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Immunohistochemistryusing in detection of Frequency of P16INK4A tumor suppresser gene overexpression in woman infected with cervical carcinoma in mid Euphrates

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Abstract : This study was designed as a retrospective research. A total number of (56) formalin-fixed, paraffin embedded cervical tissues were included. 27 with malignant cervical carcinoma (CC) and 16 with benign cervical tumors, 13 apparently healthy cervical tissues were used as a control group. The age of these individuals (patients and control groups) ranged between 23 and 73 years. They were collected from the pathological archives of teaching laboratories at many different private histopathologicallaboratories in Babil, AL-Najaf, Kerbela, AL-Qadisiya, , during the period from April 2015 to January 2016. After sectioning of these cervical biopsies and staining by hematoxyline and eosin, a final definitive histopathological diagnosis was done by a consultant histopathologist. The study was designed as Immunohistochemical study to demonstrate the expression state of tumor suppressor genes (P16) in those tissues with cervical carcinoma, benign tumors as well as apparently healthy cervical tissues. The obtained results are summarized as follows: the most affected age of the patients with cervical carcinoma (68-83 years)wererepresented by 100% while the most affected age of the benign tumors group and control was (20-35) were represented by 53%.40% respectively . The most common histopathological type among all studied archived cervical carcinoma was squamous cell carcinoma 66,6% followed by the adinocarcenoma 33.3%. Over expression of p16 was detected by IHC in 48.1% (13 out of 27) cervical cancer cases and in 43.7%(7 out of 16) benign cervical tumor group, while none of control group showed P16- over expression. A high percentage 46.2% (6 out of 13) in cervical cancer while 42.9% (3 out of 7) found in benign cervical tumor group has a moderate score (score II).Inthe present study the highest percentage of p16 expression showed within cervical cancer cases was 53.8%, and 42.9% within benign cervical tumor group that have moderate signal intensity. The present study showed the high percentageof over expression of p16 was 52% found within age group (36-51 years) followed by 36%, 34%, 25% in age group (52-67years), (20-35years), (68-83years) respectively. The present study showed the high percentage 50% of p16 INK4A overexpression found within squamous cell carcinoma.

Introduction

The tumor suppressor gene p16INK4A belongs to asignificant group of cycline kinase inhibitor genes which negatively regulate the Rbprotein and subsequently the G1 phase of cell cycle its located on chromosome 9p21[1](Brooks *et al*., 2010).Inactivation of p16INK4A gene is understood to be an early genetic event incervicaltumorigenesis with later expansion of the cell cycle from G1 to Sphase The effect of loss of

p16INK4A geneexpression appeared through allowing cell growth and transformation byinactivating (phosphorylating) Rb protein[2](Clifford *et al*., 2008).

The cancer is a second greatest common disease in females worldwide, it is the main cancer of women in most developing countries, wherever 80% of cases occur[3] (Hesselink ., 2007).The risk of cervical cancer is more in women aging 40 years of age or older and those who smoke, take contraceptive drugs for more than 5 years, have the history of multiple sex partners or immunosuppression[4] (Kufe*et al*, 2003). However, infection by certain types of human Papilloma virus (HPV) has been considered as the most significant risk factor for the development of cervical cancer[5,6](Kjaer*et al*, 1996; Kraus *et al*, 2006).It appearances that the incidence of most types of tumors is increased in Iraq at multifoldkey to transformation are the E6 and E7 oncoproteins , which work to disrupt cell-cycle regulation , inhibit apoptosis and stimulate cell-cycle progression by binding inhibiting the P53 and,p16,RB p110 tumor suppressor genes, respectively[1] (Brooks *et al*., 2010). Immunohistochemistry was performed for revealing of tumor suppresser gen p16,Objective: To determine the frequency of p16INK4a expression in uterine cervical biopsies with or without dysplasia. Therefore, p16INK4a Immunohistochemistry (IHC) provides valuable additional information in the interpretation of cervical histology with resultant improvement in definitive identification of dysplastic lesions and reduction of inter-observer disagreement in conventional histology.

Methods:

Fifty (56) formalin-fixed, paraffin-embedded uterine cervical tissue blocks from patients who had undergone hysterectomy or punch biopsy from the cervix , Among these 56 cervical biopsies (43)Malignant and benign blocks were collected from the archives of histopathology laboratories of different general hospital in Mid Euphrates as will as many private laboratories.

