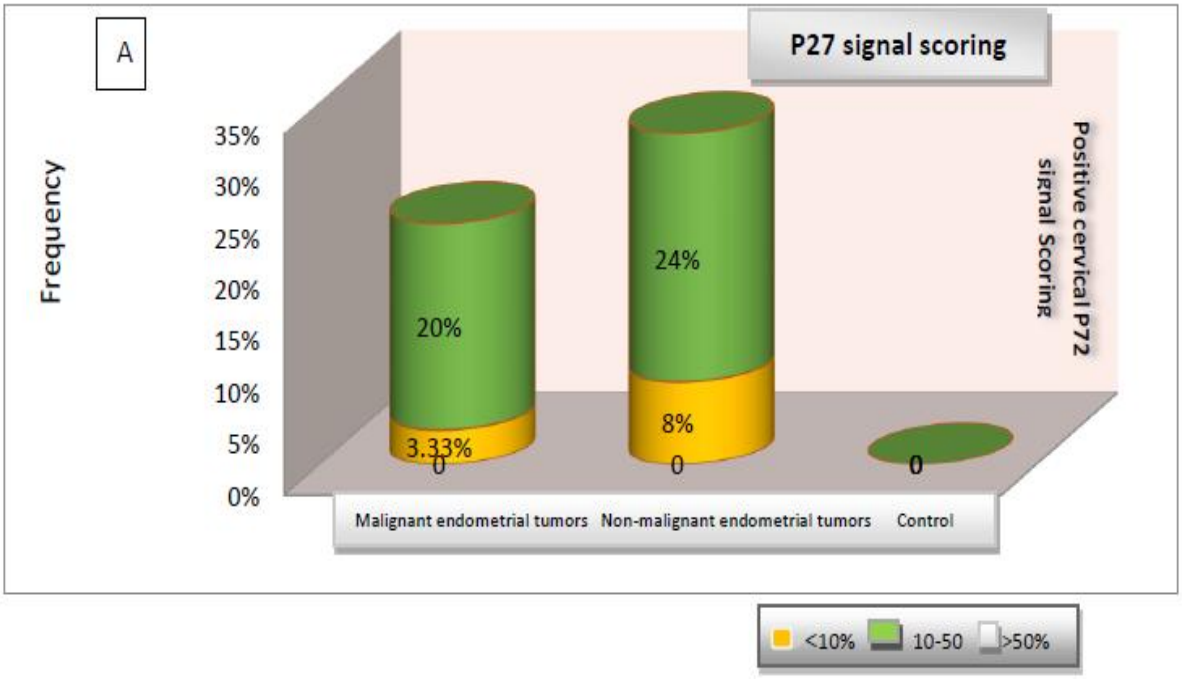
The results of P27 staining in cervical squamous cell nuclei reveald the out of 7/30 (23.3%) cases in the cervical lesions of malignant endometrial tumors group while out of 8/25 (32.0%) cases in the cervical lesions of non-malignant endometrial tumors group have this protein expression .No detection of P27 in control group has observed .No significant different in the cervical lesions between malignant and non malignant endometrial tumor groups (Table 4).

