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Occurrence of RB- tumor suppressor gene overexpression in woman suffering with cervical carcinomain mid Euphrates

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Abstract : The retrospective research was designed as immunohistochemical study to demonstrate the expression state of tumor suppressor genes (RB) in (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 histopathological laboratories in Babylon, 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 obtained results are summarized as follows: The mean age of the patients with cervical carcinoma (54 $\pm$ 11.22years) was higher than the mean age of the benign group (38.8$\pm$9.85 years) and the mean age of those females in the group of healthy control (35.5$\pm$8.64years). There are significant differences ($p< 0.05$) between different groups according to age. The well differentiated grade of CC constituted 52%, whereas those with moderately and poorly differentiated grades CC constituted 37%and 11% respectively. Over expression of RB - TSG was detected by IHC in 59.3 % of cervical cancer cases and in 43.8% of benign cervical tumor group, while none of control group showed RB- over expression. A high percentage 43.6% showed of cervical cancer have a moderate score (score II). Flowed by 37.5% and 18.8% were found to have low score (score I) and strong score (score III), respectively. While, in benign cervical tumor group, the highest percentage 57.1% were found to have moderate score (score II) and 28.6%, 14.3% have strong score (score III) and low score (score I) respectively.
The highest percentage of RB protein expression it was found within poorly differentiated that have 100% (3 out of 3 cases). Followed by 60% (6 out of 10cases), 57.1 %(8 out of 14 cases) within moderately, well differentiated respectively.
Keywords: RB- tumor, gene overexpression, cervical carcinomain, mid Euphrates.

Introduction:

The retinoblastoma (Rb) gene mapped on chromosome 13q14.2, encodes a 110 kDa nuclear phosphoprotein, and regarded as important regulator of the cell cycle at G1-S transition, that is at the convergence of several positive and negative regulatory pathways and are often referred to collectively as the Rb pathway[1]. Disruption of the Rb pathway function through direct Rb mutation or mutation of upstream regulator of Rb, such as p16INK4a, is thought to occur in the vast majority of human cancer including cervical cancer[2,3].

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[4].

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[5]. 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[6,7].

Objective: To determine the frequency of RB-overexpression in uterine cervical biopsies with or without dysplasia. Therefore, RB-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:

Among fifty six (56) formalin-fixed, paraffin-embedded uterine cervical tissue blocks from patients who had undergone hysterectomy or punch biopsy from the cervix , (43) malignant and benign blocks were collected from the archives of histopathology laboratories of different general hospital in mid - Euphrates as well as many private laboratories. With thirteen cervical 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)[8].

Immunohistochemical staining was performed using the primary mouse monoclonal antibodies against RB-TSG, dilution 1:40 (clone E6H4, dakocytomation), immunostaining for RB-TSG: 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. The immunoreactivity for all cell cycle regulatory proteins investigated in this study was evaluated as strong, moderately and weak according to the values of median. Statistical analysis was performed by SPSS statistical software package version 20, using the $x2$ test t- test and 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 mean age was constituted (54 $\pm$ 11.22 years). While in benign cervical tumor and healthy control groups were(38.8 $\pm$ 9.85 years) control (35.5$\pm$ 8.64 years, significant differences ($p< 0.05$)revealed between different groups according to age (table1).

Table (1): Distribution of cervical tumor patients according to their age

Maximum	Minimum	S.E	S.D	Mean age	N	The Patients
73	23	2.1	11.22	54	27	Malignant cervical tumors
53	25	2.4	9.85	38.8	16	Benign cervicaltumors
48	23	2.3	8.64	35.5	13	Healthy cervical tissues control
(P <0.05)						Statistical analysis

the results of present study show that well differentiated grade cervical carcinomas constituted 52% (14 of total 27 cases) , whereas cases with moderately and poorly differentiated grades constituted 37% (10 out of 27 cases) and 11% (3 out of 27 cases) respectively. Fig (1)