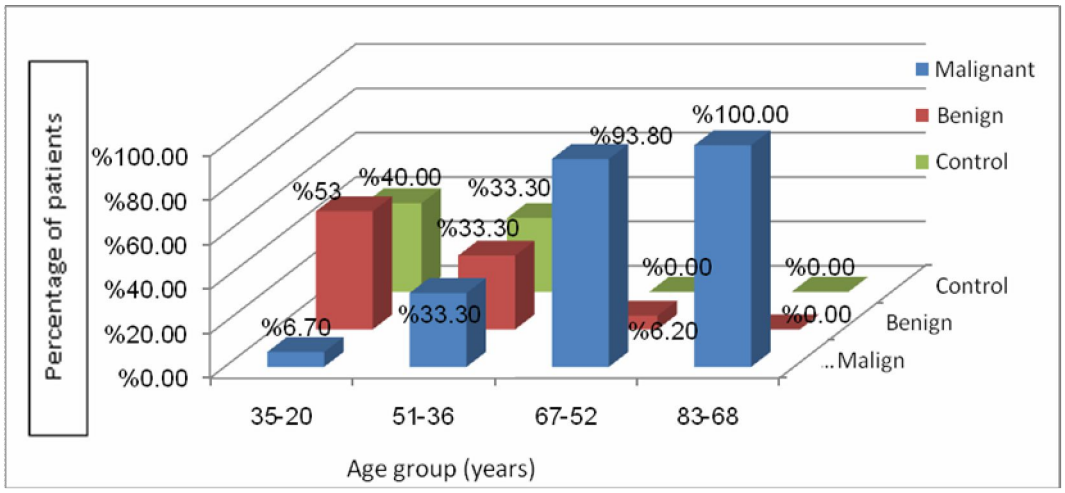
In additionthirteencervical tissue blocks without any significant pathological changes have been obtained from patients sustained hysterectomies for uterine bleeding and were included as apparently healthy control group. Sections of 4-µm thickness were cut from the paraffin blocks for hematoxylin-eosin staining and a detailed histopathological classification was assigned according to the criteria of the WHO (2011).

Immunohistochemistry.

Immunohistochemical staining was performed using the primary mouse monoclonal antibodies against p16INK4a, dilution 1:40 (clone E6H4, dakocytomation), immunostaining for p16INK4a: After routine de paraffinization in xylene and rehydration through serial dilutions of alcohol the sections were subjected to heat-mediated antigen retrieval for 15 minutes in citrate buffer (pH 6.0). To minimize nonspecific binding, blocking was performed with1% BSA at RT for 30 minutes. The primary antibodies were applied overnight at 4 °C followed by Envision visualization mouse system (dakocytomation). 3,3-diaminobenzidine (DAB) was used as the chromogen for 5 minutes and haematoxylin, as a counterstain. Stained sections were dehydrated and mounted in xylene. The percentage of immunopositive cells was evaluated (labeling index — LI). The immunoreactivity for all cell cycle regulatory proteins investigated in this study was evaluated as strong and weak according to the values of median. Statistical analysis was performed by SPSS statistical software package version 20, using the $x2$ test t-testand ANOVA for comparing and finding any relations between HPV positivity and other variations. Statistical significance was assumed at the $P<0.05$ level.

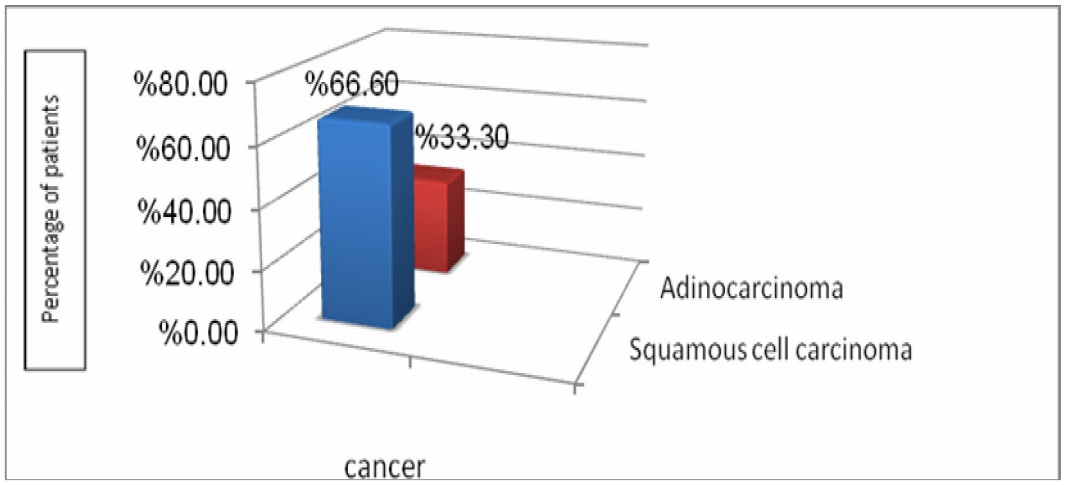
Results:

The archival specimen collected in this study was related to cervical tumor patients whom ages were ranged from twenty three years to seventy three years. In malignant cervical tumors, the most commonly affected age stratum was (68-83) years which constituted 100%,while the age stratum (52-67 years) was constituted 93.8% , followed by 33.3%, 6.7% for age stratum (36-51 years) and (20 -30 years) respectively. In benign cervical tumors, the most affected age stratum(20 - 35years) constituting 53% followed by 33.3 % for age stratum was (36 -51 years) and 0% in age stratum (52-67 years).in control cases the most affected age stratum(20 - 35years) constituting 40 % flowed by 33.3% in age stratum(36-51),Statistical comparison of these age strata revealed significant differences ($p< 0.05$) between different groups according to age (fig1).