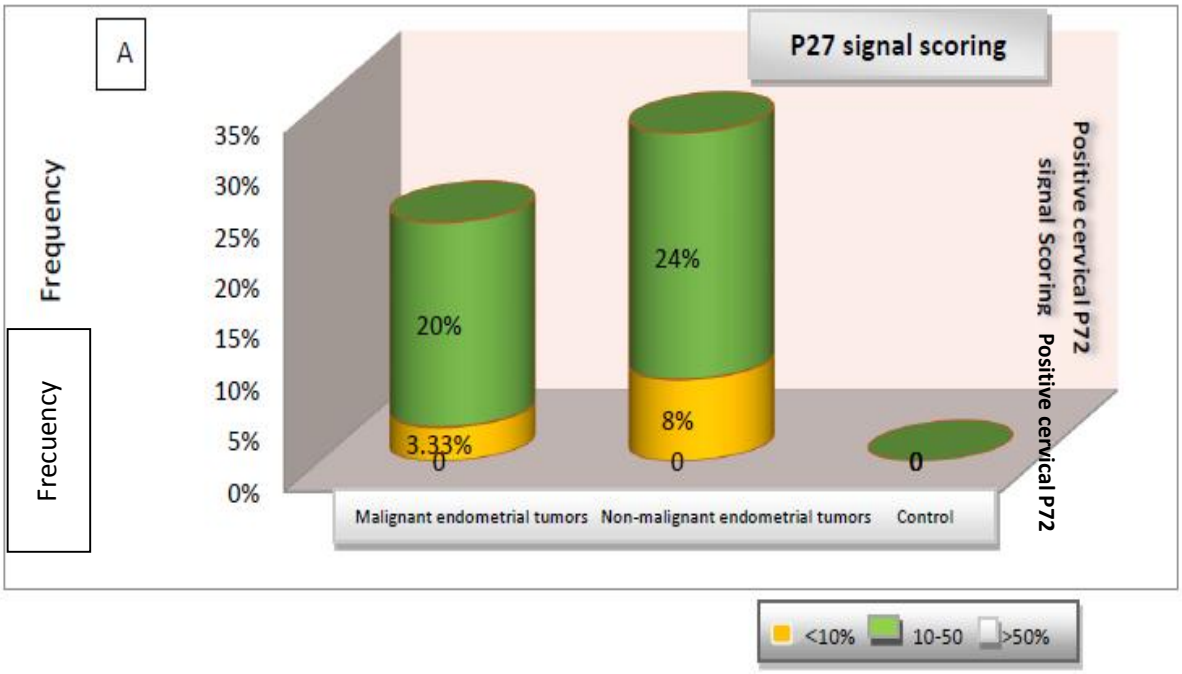
Table(4): Immunohistochemical results of P27 expression in the cervical lesions among the study groups.

Cervix IHC signal results		Malignant endometrial tumors		Non- malignant endometrial tumors		Control endometrial Tissues	
		No	%	No	%	No	%
P27 IHC Signal results	Positive	7	23.3	8	32.0	-	-
	Negative	23	76.7	17	68.0	15	100.0
	P compared to NT		-		-		-

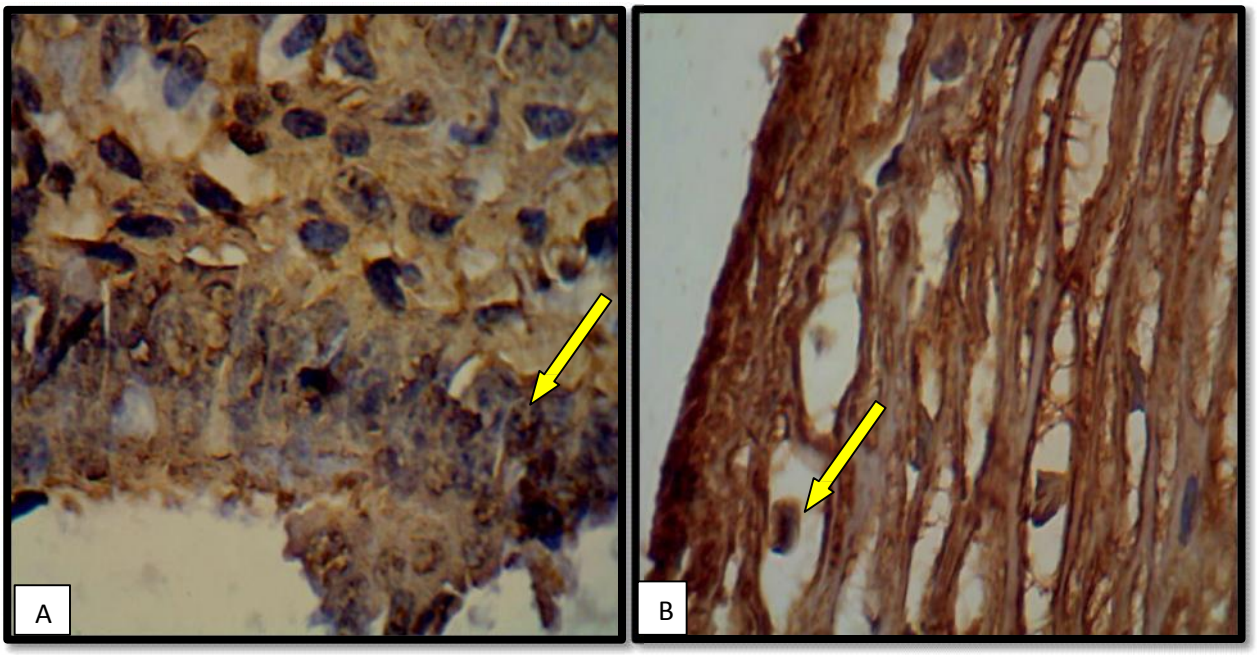
No Significant difference between proportions using Pearson Chi-square test at 0.05 level.
P:p-value, NT:non-malignant endometrial tumor, CT:Control endometrial tissues.