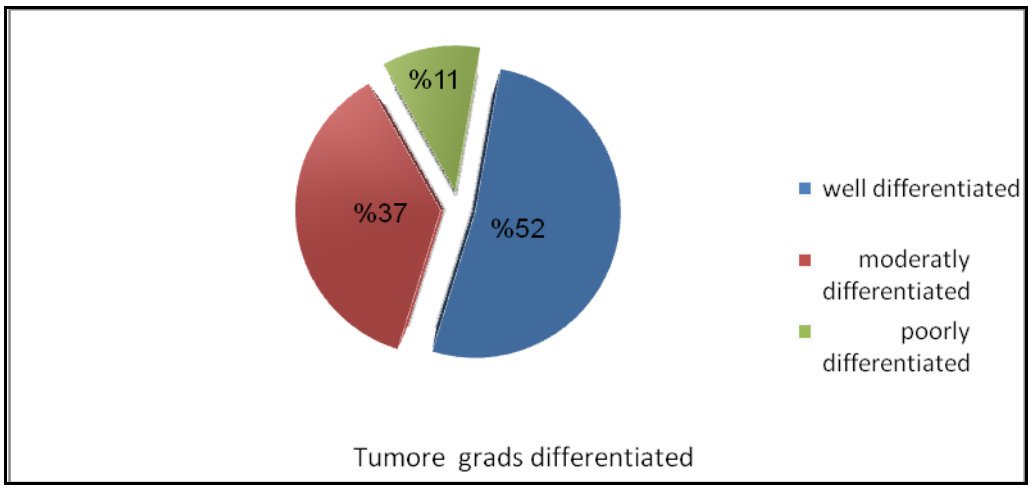


Fig (1) :Tumor grading of cervical carcinoma groups

The statistical analysis of grading distribution of cervical carcinoma shows significant differences ($p<0.05$) between well and poorly differentiated grade, while non-significant difference was noticed between well and moderately differentiated cervical carcinomas . Over expression of mutated RB- protein was detected as a brownish discoloration at nuclear localization (Figure 2). Over expression of RB-TSG was detected in 59.3% (16 out of 27) cells with cervical cancers and in 43.8% (7 out of 16) cases with benign cervical tumor, while non-cases of control group showed RB- over expression. A highest percentage 57.1 % (4 out of 7 cases), 43.6 %(7 out of 16 case) was involving cases with benign, malignant cervical tumor that have moderately score (score II) while none of control group showed positive signal, significant differences($p<0.05$).

Were found on comparing the results (according to score) between cervical cancers, benign tumors and control group, (table 2)

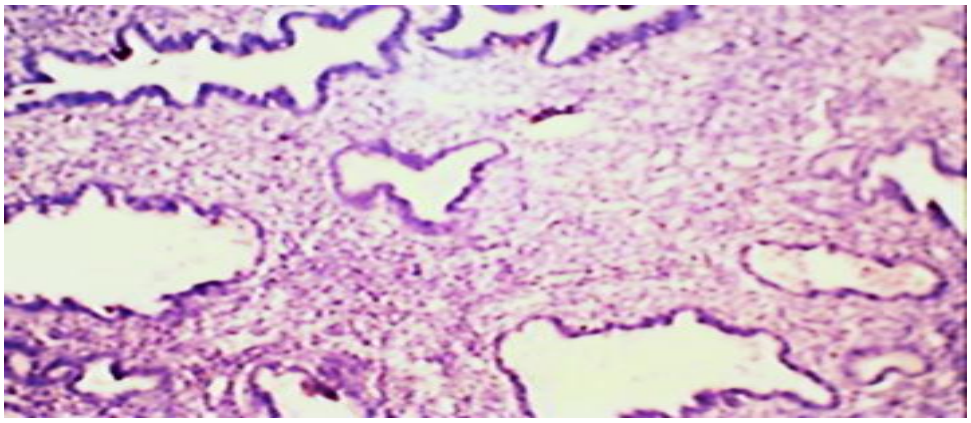
Table (2): Distribution of immunohistochemistry results for Rb protein according to signal scoring

Chi-square &p value	Healthy cervical tissues (n=13)		Benign cervical tumors (n=16)		Malignant cervical cancers (n=27)		Rb over expression	
	%	N	%	N	%	N		
1.3* Sig.	100	13/13	56.3	9/16	40.7	11/27	Negative	
	0.0	0/13	43.7	7/16	59.3	16/27	Positive	
	0.0	0/0	14.3	1/7	37.5	6/16	I	Scoring
	0.0	0/0	57.1	4/7	43.6	7/16	II	
	0.0	0/0	28.6	2/7	18.8	3/16	III	