Fig(1) Distribution of studied groups among the age (years) strata.

Seventy-two cases of cervical cell carcinoma were included in this study. Clinicopathological assessment revealed that 66.6% (18 out of 27 cases)patients were with squamous cell carcinoma and 33.3% (9 out of 27 cases) were with adenocarcinoma, Statistical analyses revealed significant differences ($p< 0.05$) between malignant sample groups according to type of cancer (Fig. 2).



Fig(2) Histopathological types of the presented cervical carcinoma patients

Over expression of mutated P16 protein was detected as a brownish discoloration at nuclear localization (Figure 3). Over expression of p16 was detected in 48% (13 out of 27) cells with cervical cancers and in 43.7% (7 out of 16) cases with benign cervical tumor, while non-cases of control group showed P16- over expression. A highest percentage 46.2 % (6 out of 13 cases) was involving cases with malignant cervical tumor that have either strong score (score III) ,30.8%(4 out of 13) have low score (score I).While, in benign cervical tumor group, 42.9 % (3 out of 7) were found to have low score (score I) and none of control group showed positive signal. Statistically, significant differences($p<0.05$) were found on comparing the results (according to score) between cervical cancers, benign tumors and control group ,(Table 1).

Table (1): Distribution of immunohistochemistry of P16protein according to the signal scoring.

P16 over expression		Malignant cervicalcancers (n=27)		Benign cervical tumors (n=16)		Healthy cervical tissues (n=13)		P
		N	%	N	%	N	%	
Negative		14/27	51.9	9/16	56.3	13/13	100	
Positive		13/27	48.1*	7/16	43.7*	0/13	0.0	
Scoring	I	4/13	30.8	3/7	42.9	0/0	0.0	**0.005***
	II	3/13	23	2/7	28.6	0/0	0.0	
	III	6/13	46.2	2/7	28.6	0/0	0.0	**0.236 N.S**

*significant

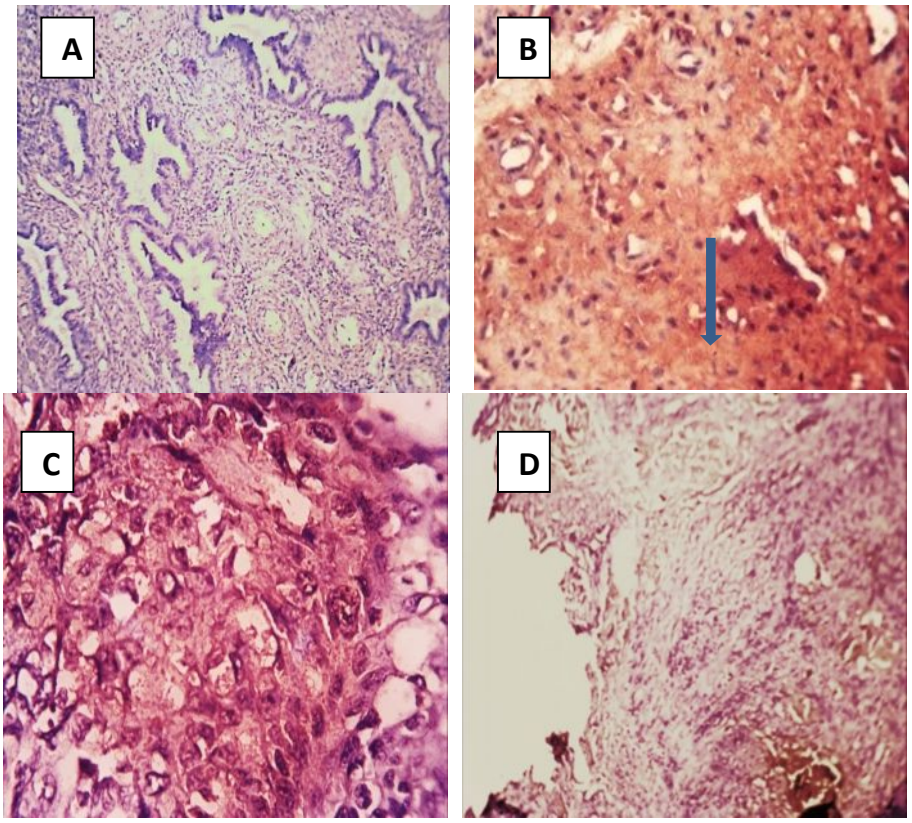


Fig (3) Immunohistochemical staining of P16 protein over expression using biotinylated -labeled anti-P16 protein atibody, stained by DAB-chromogen (Brown) and counter stained by mayer'shematoxyline (Blue). A. cervical cancer with negative P16 –ICH reactions(40X) B. Positive P16 –ICH reaction with strong score and high signal intensity (40X). C. Positive P16 –ICH reaction with moderate score and high signal intensity (40X). D. Positive P16 –ICH reaction with low score and high signal intensity (40X).