P27 expression show highly (20%)in score 3 in the cervical lesions of malignant endometrial tumor with moderate intensity predominated constituted (10%), and in score 3 of cervical lesions of non-malignant tumor (24%) with high intensity was predominated constituted (16%) but no expression for p27 in control groups.There were no statistical significant differences (P>0.05) according to score and intensity between the study groups (Figure 5,6,7).

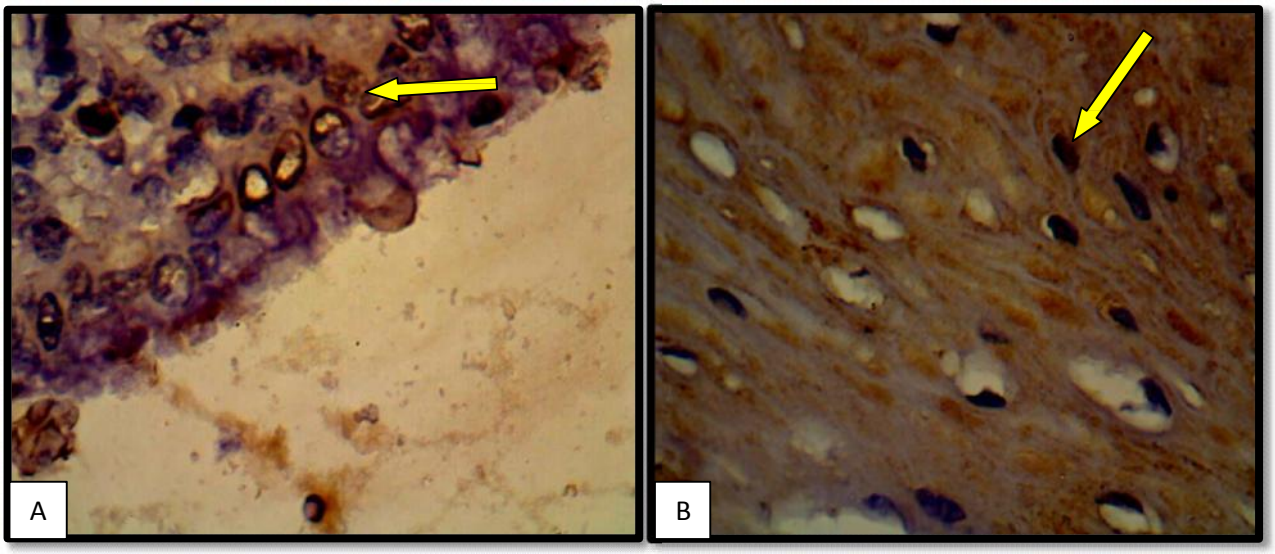




Figure(5):A-Frequency distribution of IHC-results for P27 according to signal score in the cervical lesions B-Frequency distribution of IHC-results for p27 according to signal intensity in the cervical lesion.



Figure(6):Microphotographs of IHC positive staining for P27 in cell nuclei (yellow arrow) of cervical tissues in : A- Chronic cystic cervicitis shows score 2 with moderate intensity (1000x). B-Normal cervix tissue shows score 3 and high intensity (1000x).



Figure(7):Microphotographs of IHC positive staining for P27 in cell nuclei (yellow arrow) of cervical epithelial tissue in : A- chronic cystic cervicitis shows score 2 with moderate intensity(1000x). B-normal cervix tissue shows score 2and low intensity (400x).

4- Association between p21,P27 proteins expression and HR-HPV16/18 in the cervical lesions.

The percentage of positive results for both p21 and HR-HPV16/18 in the cervical lesions of malignant endometrial tumors was 3/30 (10%). While in the cervical lesions of non-malignant endometrial tumors group was 6/25 cases(24%) and in control group was 1/15(6.7%). There was an association between p21 expression and viral infection among malignant endometrial tumors group in the cervical lesions but no significant association was observed between p21 expression and HR-HPV16/18 infection among other groups. (Table 5).

Table (5): Association between P21 expression and HR-HPV16/18 infections in the cervical lesions among the study groups.