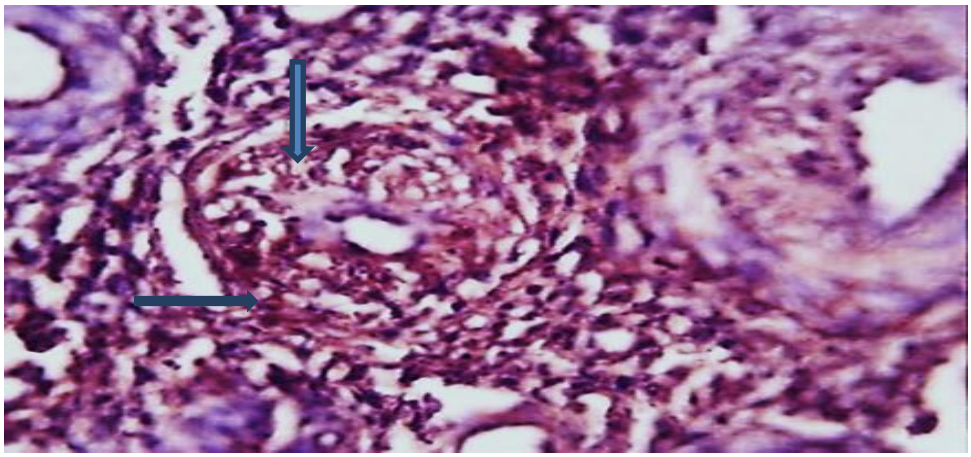
Among (27) malignant cervical tumors, 16 cases (59.3%) showed positive RB –IHC reactions, a percentage of 56.3% (9 cases out of 16) was found to have moderate signal intensity while each weak and strong signals intensities constituted 25% (4 out of 16),18.8% (3 out of 16) respectively. In benign cervical tumor 43.7% (7 out of 16 cases) showed positive RB –IHC reactions, were 42.9% (3 cases out of 7) has strong signals intensity, and 28.6% (2 out of 7) showed in both weak and moderately differentiated. None of healthy cervical tissues showed RB—IHC reaction, significant differences ($p <0.05$) were found on comparing studded group of cervical tumors with the control group table (3)

Table (3): Frequency distribution of immunohistochemistry results for RB protein according to signal intensity

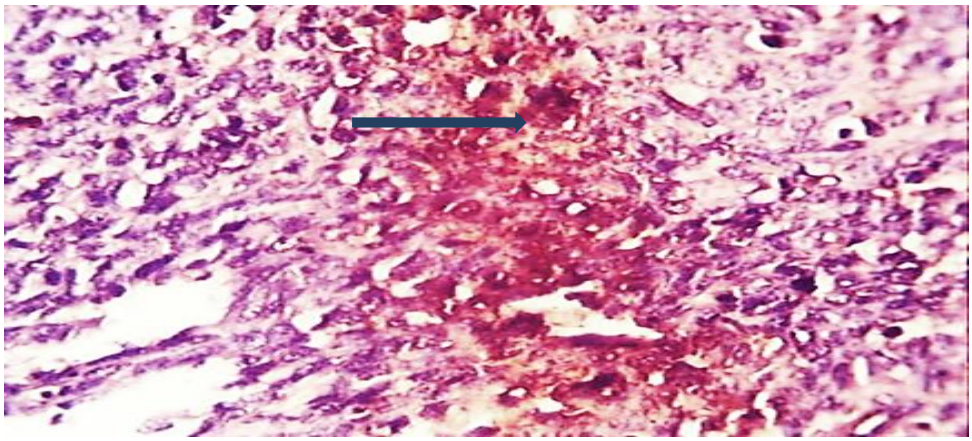
Chi-square &p value	Healthy cervical tissues (n=13)		Benign cervical tumors (n=16)		Malignant cervical cancers (n=27)		Tissue Rb intensity	
	%	N	%	N	%	N		
1.3*Sig	100	13/13	56.3	9/16	40.7	11/27	Negative	
	0.0	0	43.7	7/16	59.3	16/27	Positive	
	0.0	0	28.6	2/7	25	4/16	Weak	Intensity
	0.0	0	28.6	2/7	56.3	9/16	Moderate	
	0.0	0	42.9	3/7	18.8	3/16	High	