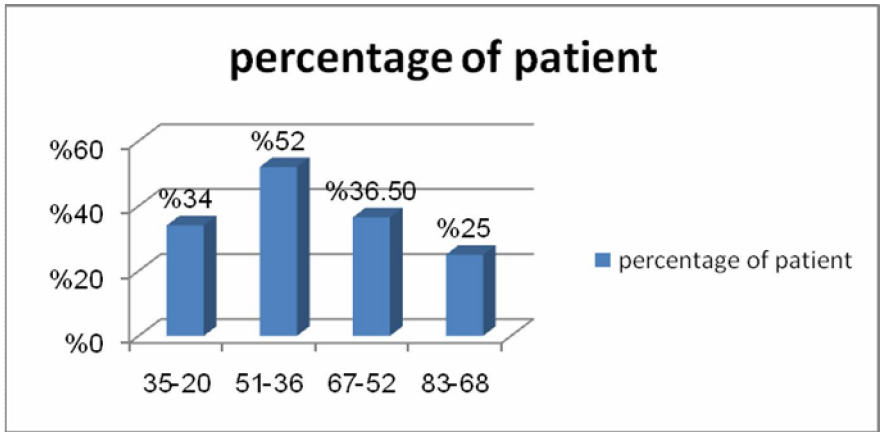
Twenty cases out of 56 (35.6 %) showed positive IHC- reactions of p16 over expression.None of healthy cervical tissues showed positive P16-IHC reaction. The highest positive P16-IHC reactions in those with malignant cervical cancer group were showed moderate signal intensity 53.8% (7 cases out of 13) while 30.8% (4 out of 13) foundwithinstrong signal intensity . In benign cervical tumor group, the percentage 42.9% (3 cases out of 7) have moderate and strong signal intensity. Statistically, non-significant differences ($p > 0.05$) were found on comparing each group of cervical cancers and benign cervical tumors to the control group (Table 2)

Table (2) : distribution of immunohistochemistry of P16 protein according to the signal intensity.

P16 over expression		Malignant cervical cancers (n=27)		Benign cervical tumors (n=16)		Healthy cervical tissues (n=13)		P
		N	%	N	%	N	%	
Negative		14/27	51.9	9/16	56.3	13/13	100	
Positive		13/27	48.1*	7/16	43.7*	0/13	0.0	**0.002***
Scoring	Weak	2/13	15.4	1/7	14.2	0/0	0.0	
	Moderate	7/13	53.8*	3/7	42.9*	0/0	0.0	**0.148 N.S**
	High	4/13	30.8	3/7	42.9	0/0	0.0	

- **Significant P16 overexpression according to age groups**

The highest percentage of P16 overexpression found in age group(36-51years) was represented 52% ,followed by 36% ,34% ,25% showed within age group (52-67 years),(20-35years), (68-83years) respectively. Statistically theP16overexpression has no significant correlation to age. Table(3)



Fig(3): Occurrence of Immunohistochemistry for P16INK4A protein according to age groups

Over expressed-P16 protein in relation to cervical cancer types

The highest percentageof P16 – IHC reaction showed within squamous cell carcinoma were represented 50% (9 out of 18 cases) while adenocarcinoma was represented 44.4% (4 out of 9 cases), 75% (3 out of 4 cases) of adinocarcinoma that have high score (score III) while 44.4 %(4 out of 9 cases), 33.4 % (3 out of 9 cases) in squamous cell carcinoma was showed within score (score III, II) respectively.Statistically, non – significant differences ($p>0.05$) was found between cervical cancer types and their P16-protein over expression.Table (4).

Table (4): The Relation of P16 – overexpression with cervicalcancertyping

P16 over expression		Squamous cell carcinoma (n=18/27)		Edenocarcinoma (n=9/27)		P
		N	%	N	%	
Negative		9/18	50	5/9	55.5	
Positive		9/18	50	4/9	44.5	
Scoring	I	2/9	22.2	0/4	0.0	
	II	3/9	33.4	1/4	25	**0.245 N.S**
	III	4/9	44.4	3/4	75	

Discussion:

Thepathological and clinical data of Cervical Cancer were measured to explore this challenging group of malignancies in relative to their existing information. look over the 56 cases which were included in this study, it was establish the age of the patients with cervical tumor and apparently healthy control was ranging between 23-73 years fig1.The current results are reliable with those reported world-wide where these cervical cancer commonly affecting females over forty years of age[7] (Goodman , 2002).Moreover, it was observed that the percentages of cervical cancer cases are improved with the proceeding of age of patients. These results could reflect that age is an significant risk factor in cancer changes affecting cervical epithelial tissues lesions. In present study the results could have their significance when identifying that the age of the cervical cancer patients is an strong factor both for the incidence and management of the disease[8] (American Cancer Society, 2009).