Pathological Type	IHC P21 results	HR-HPV		P value
		ISH Negative results	ISH Positive results	
Malignant endometrial tumors (30)	Negative	21 70%	5 16.7%	0.019*
	Positive	1 3.3%	3 10%	
Non malignant endometrial tumors (25)	Negative	10 4%	4 24%	0.188
	Positive	5 20%	6 24%	
Control tissues (15)	Negative	10 66.7%	3 20%	0.423
	Positive	1 6.7%	1 6.7%	

*Significant difference between proportions using Pearson Chi-square test at 0.05 level. ** No statistical analysis.

The percentage of positive results for both p27 and HR-HPV16/18 in cervical lesions of the malignant endometrial tumors was noticed 4 out of 30 (13.33%).while in the cervical lesions of non-malignant endometrial tumors was found in 4 out of 25(16%).Significant association was revealed between P27 expression and HR-HPV16/18 infection in the cervical site of malignant endometrial tumor group while no significant association was revealed between p27 expression and HR-HPV16/18 infection in non-malignant endometrial groups(Table 6) .

Table (6): Association between P27, HR-HPV in the Cervical lesions among study groups.

Pathological types	P27 IHC results	HR-HPV		Chi-Square test
		Negative ISH results	Positive ISH results	
Malignant endometrial tumors (30)	Negative	19 63.3%	4 13.3%	0.037*
	Positive	3 10%	4 13.3%	
Non-malignant endometrial tumors (25)	Negative	11 44%	6 25%	0.317
	Positive	4 16%	4 16%	
Control endometrial tissues (15)	Negative	11 73%	4 26.7%	No S.**
	Positive	0 0%	0 0%	

*Significant difference between proportions using Pearson Chi-square test at 0.05 level.
** No statistical analysis.

Discussion

1-Molecular detection of HR-HPV16/18 infection in cervical lesions:

This study demonstrated that HR-HPV16/18 DNA was found mostly in non- malignant endometrial tumors (40%) with punctate form(Table 1,2)(Figure 1). We agree with most relevant studies about the association of this virus with cervical lesions in studies from different countries (Huang *et al.,* 2010; Juckett *et al.,* 2010;Weyn 2011 ,Kashani *et al.,* 2014) and also in many Iraqi studies (Mohammed Ali, 2001;Al-azzawi, 2006;Fahad *et al*., 2011, Aziz &AL-Irhayim, 2013).

Several studies have recognized HR-HPV16/18 as the sexually transmitted etiological agents of cervical neoplastic lesions and subsequently cervical carcinoma (Hammouda *et al.,* 2004) where the HR-HPV 16/18 are the most commonly detected HR-HPV types in these cervical lesions (Bahnassy *et al.,* 2006).

Progresses in cervical cancer research have provided an evidence that HPV E6 & E7 oncoproteins induce immortalization of the cells through their inhibitory effects on the tumour suppressor proteins (Rb and P53) and disturbing cell cycle control (Baldwin *et al.,* 2006). Also experimental evidence has suggested a link between steroid hormones, HPV and cervical lesions and the main linking is the steroid receptor coactivator 1 (SRC-1) .

They showed that HPV E7 can dysregulate the function of (SRC-1) transcriptional complexes by relocalies (SCR-1) to the cytoplasm, removing it from its transcriptional target and that may disrupt the balance of co-activators and co-repressor or promoters leading to dysregulation of gene expression, possibly contributing to the process of cervical carcinogensis. (Mu- nger *et al.,* 2004).

2- Detection of P21 expression in cervicals lesions (Tables 3)(Figures 2,3,4):-

The present results show a significant differences in the P21 expression in cervical lesions of malignant endometrial tumors as compared with control tissues group among hysterectomized patients . The Possible explanations in alter in p21 expression and impaired in its function include inactivating mutations , mutation of its targets , overexpression of its target , or overexpression of proteins in more downstream pathways in cell cycle (Kim & Zhao, 2005).Others mention that p21 inactivation was by HPV16/18 E6 oncoprotein (Ganguly &Parihajj, 2009;Tagle *et al.*, 2014) .E6 induced degradation of p53 by 26S proteasome causes DNA damage and chromosomal instability and as aconsequence p21 gene transcription inhibited since p21 gene is regulated through p53-dependent and independent pathway,through platelet-derived,fibroblast and epidermal growth factors(Matsumoto *et al*., 2006) .Also, Tagle *et al.,* 2014 have deen reported that HPV E7 oncoprotein can target p21 for degradation during carcinogenesis in various tumors.