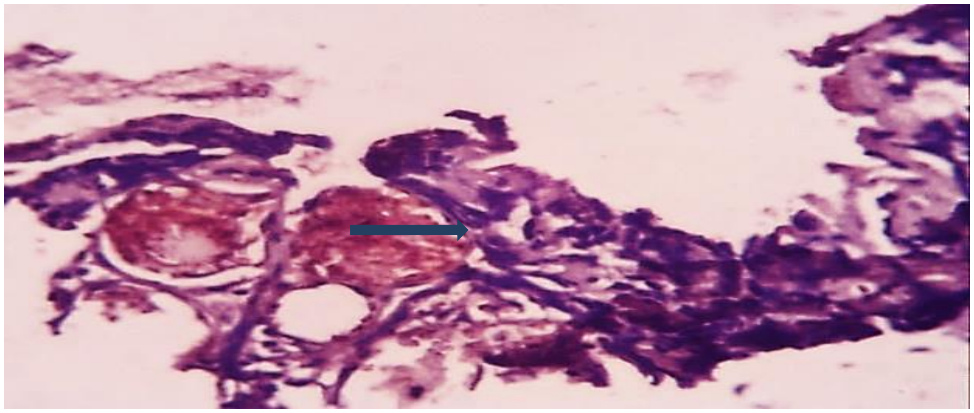
A



B



C



D

Fig. (2): Cervical carcinoma showing the results of immunohistochemical staining of Rb protein expression usingBiotinylated -Labeled anti-Rb protein antibody, stained by DAB-Chromogen (Brown) and counter stained by mayer's hematoxyline (Blue). A. **cervical cancer with negative Rb –ICH reactions (40X).B .positive Rb –ICH reaction with strong score and high signal intensity (40X).C .positive Rb –ICH reaction with moderate score and high signal intensity (40X). D. Positive Rb –ICH reaction with low score and high signal intensity (40X).**

Our result revealed, the highest percentage of positive RB-IHC reactions it was found within poorly differentiated that have 100% (3 out of 3 cases). Followed by 60% (6 out of 10 cases), 57.1 %(8 out of 14 cases) within moderately, well differentiated respectively. The overall Rb –IHC scoring according to tumor grading showed significant ($p<0.05$) differences table (4).

Table (4): Relationship of RB-IHC scoring with grading of cervical carcinoma

Rb signal			Tumor grade			Chi-square & p value
			Well differentiated n=(14)	Moderately differentiated N= 10	Poorly differentiated N=(3)	
Rb signal	Positive	Count	8	6	3	1.5*Sig.
		% within tumor grade	57.1%	60%	100%	
	negative	Count	6	4	0	
		% within tumor grade	42.9%	40%	0.0%	
Total		Count	14	10	3	
		% within tumor grade	100%	100%	100%	

Discussion:

Invasive cancer of the uterine cervix is one of the major killer's cancers that affect women across the world, also its lines as the most significant of all the 10 greatest common female neoplasms in developing countries, whereas it defenses as the fifth in western developed nations, however the disease continues to be the second most common cancer in women world-wide, The tragedy behind the thousands of deaths occurring each year from the disease is the lost opportunity of them being prevented[10].

Distribution of cervical cancer patients according to their age

The experimental management pattern of cervical cancer will help us to recognize the appropriate age for cervical cancer screening[11].

On reviewing the 56 cases which were included in this study, it was found the age of the patients with cervical tumors was ranging between 23-73 years with mean age was (48.5± 12.9 years).Among cervical cancer, benign tumor cases the mean age (54± 11.22),(38.8± 9.85) respectively. While the mean age among control group was (35.5 ± 8.64) (Table 1).These results are consistent with those reported world-wide where these cervical tumors usually affecting females over forty years of age[12].

Also, on reviewing (table 1), it was noticed that the percentages of CC cases are increased with the proceeding of age of patients. The present results could have their importance when realizing that the age of the cervical cancer patients is an important factor both for the occurrence and management of the disease[13].