Overall, aging increase the incidence of the malignant changes in cervical epithelial tissues[9,10] (Grey and Lila. 1994;Koyama *et al*., 2007).In current study, the peak age frequency was 68-83,52-67 years which constituted 100%, 93.8,respectively (Figure 1).Asimilar peak frequency was recorded in other reports from other country that older women have increased rates of cervical cancer[6] (Kraus*et al*, 2006).This is similar to another study by Aslani*et al,* where patients age ranged from 20 to 80 years[11] (Hong *et al* ,2010).

Therecentnumbersthatshowyoungerwomenbeing affected moreby the disease has led many professionals tomodify ages to include older and younger women, the most recommended ages for screening are between20- 70 years of age even though some researchers suggest that screening under the age of 25 is poor use of resources (Paul *et al*,1991).The current findings of high percentage of cervical cancer with the increasing of the patients age could be correlated by many other factors which improve the appearance of malignant cervical tumor in old age group through the proceeding of age such as genetic predisposition ,hormonal factors and changes in life style which characterized by a highly caloric diet-rich in fat , and refined carbohydrate which in turn are reinforced by many previous studies done by[13]

Squamous cell carcinoma is the cervical cancer with the most incidences; while the incidence of adenocarcinoma of the cervix has been increasing in recent decades[14].(Kumar,*2007).* In the present study, squamous cell carcinoma was found to be the most common type (66.6%) among the group of cervical cancers patients.This result means that the woman is likely to have an invasive cancer, in contrast the type ofglandular cell abnormalities adenocarcinoma constituted 33.3%, Cancers of the glandular cells are called *adenocarcinomas*,in some cases, the pathologist examining the cells can tell whether the adenocarcinoma started in the endocervix, in the uterus (endometrium), or elsewhere in the body[8](American Cancer Society, 2009).

Occurrence of p16 INK4A among cervical tissues tested:

One of the most significant themes still unresolved in the cure and management of Cervical Cancer is our inability to define earliest changes that lead to the disease and target it for treatment. Thus, the documentation of reliable biomarkers such as p16 tumor suppressor gene, treatment represents an crucial requirement not only for translational academic but also for clinical oncologists[15](Buchynska *et al*, 2007).The p16INK4A gene is exclusively the cell cycle regulating genes and encodes a nuclear protein p16 , that phosphorylate the retinoblastoma gene product (pRb), thus blocking G1-S cycle progression. This gene when inactivation stimulates cell proliferation, therefore is found in many diverse types of carcinomas such as gastric carcinoma, bladder tumor, glioma, breast cancer, cervical cancer and head and neck tumors[16] (Chen,*et al*.,2004).

Immunohistological expression of $p16^{INK4a}$ was seen only in dysplastic/neoplastic cells, and was never observed in normal cervical epithelium. Thus, $p16^{INK4a}$ expression appears to be a strong, specific and subtle biomarker of cervical neoplasia, confirming the results of previous smaller series[17,18](*Klaeset al* 2001,,*Drayet al* ,2005). Although other pathways cannot be ruled out, increased expression of $p16^{INK4a}$ in the setting of CC probably occurs mainly as a result of inactivation of RB by high-risk HPVs. Incidental support for this evidence comes from the observation that increasingly high $p16^{INK4a}$ expression scores were seen in cervical specimens showing invasive carcinoma, lesions known to be closely associated with high-risk HPV infection[19]*(Ostor ,1993).*

In current study, we found the positive signalsof P16-IHC in malignant cervical tumor were 48.1 %, followed by 43.7% in benign cervical tumor, and 0.0% in healthy cervical tissues (table 1). Among the 27 cervical cancer cases in this study, 14 (51.9%) were negative for p16 protein immune-expression.These findings are in agreement with reports published by Lana Lesnikova*et al* ,2009 [20] who demonstrated that p16 overexpression in CC cases have 98.5% the differences in p16 expressing between this finding and our finding may be due false positive or false negative result or due to poorly preservative of formalin fixed paraffin embedded tissue. The non- expression rate of p16 protein also correlated with the presence of metastatic disease at diagnosis and development of distant recurrence disease. The concordance between the P16 protein expression and high grade cervical abnormality is documented by[17] Klaes*et al*,(2001) who demonstrated that most if not all HSILs were P16 INK4A positive , which is in agreement with results reported by **Nicol***et al*., (2012)[21] where P16 expression was observed in 19/35 (54.3%) .In present study P16INK4a positivity in cervical cancer wasrelativelyagreementto the findings of between 50% and 60% in the studies reported by Agoff*et al* and Klaes *et al* (Agoff*et al*, 2003;Klaes *et al*,2001)[17,24].