3-Detection of P27 expression in cervial lesions among the study groups

(Table 4)(Figure 5,6,7):- Very low expression of P27 was observed in the malignant as well as non-malignant endometrial tumor while control group showed no expression of p27 . In hystersctomised patient who have a cancer invasion from the endometrium sites to the cervix,a low expression of P27 was observed which was in agreement with several other studies (Alfsen, 2003 ; Jo &Kim, 2005 , Bahnassy *et al*., 2007 ; & Huang *et al.,* 2010).

In the cancer, P27 has often inactivated via impaired synthesis, accelerated degradation, mislocalization or proteolysis of P27 by oncogenic activation of various pathways(including e.g: receptor tyrosine kinases) which make cancer cell undergo rapid division and uncontrolled proliferation. Also, over expression of EGR in epithelial cancer cell played a role in proteolysis of P27 (Ramasubramian *et al*., 2013). Investegators have reported a lower level of P27 in cervical carcinoma might be explained as a direct consequence of the increase of cell proliferation (Kim & Zhao., 2005). Others researcher found P27 expression is often be detectable only in a small percentage of cervical cancers more than normal epithelium and precancerous lesions, but no relationship was observed with the proliferative activity and these observation are suggesting that deregulated expression of P27 might contributed to tumor formation, through mechanism other than increased cell proliferation (Pfert *et al*., 2003).

4-Association between P21 expression and HR-HPV16/18 infection in the cervical lesions (Table 5):

Regarding expression of P21 in relation to the HR-HPV 16/18 infection the current study has revealed a significant association between P21 expression and HR-HPV 16/18 infection of cervical sites among the malignant endometrial tumors as compared to other groups. The present results are in agreement with (Huang *et al.*, 2010) who found that P21 has strong association with HR-HPV 16/18 positive cervical carcinoma.One of the mechanism by which HR-HPV 16/18 interferes with normal cell cycle is represented by the binding of the E6 oncoprotein of HR-HPV to the P53 and inactivate it by proteosomal degradation where this overcomes the G1/S chechpoint. The down regulation of the cell cycle by P53 which acts as a tumor suppressor protein and has the capacity to reduce the expression of P21. The blockage of the P53 function leads to inactivity of P21 (Bahnassy *et al*., 2007)

5- Association of P27 expression with HR-HPV16/18 infection in different cervical lesions (Table 6):-

The current study has found significant association of P27 expression with HR- HPV 16/18 infections in cervical lesions among the malignant endometrial tumor cases only, represented by several lesions such as cervical cancer invasion from endometrium sites, chronic cystic cervicitis. These results match with other studies which showed changes in the expression of P27 in HR-HPV infected cells. (Bahnassy, 2007; Jayshree, 2009 ;Satncule *et al*., 2013).

Several studies reported association between P27 expression and HR-HPV 16/18 infection in cancers of uterine cervix. They suggested that the increase in level P27 protein could be due to a distrupted of P27 function in the presence of HR-HPV 16/18 oncoprotein E7 (Dowen *et al*., 2003). This oncoprotein inactivated P27 and disassociated it from the cyclin-CDK complexes (Satncule *et al*., 2013) . This removing will lead to prevent P27 phosphorylation and increase the level of P27 (Dowen *et al*., 2003). Also, viral oncogene E6 is shown to bind to P53 where it inactivates it by proteosomal degradation mediated by E6-AP leads to down regulation of p27,which are targets of p53.In addition to E7 can antagonized the ability of p27 to block cyclin E associated kinase (Jayshree *et al*., 2009).

In general, in HR-HPV 16/18 associatied cervical carcinoma, the situation is less clear since some studies showed that the tumor suppressor activity of these proteins (P27, P21) is over comed through the action of the viral oncogenes E6/E7 without any

change in their expression level, others showed that HR-HPV types impair the function of P21 but not the expression by rendering them in sensitive to cyclin-CDK complex formation whereas P27 is usually dow-regulated (Bahnassy *et al*., 2007).In conclusion we can say:-

- No significant difference of HR-HPV16/18 infections among cervical lesions for all study groups .
- There are a significant difference in the P21 expression in cervical tissues of Malignant endometrial groups .Also there is a significant correlation between p21 expresion and viral infection in this group. Low expression of P27 in all study groups with significant correlation was revealed between p27 expression and HR-HPV16/18 infection in the cervical lesions of Malignant endometrial groups only.