However, the result relatively in agreement with other studying In Indonesia where showed that the highest incidence was in the age group of 55 - 64 years ,were it was reported that malignant tumors have increased with the proceeding of age of patients[14].

Likewise, the mean age in our study compatible to the mean age found by Missaoui *et al* (2010) [15] in Tunisia , Badar*et al* (2007) [16] At Muslim – community, were the mean age of these studies 52.1 and 49.2 years old respectively. Moreover, Sando *et al*.(2014)[17] reported the mean age was (52.73 ± 3.82 years), Engbang *et al*.(2015) [18] in his research revealed the mean age was (51.22 ± 11.93 years old), and Elmajjawi *et al*. Morocco, in his series of 696 cases that had an average age of 50 years[19].

Also, the result found that cervical cancer was higher at elderly ages. This might be caused by deficiencies in screening programs that has been very recently used in Iraq These findings of high percentage of cervical cancer with the increasing of the patients age could be associated by many other factors which enhance the appearance of malignant cervical tumor in young 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 (11). Aging is also a risk factor for persistent infection. The rate of persistent high-risk infection for women older than age 55 is 50%, while the persistent rate of women younger than 25 years old is 20% , Cervical cancer is extremely rare in women younger than age 20[20].

However, in contrast to our result many other studies at developed countries showed the increase in cervical cancer incidence at younger age[21,22]. England and Wales reported a decrease cervical cancer prevalence in the age group of 45 - 64 years and 55 - 64 years and a significantly increase in younger age groups, 35 - 39 years and 40- 44 years. Moreover, Switzerland another country with successful screening programs, especially at 20 - 44 years old, reported a decrease in cervical cancer occurrence in 30 - 64 years old group. So the incidence of cervical cancer at younger age may be contributed by early exposure of sexual activity, early menarche, multiplexes partner, sexual promiscuity, HPV infection, a high incidence of sexual transmitted disease, smoking, and oral contraception[23,24].

Furthermore, other factor that donates the earlier finding is screening programs in developed countries which have been carried out sufficiently, allowing early detection of cervical cancer at early stage[25]. On over view the dissimilarity in the mean age and the highest age group prevalence of cervical cancer patients among our study and studies in countries with sufficient screening programs revealed that the screening programs in Iraq have not been conducted sufficiently. The presence of cervical cancer diagnosed in post-menopausal age indicated that cervical cancer screening should not be stopped in menopausal age. So a report that women who

are screened every three years before 50 years, did not develop cervical cancer, but women who did not undergo regular screening, may still develop cervical cancer after the age of 50[26].

Histopathological grading of cervical carcinoma

This study has depended on that popular grading system to fix or document the histopathology grading of our series of cervical cancers. Grading of the presented malignant cases were assessed according to the WHO grading system of cervical carcinoma , revealing that grade I(well differentiated grade) was reported in 14 (52%) cases, grade II(moderately differentiated grade) in 10 (37%), while those of grade III (poorly differentiated grade) were 3 (11%) cases (Figure 1).

Likewise Abdul-Hussein A., (2011)[27] reported similar percentages in the Iraqi patients were 67. 44% found in grade I, 25.58% in grade II and 6.98% in grade III respectively, this may be due to early detection of cervical neoplasm change. However, Gichangi*et al*., (2002)[28] of their studied CC grading revealed 13% as well-differentiated, 28% as moderately-well differentiated and 48% of the histological subtypes were reported as poorly differentiated this result are incompatible to our results. Also our study dissimilar to the result finding byKlaes*et al*. (2002)[29], Bergeron *et al*. (2010)[30], Galgano*et al*. (2010)[31] Which reported the poorly differentiated constituted 72%,76.6%,75% respectively.

Viladiu*et al*. (1977) [32] identified clinical grad as the only independent prognostic factor by contrast advanced grad was identified as a prognostic factor in our study. The survival rate of advanced grad patients is also less. The prognostic models of age and grad are powerful in predicting death and relapse. Longest survival was for patients with early stage disease, younger patients and after primary surgery. We found International Federation of Gynecology and Obstetrics (FIGO) stage, grade and lymph node metastases of significant prognostic value for survival in cervical adenocarcinoma[33].