In the current study, p16 immunoreactivty was calculated depending on the scoring. That is used by several studies[22] (*Tam SW,1994*).In the present study, the highest percentage of P16-IHC score signal found in high score (III).was (46.2%) were found within cervical cancer and in low score(I) 42.9% within benign tumor. This result was agreement with study of Maxwell *et al*., (2010)[23] that found the high percent in strong scoring (III).According to the intensity signals, it was detected the highest percent of p16-INK4A in the moderate intensity (53.8%) for malignant cervical tumor, followed by 30.9% in the strong intensity. while In the benign cervical tumor it was found the highest percent of p16INK4A in the moderate and strong intensity in (42.9%). while none detected any signals in control group (Figure: 4-3).

The score as 1, 2, or 3 was considered as p16 -positive in order to show the differentially expressed levels of p16INK4A between neoplastic spindle cells and non-spindled cells. In the present study, the score of p16 INK4A expression was determined based on both the intensity and the percentage of positive tumor cells. According to the intensity, staining in the nuclei of cancer cells was determined as the positive hybridization signal[24]. (Agoff*et al*, 2003).

In present study found the high percentage of p16 INK4A over expression found within age group 36--51 years was 52% followed by 36% in age group (52-67 years) Zhang, (2009) found the high percentage in median of age as 59.6% (range, 20–89 years). years this result is consistent with our result where the high percentage of P16 ranging between age group (36-51 years) and age group (52-67) years (Figure 3). This result explained by (Hend,*et al*.,2006)[25] as high expression of P16 was more frequently detected in adult CC patients form than in young age. The present study showed the high percentage ofp16 INK4A over expression found within squamous cell carcinoma were represented as 50% while in adenocarcinoma the p16INK4A expression have 44.4% By analogy, these observations are supported by the findings of Reed, *et al*,(1996)[26] where them found that 70% of poorly differentiated squamous cell carcinoma carcinoma have abnormalities in the expression of P16.[27-51]

Conclusion:

Weestablish thathighest age – specific frequency was noticed in elderly aged- patients with cervical malignant age group of (52–67 years),(67-83years) and in benign cervical tumors in age group(20 – 35 years).Alsoa majority cervical carcinomas are pronounced by strong expressionp16INK4a proteins, although in need of corroboration on a larger pool of cases, show that p16INK4amay represent promising tool toward the identification of patientswith poorerprognosis who may benefit from more aggressive therapy and HPV screening.

References:

1. Brooks, G. F; Carroli, K. C. ;Butel, J. S. and Morse, S. A. (2010)Jawetz, Melnick and Adelberg, S.Medical Microbiology . 24 ed.McGraw Hill Companies.USA.
2. Clifford, G. M. andFranceschi, S. (2008). Human papilloma virus types among women infected with HIV : J. ameta-analysis.AIDS, v,20 ,p, 2337-2344.