References

1. **Abukhdeir AM and Park BH** :p21 and p27:roles in carcinogenesis and drug resistance .Expert Rev Mol Med. 2009; 10:2-7.
2. **Alfsen GC**, Reed W, Sandstad B, Kristensen GB, and Abeler VM.:The Prognostic Impact of Cyclin Dependent Kinase Inhibitors p21WAF1, p27Kip1, and p16INK4/MTS1 Adenocarcinomas of the Uterine Cervix .CANCER . 2003 ; 98 (9)
3. **Abbas T, and Dutta A**:p21 in cancer: intricate networks and multiple activitiesNat Rev Cancer. 2009 Jun; 9(6): 400–414.
4. **Aziz DG**, **and Al-Irhayim BAK** :HPV in squamous cell carcinoma: An Immuno-histochemical study.Ann. Coll. Med. Mosul 2013; 39 (1): 19-24.
5. **AL-Azzawi MKK** ,Mhamood MM , and Mohammed Ali SH :molecular detection of Human pappiloma virus associated with uterine cervical carcinoma in Iraq female patients.Al-Mustansiriyah University,College of Science 2006.
6. **Baldwin A**, Huh KW, and Mu¨nger K :Human Papillomavirus E7 Oncoprotein Dysregulates Steroid Receptor Coactivator 1 Localization and Function .jour.vir, 2006;80(13): 6669–6677.
7. **Bahnassy AA**, Zekri ARN, Saleh M,Lotayef M, Moneir M and Shawki O :The possible role of cell cycle regulators in multistep process of HPV-associated cervical carcinoma. BMC Clinical Pathology 2007, **7**:4 .
8. **Ciechanover A**.The ubiquitin proteolytic system and pathogenesis of human diseases: a novel platform for mechanism-based drug targeting .Biochemical Society Transactions ,2003;31(2).
9. **Dellas A,** Jundt G, Sartorius G:Combined PTEN and p27kip1 Protein Expression Patterns Are Associated with Obesity and Prognosis in Endometrial Carcinomas .Clin Cancer Res, 2009;15:2456-2462**.**
10. **Denicourt C & Dowdy SF** : Cip/Kip proteins: more than just CDKs inhibitors.GENES & DEVELOPMENT ,2004;18:851–855 .
11. **DeFilippis RA,** Goodwin E C, Lingling Wu, and DiMaio D :Endogenous Human Papillomavirus E6 and E7 Proteins Differentially Regulate Proliferation, Senescence, and Apoptosis in HeLa Cervical Carcinoma.Jor.Viro. 2003;77(2):1551–1563
12. **Dowen SE,** A. Scott A, Mukherjee G and Stanley MA:Over expression of skp2 in carcinoma of the cervix dose not correlated inversely with P27 expression Int. J. Cancer: 2003,105, 326–330.
13. **Ganguly N and Parihajj SP** :Human papillomavirus E6 and E7 oncoproteins as risk factors for tumorigenesis . Indian j. Biosci.2009 ; 34(1), 113–123.
14. **Fahad RO**, Abdulbaqi S and Hasony HJ :The association of Human Papilloma Virus with cervical neoplasm in basrah. Bas J Surg, March , 2011; 17.
15. **Hammouda D,** Mun N , Herrero R , Arslan A , Bouhadef A , Oublil M, Djedeat B , Fontanie‘re B, Snijders P , Meijer C and Franceschi S :Cervical Carcinoma in Algiers, Algeria: Human Papillomavirus and Lifestyle Risk Factors . Int. J.Cancer,2005; 113, 483–489.
16. **Huang LW** ,Seow,KM ,Lee,CC ,Lin YH ,Pan HS and Chen HJ :Decreased p21 Expression in HPV-18 Positive Cervical Carcinomas Pathology &Oncology Research,.2010; 16(1):81-8.
17. **Jayshree RS,** Sreenivas A, Tessy M andKrishna S:Cell intrinsic & extrinsic factors in cervical carcinogenesis : .J Med Res , 2009;130: 286-295.
18. **Jo H**, and Kim MJW :Implications of HPV infection in uterine cervical cancer .Cancer Therapy ,2005;3: 419-434.
19. **Juckett G** and Ttman-adams H :Human Papillomavirus: Clinical Manifestations and Prevention. American Family Physician Web site at www.aafp.org/afp.
20. **Kashani** E M, Bouzari M , Talebi AR , and ZavarehFA :Detection of Human Papillomavirus in Chronic Cervicitis, Cervical Adenocarcinoma, Intraepithelial Neoplasia and Squamus Cell Carcinoma . J Microbiol. 2014; 7(5):
21. **Kamura T,** Hara T, Matsumoto M, Ishida N, Okumura F, HatakeyamaS, Minoru Yoshida M, Nakayama K and Nakayama K