The evaluation of RB-tumor suppressor genes among malignant and benign cervical tumors:

The definition of immunhistochemical staining an altered Rb expression differs greatly between studies. Various investigators considered that all nuclei in tumor cells must be negative to classify a tumor as having an altered Rb expression[20]. While, others regarded tumors with areas of positively and negatively stained tumor cell nuclei as having an altered Rb expression[34]. Deletion and Mutations of the RB gene are established in retinoblastoma moreover, loss of RB function has been described in a variety of human malignancies The tumor suppressor gene retinoblastoma is functionally deactivated in a large fraction of human cancers[35-37].

In the present study, Rb protein was detected by IHC test in 59.3% of malignant cervical tumors, 43.8% of benign cervical tumors but no signal was reported in the tissues of control group, our result showed overexpression of pRb in majority of malignant and benign cervical tumors as compared with normal cervical squamous epithelium. Statistical analysis showed significant differences in pRb expression between normal and cervical tumor (Table 2).

Likewise, Skomedal et *al*.,(1999)[38] has reported Rb expression in all 74(100%) primary carcinomas of cervical analyzed , also, Norniani *et al*., (2003)[39] showed Rb expression in 64/65 (98.5%) these result very higher than our result. The reduced of Rb led to the improved expression of E2F target genes, suggesting that the Rb protein present in human cervical carcinoma cell line is at least partially functional[37]. This, in synergy with the inactivation of another cell cycle regulator, p105/Rb, allows for repeated cell division[1].

In addition to chromosomal loss and mutation, also Rb can be inactivated in tumors by the loss of one allele and hypermethylation of the other alleles[41]. Meantime, tumors with high cyclin E / low cyclin D1 are either Rb inactivated or seem to bypassRb pathway in growth control[42].

In a study carried by Wang and Lu (2004)[43], found that the loss of nuclear Rb expression in small cell carcinoma is not evident may suggest that Rb protein is deregulate through two different mechanisms in HPV related tumors. Rb deregulation is achieved through accelerated protein degradation as evidenced by a negative nuclear staining or Rb is inactivated by the HPV E7 oncoprotein at the functional level and thus the nuclear Rb protein detectable immunohistochemically. In present study, the majority of the Rb positive cases showed a heterogeneous staining pattern. The intensity of the nuclear staining varied from cells to cells with variable staining proportion of cells having an unstained nucleus. Similar finding have been observed and reported by

Zur Hausen, (2002)[44]. This variation in staining probably resulted from asynchronous progression of the cells through the cell cycle[39].

Moreover, the result revealed significant association could be detected with different grades of malignant tumors. These results are in contrary with other studies such as Liu *et al.,* 1994[45] and Lie *et al*, 2010[46] who found that no- significant relation between the alteration of RB-gene and grading of ovarian cancers. Moreover, The E6and E7 its two early HPV genes, play essential role in tumor formation. There is integration of viral DNA into host DNA which is the serious step in cervical carcinogenesis. As E6 and E7 are the viral oncoproteins, uncontrolled expression of these proteins are seen when there is inactivation of host tumor suppressor genes (p53 & pRb). This will result in loss of normal maturation sequence, causing persistent, proliferative HPV infection. This will further result in transformation to high grade dysplasia and progresses to invasive carcinoma[47,48].

Conclusion:

We establish that highest age – specific frequency was noticed in elderly aged- patients. Alsoa majority cervical carcinomas are pronounced by strong expression RB-TSG proteins, although in need of corroboration on a larger pool of cases, show that Rb-TSG may represent promising tool toward the identification of patients with poorer prognosis who may benefit from more aggressive therapy and HPV screening.