3. HesselinkA.T.(2007) High-risk human papillomavirus testing in cervical screening .Netherlands Organization for Health Research and Development; grant 2200.0147.
4. Kufe, DW, Pollock RE, Weichselbaum RR, BastR C, Gansler TS, Holland JF, Frei E (2003).Section 3, Cancer etiology, Papillomaviruses and Cervical neoplasia. In: Cancer Medicine.
5. Kjaer SK, van den Brule AJ, Bock JE, Poll PA, Engholm G, Sherman ME, Walboomers JM, Meijer CJ (1996) Human papillomavirus: the most significant risk determinant of cervical intraepithelial neoplasia. Int J Cancer 65, 601-606.
6. Kraus I, Molden T, Holm R, Lie AK, Karlsen F, Kristensen GB, Skomedal H (2006) Presence of E6 and E7 mRNA from Human Papillomavirus Types 16, 18, 31, 33, and 45 in the Majority of Cervical Carcinomas. J ClinMicrobiol44, 1310-1317.
7. Goodman, A. Human papillomavirus infections in incarcerated women . H.E.P.P. News (2002). 5 (1). (Internet).
8. American Cancer Society, 2009.Also available online 7. Last accessed January 6, 2010.
9. Grey L and Lila R. Immunohistochemistry.In: Ulrikav.Mikel. Advanced laboratory method in histology and pathology, Armed forces institute of pathology.American registry of pathology, Washington, DC, 1994, 2:1-30.
10. KoyamaT. ,Tamai K. ,Togashi K.(2007) Staging of carcinoma of the uterine cervix and endometrium.EurRadiol ,17:2009-2019.
11. Huang MZ,Li HB, NieXM,etal.An analysis on the combination expression of HPV L1 capsid protein and p16INK4a in cervical lesions. Diagn Cytopathol.2010;38:573–578.
12. Paul, C., Bagshaw,S., Bonita,R.,Durham,G.,Fitzgerald,N.,Jones,R.,Marshall,B., McAvoy, B. (1991). Cervical screening recommendations: a working group report. New Zealand Medical Journal, 104, 291-295.
13. De Villiers E. M. ,Fauquet C. , Broker T. R. , Bernard H. U. and zurHausen H.(2004) Classification of papillomaviruses. Virology; 324, 17–27.
14. Kumar, V. ; Abul , K. A.; Nelson, F . and Richard, N. M. (2007).Robbins Basic pathology 8th Ed. Saunders, an imprint of Elsevier Inc .
15. BuchynskaLG, Nesina IP, Yurchenko NP, et al.(2007)Expression of p53, p21WAF1/CIP1, p16INK4a and Ki-67 proteins in serous ovarian tumors. ExpOncol; 29: 49–53.
16. Chen, C.A. and Hsieh, C.Y. (2004) Recent advances and problems in primaryTherapy for cervical cancer in Taiwan. J Formos Med Assoc, 103, 511-518
17. Klaes R, Friedrich T, Spitkovsky D, Ridder R, Rudy W, Petry U, Dallenbach-Hellweg G, Schmidt D, von KnebelDoeberitz M: Overexpression of p16(INK4A) as a specific marker for dysplastic and neoplastic epithelial cells of the cervix uteri. Int J Cancer. 2001, 92 (2): 276-284. 10.1002/ijc.1174.
18. Dray M, Russell P, Dalrymple C, Wallman N, Angus G, Leong A, Carter J, Cheerala B: p16(INK4a) as a complementary marker of high-grade intraepithelial lesions of the uterine cervix. I: Experience with squamous lesions in 189 consecutive cervical biopsies. Pathology. 2005, 37(2): 112-124. 10.1080/00313020500058607.
19. Ostor AG: Natural history of cervical intraepithelial neoplasia: a critical review. Int J GynecolPathol. 1993, 12 (2): 186-192. 10.1097/00004347-199304000-00018
20. Lana lesnikova , Marianna ladan, Stephen Hamilton Dutoit and jernkoch,(2009) p16 as diagnostic markerof cervical neoplasiatissuemicroarray study of 796 archival specimen (2009) .10.1186/1796-22
21. Nicol AF, Golub JE, Lapa JR et al. An evaluation of p16 expression in cervical intraepithelial neoplasiaspecimens , including women with HIV-1. 2012;107(August):571–7.63.
22. Tam SW, Shay JW, Pagano M: Differential expression and cell cycle regulation of the cyclin-dependent kinase 4 inhibitor p16Ink4. Cancer Res. 1994, 54 (22): 5816-5820.
23. Straton, J. A.Y.,Sutherland,G.J.,C..(1995), Cervical cancer screening for hospital inpatients: report of an intervention study. Australian Journal of public health 19(3)288-293 Zhang LL, Zheng CQ: Expression of p16 and CDK4 in colonic carcinoma and pericancerousmucose. J Shenyang Medical College 2009; 11:74–76.
24. AgoffSN, Lin P, Morihara J, Mao C, Kiviat NB, Koutsky LA: p16(INK4a) expression correlates with degree of cervical neoplasia: a comparison with Ki-67 expression and detection of high-risk HPV types. Mod Pathol. 2003, 16 (7): 665-673. 10.1097/01.MP.0000077518.78046.0C.
25. HendBraham ,MounirTrimeche , Sonia Ziadi . CD10 expression by fusiform stromal cells in nasopharyngeal carcinoma correlates with tumor progression. Virchows Arch, 2006; 449: 220–224.