Cytoplasmic: ubiquitin ligase KPC regulates proteolysis of p27Kip1 at G1 phase NATURE CELL BIOLOGY ,2004;6 (12).
22. **Kim YT** and Zhao M :Aberrant Cell Cycle Regulation in Cervical CarcinomaYonsei Medical Journal,2005; 46(5):597 – 613.
23. **Lahav-Barat S**, Ben-Izhak O , Sabo E , Ben-Eliezer S , Lavie O , Ishai D , Ciechanover A and Dirnfeld M:Decreased level of the cell cycle regulator p27 and increased level of its ubiquitin ligase Skp2 in endometrial carcinoma but not in normal secretory or in hyperstimulated endometrium. Molecular Human Reproduction ,2004 ;10(8):567–572.
24. **Lu W,** QuJ, Lan LIB, Lu C, Yan Q, Wu XM, Chen XY and Wan XP:Overexpression of p21-activated kinase 1 promotes endometrial cancer progression.ONCOLOGY REPORTS,2013; 29: 1547-1555, 2013.
25. **Matsumoto D :** Are cellular differences in emotion regulation mediated by personality trials?. J cross- callular psychology ,2006; 37 (4): 421-437**.**
26. **Mohammed Ali SH**:Molecular Biological Studies of Human Papilommavirus infection in Patients with Cervical Neoplasia.Medical Colleges of Al-Nahrin University ,(2001).
27. **Mu¨nger K ,** Baldwin A, Edwards KM,Hayakawa H , Nguyen CL, Owens M, Grace M, and Huh KW Mechanisms of Human Papillomavirus-Induced Oncogenesis. J .viro, 2004:11451–11460 .
28. **Pfert UG,** Kullmann M and Hengst L :Cell cycle-dependent translation of p27involves a responsive element in its 50-UTR thatoverlaps with a uORF .Human Molecular Genetics, 2003;12(14):1767–1779.
29. **Satncule SUR**,Caeusu M, Ceaus Z, and Usic V :mmunofluorescence expression of Ki-67, p53 and cyclin inhibitors (p16ink4a, p21 and p27) in low-grade cervical lesions versus high-grade cervical lesions. Research study on cell cultures . Rom J Morphol Embryol ,2013; 54(3):725–734.
30. **Sherr CJ and Roberts JM** :CDK inhibitors: positive and negative regulators of G1-phase progression. GENES & DEVELOPMENT ,1999;13:1501–1512
31. **Siu MKY**,Wong ESY ,Chan HY, Kong SHD ,Woo NWS, Tam KF,Ngan YSH, Chan QKY, Chan DCW, Chan KYK and Cheung A NY Differential expression and phosphorylation of Pak1 and Pak2 in ovarian cancer: effects on prognosis and cell invasion Int. J. Cancer,2010;127 :21–31.
32. **Tagle DKJ**, Sotelo DH,Illades-Aguiar B,and Leyva-Vazquez,:Expression of E6, p53 and p21 proteins and physical state of HPV16 in cervical cytologies with and without low grade lesions. Int J Clin Exp Med ,2014;7(1):186-193.
33. **Weyn Ch**, Vanderwinden JM, Rasschaert J, Englert Y, and FontaineV: Regulation of human papillomavirus type 16 early gene expression in trophoblastic and cervical cells Virology 2011; 412:146–155.

Access this Article Online	
Quick Response Code	Website: www.icrcps.com
	Subject: Viral Genetics

How to cite this article:
Saad Hasan Mohammed Ali, Basim Shehab Ahmed, Isra'a M. Al-Sudani and Sura Dhafer AL-Aziz (2016). Immunohistochemical Targeting of p21WAF1 and p27Kip1 Gene Expression in the Cervical Cancers Tissue Infected with High Oncogenic Risk HPV. Int. J. Curr. Res. Chem. Pharm. Sci. 3(4): 1-14.