References:

1. Sherr, C, and Roberts, J .M:CDK inhibitors positive and negative regulators of G1-phase progression .Genes Dev(1999) .13, 1501–1512.
2. Weinberg RA The retinoblastoma protein and cell cycle control. (1995). Cell, 81: 323-330.
3. Chatterjee S ,DatarR,Youssefzadeh D, George B,Goebell P, Stein J, Young L,Shi S-R, Gee C,Groshen S, Skinner D and Cote R Combined effects of P53, P21, and PRb expression in the progression of bladder transitional cell carcinoma. J ClinOncol, (2004a).l22(6): 1007-1013.
4. Hesselink A.T:High-risk human papillomavirus testing in cervical screening .Netherlands Organization for Health Research and Development; .(2007) grant
5. Kufe, DW, Pollock RE, Weichselbaum RR, BastR C, Gansler TS, Holland JF, Frei E ,Section 3, Cancer etiology, Papillomaviruses and Cervical neoplasia. In: Cancer Medicine(2003)..
6. Kraus I, Molden T, Holm R, Lie AK, Karlsen F, Kristensen GB, Skomedal H Presence of E6 and E7 mRNA from Human Papillomavirus Types 16, 18, 31, 33, and 45 in the Majority of Cervical Carcinomas. J ClinMicrobiol(2006) 44, 1310-1317.
7. Kjaer SK, van den Brule AJ, Bock JE, Poll PA, Engholm G, Sherman ME, Walboomers JM, Meijer CJ :Human papillomavirus: the most significant risk determinant of cervical intraepithelial neoplasia. Int J Cancer(1996) 65, 601-606.
8. WHO/ICO (2011) Information center on HPV (http/www.who.int/hpv center).
9. Goodman, A. Human papillomavirus infections in incarcerated women . H.E.P.P. News (2002) . 5 (1) . (Internet).
10. Mark I. , Hunter MD. , Christine H. ,Holschneider MD. (2002) : Cervical intraepithelial neoplasia: Etiology, diagnosis, and natural history ,998-6374.
11. Bhurgri Y, Nazir K, Shaheen Y, Usman A, Faridi N, Bhur-gri A.(2007).Patho-epidemiology of Cancer Cervix in KarachiSouth. Asian Pac J Cancer Prev.; 8: 357-62.
12. Jean, S. ; Major, D.; Rochette, L., and Brisson ,J.(2005).Screening mammography participation and invitational strategy: the Quebec Breast Cancer Screening Program, 1998-2000.J hronic Dis Can. 26(2-3):52-8.
13. American Cancer Society, 2009.Also available online 7. Last accessed January 6, 2010.
14. Aziz MF.(2008). Epidemiologic Reports in Indonesia. In 7 th Ko-rea-Japan Gynecologic Cancer Joint Meeting;, Seoul7.
15. Badar F. Cervical Carcinoma in a Muslim Community. Asian Pac J Cancer Prev. (2007); 8: 24-68
16. Missaoui N, Hmissa S, Trabelsi A, Frappart L, MokniM,Korbi S. (2010);Cervix cancer in Tunisia: Clinical and Pathologi-cal Study. Asian Pac J Cancer Prev.; 11(1): 235-8