26. Reed AL, Califano J, Cairns P, Westra WH, Jones RM, Koch W, Ahrendt S, Eby Y, Sewell D, Nawroz H, Bartek J, Sidransky D: High frequency of p16 (CDKN2/MTS-1/INK4A) inactivation in head and neck squamous cell carcinoma. Cancer Res 1996, 56:3630-3633 .
27. Al-Hamairy , A , K., 2016,Effect of lipopolysaccharide extracted from escherichia coli on the growth and development of promastigote of leishmania tropica (in vitro study), International Journal of PharmTech Research,9(4), 377-386.
28. Al-Gazally, M. E.; Al-Awad, A. S.; Kzar, H. H., 2016. Assessment of antioxidant status in different genotypes/phenotypes at codon 72 of TP53 gene for patients with sporadic colorectal cancer in Babylon province, International Journal of PharmTech Research, 9: 280-286.
29. Kareem, A.; Abd Alrazak, N.; Aljebori, K. H.; Aljebori, A. M.; Algboory, H. L.; Alkaim, A. F., 2016.Removal of methylene blue dye from aqueous solutions by using activated carbon/ureaformaldehyde composite resin as an adsorbent, Int. J. Chem. Sci., 14: 635-648.
30. Aljeboree, A. M.,Adsorption of crystal violet dye by Fugas Sawdust from aqueous solution, International Journal of ChemTech Research, 2016. 9(3): p. 412-423.
31. Al-Gubury, H.Y.The effect of coupled titanium dioxide and cobalt oxide on photo catalytic degradation of malachite green, International Journal of ChemTech Research, 2016. 9(2): p. 227-235.
32. Al-Terehi, M., , Al Saadi, A. H., Zaidan, H. K., and Al-Harbi, S. J., 2016.Protective effects of Glycyrrihza glabra plant extract against cyclophosphomide in kidney and liver tissues in white albino rats, International Journal of ChemTech Research, 9: 402-406.
33. Omran, A. R.; Baiee, M. A.; Juda, S. A.; Salman, J. M.; Alkaim, A. F., 2016.Removal of congo red dye from aqueous solution using a new adsorbent surface developed from aquatic plant (Phragmites australis), International Journal of ChemTech Research, 9: 334-342.
34. Alkaim, A. F.; Hussein, F. H., 2012.Photocatalytic degradation of edta by using TiO 2 suspension, International Journal of Chemical Sciences, 10: 586-598.
35. Al-Saadi, A. H.; Zaidan, K. I.; Mohammed, T.; Zaidan, H. K.; Al-Terehi, M., 2015.Dental sex determination by multiplex PCR in iraqi samples, Research Journal of Pharmaceutical, Biological and Chemical Sciences, 6: 1572-1577.
36. Alshirifi, A. N.; Alhameedi, D. Y., 2016., International Journal of ChemTech Research, 9: 712-722.
37. Al-Gubury H. Y., and Al-Murshidy G. S.Photocatalytic decolorization of brilliant cresyl blue using zinc oxide, 2015. International Journal of PharmTech Research, 8: 289-297.
38. Al-Gazally, M. E., Obed, A. F., and Al-Saadi, A. H., 2016.Effect of ACE gene polymorphism of Iraqi patients on ischemic stroke, International Journal of ChemTech Research, 9: 424-429.
39. Alkaim A. F., and Alqaragully M. B., 2013.Adsorption of basic yellow dye from aqueous solutions by activated carbon derived from waste apricot stones (ASAC): Equilibrium, and thermodynamic aspects, Int. J. Chem. Sc., 11: 797-814.
40. Al-Dahmoshi, H. O. M.; Al-Saffar, A. K. H.; Al-Khafaji, N. S. K.; Ali, J. A.; Muttaleb, W. H., 2016.Non invasive markers to predict liver damage among patients with chronic HBV, Hilla City-Iraq, International Journal of PharmTech Research, 9: 388-393.
41. Ai-Mashhedy, L. A. M.; Fijer, A. N., 2016.Acute toxicity of food additives tartrazine and carmoisine on white male mice, International Journal of PharmTech Research, 9: 364-367.
42. Al-Alaq, F. T.; Abdulazeem, L.; Al-Dahmoshi, H. O. M.; Al-Khafaji, N. S. K.; Al-Wesawei, Y. A. R., 2016.PCR-based investigation of oxygenase among crude oil degrading bacteria in Hilla city, Iraq, International Journal of PharmTech Research, 9: 284-291.
43. Jabbar, A. Z.; Hadi, A. G.; Sami, F., 2014.Removal of azo dye from aqueous solutions using chitosan, Oriental Journal of Chemistry, 30: 571-575.
44. Al-Terehi, M.; Zaidan, H. K.; Al-Mamoori, A. M. J.; Al-Saadi, A. H.; Harjan, I., 2015.Effective of different factors on trace elements concentrations in Iraqi lactating mother'smilk, International Journal of PharmTech Research, 8: 151-157.
45. Raheem, H. Q.; Al-Thahab, A. A.; Abd, F. G., 2016.Different methods for detection sliver nanoparticles produced by proteus mirabilis bacteria, International Journal of PharmTech Research, 9: 368-376.
46. Hadi, A. G.; Humedy, E. H.; Saddam, N. S.; Abd-Alameer, F. S., 2016.Spectrophotometric study of complex formation between hematoxylin and Al3+ and Fe3+ ions, International Journal of PharmTech Research, 9: 292-298.
47. Abdulrazzak, F. H., 2016.Enhance photocatalytic activity of TiO2 by carbon nanotubes, International Journal of ChemTech Research, 9: 431-443.

48. Hadi, A. G., 2016.Removal of Fe (II) and Zn (II) ions from aqueous solutions by synthesized chitosan, International Journal of ChemTech Research, 9: 343-349.
49. Karam, F. F.; Hussein, F. H.; Baqir, S. J.; Alkaim, A. F., 2016. Optimal conditions for treatment of contaminated waters with anthracene by Fenton processes in close system reactor, Journal of Chemical and Pharmaceutical Science, 9: 1111-1115.
50. Kamil, A. M.; Mohammed, H. T.; Alkaim, A. F.; Hussein, F. H., 2016. Adsorption of Congo red on multiwall carbon nanotubes: Effect of operational parameters, Journal of Chemical and Pharmaceutical Sciences, 9: 1128-1133.
51. Raheem, R. A.; Al-gubury, H. Y.; Aljeboree, A. M.; Alkaim, A. F., 2016. Photocatalytic degradation of reactive green dye by using Zinc oxide, journal of Chemical and Pharmaceutical Science, 9: 1134-1138.

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