17. Engbang, N.J.P., Mve, K.V., Tchente, N.C. and Fewou, A. (2015) Aspects histo-épidémiologiques des cancers génitauxdela femme dans la région du Littoral, Cameroun. Pan African Medical Journal, Expert Panel on External Genital Warts. Clin Infect Dis 1998;27:796–806.21, 116.
18. Sando, Z., Fouogue, T.J., Fouelifack, Y.F., Fouedjio, H.J., Mboudou, T.E. and Essame, O.J.L. (2014) Profil des canE. N. J. Paul et al. 238 cersgynécologiqueetmammaire à Yaoundé-Cameroun. Pan African Medical Journal, 17, 28
19. Elmajjaoui, S., Ismaili, N., Kharmoum, S., El kabbaj, H., Elkacemi, H., Elhassouni, K. et al. (2010) Cancer du col utérin :expérience du Maroc, à propos de 696 cas. Cancer/Radiothérapie, 14, 640-641.
20. Wright, J.D. (2014) Cervical intraepithelial neoplasia: Terminology, incidence, pathogenesis, and prevention.Editorial. Cancer of cervix can be controlled. East Afr Med J. Feb 2001:53,4.
21. Levi, J. E., Kleter, B., Quint, W. G., Fink, M. C., Canto, C. L., Matsubara, R., Linhares, I., Segurado, A.,Vanderborght, B., Neto, J. E., and Doorn, L. J. V. (2002) High prevalence of human papillomavirus (HPV) infections and high frequency of multiple HPV genotypes in human immunodeficiency virus-infected women in Brazil.The Journal of Clinical Microbiology, 40:3341– 3345.
22. Maddux HR. (1990) Invasive Carcinoma of The Uterine CervixIn Women Age 25 or Less. Int. J. Radiation OncolBiol.Phys.; 19: 701
23. De Sanjose S, Bosch FX, Munoz N, Shah K. (1997).Social differ-ences in sexual behaviour and cervical cancer. IARC SciPubl.; 138: 309-1714
24. Zhao EF, Bao L, Li C, Song L, Li YL..(2005) Changes inepidemiology and clinical characteristic of cervical cancer over the past 50 years. J First Mil Med Univ; 25(6):605-9
25. Walker JJ, Brewster D, Gould A, Raab GM. Trends in in-cidence of and mortality from invasive cancer of the ute-rine cervix in Scotland (1975 - 1994) Public Health. 1998:373-8.1
26. Van Wijngaarden W, Duncan I. (1993);Rationale for stopping cer-vical screening in women over 50. BMJ. April 10;306(6883): 967-71
27. Abdul-Hussein A., (2011):*Immunohistochemical Study and in-situ hybridization for detection of humanpapillomavirus (HPV) in Uterine Cervical Carcinoma* ., A thesis Submitted To The College Of Medicine And the Committee Of Postgraduate Studies Of Kufa University.
28. Gichangi, P.B., De Vuyst ,H., Estambale, B., Rogo, K., Bwayo, J., Temmerman, M.(2002). GynecolOncol 94, 803-10
29. Klaes R, Benner A, Friedrich T et al. (2002) p16INK4a immunohistochemistry improves interobserver agreement in the diagnosis of cervical intraepithelial neoplasia. Am J SurgPathol.;26(11)(Nov):1389–99.
30. Bergeron, C., N. Wentzensen, et al. (2006). "[The p16INK4a protein: a cytological marker for detecting high grade intraepithelial neoplasia of the uterine cervix]." Ann Pathol 26(5): 397-402.
31. Galgano MT, Castle PE, Atkins KA et al. (2010); Using biomarkers as objective standards in the diagnosis of cervical biopsies.Am J SurgPathol. 34:1077– 87.
32. Viladiu P, Bosch FX, Castellsague X, Munoz N, Escriba JM et al. (1997). Human papillomavirus DNA and antibodies to human papilloviruses 16 E2, L2 and E7 peptides as predictors of survival in patients with squamous cell cervical cancer. J ClinOncol, 15: 610-619.
33. Baalbergen A, Ewing-Graham PC, Hop WC, Struijk P, Helmerhorst TJ (2004).Prognostic factors in adenocarcinoma of the uterine cervix.GynecolOncol92(1): 262-267.
34. Cordon-Cardo C (1995). Mutation of cell cycle regulators: biological and clinical implications for human neoplasia. Am J Pathol, 147(3): 545-560.
35. Chetty R, Bramdev A, Aguirre-Arteta A, Pegoraro RJ, Sataar N. (1997) Relationship between retinoblastoma and p53 proteins in human papilloma viruses 16/18 positive and negative cancers of the uterine cervix. J Clin Pathol.;50:413–416
36. Ceccarelli C, Santini D, Cheico P, Taffurelli M, Gamberini M, Pileri SA, Marrano D. (1998).Retinoblastoma (RB1) gene product expression in breast carcinoma. Correlation with Kiñ 67 growth fraction and biopathological profile. J Clin Pathol.;51:818–824.
37. Emily , E. ;Ying , W. ; Huan, X. U.; Jack, T. ; Zilfou ; Karen, E. K .;Bruce , J. ; Aronow. S. W and Erik , S. (2007). The retinoblastoma tumor suppressor Modifies the therapeutic response of breast cancer . J Clin .Invest .117 : 218 – 228.
38. Skomedal, H., Kristensen, G.B., Lie, A.K. and Holm, R., : (1999) Aberrant expression of the cell cycle protein TP53, MDM2, p21, cdk4, cyclin D1, RB and EGFR in cervical carcinomas. GynecolOncol, , 73(2), 223-8.

39. Noraini, M.D., Si-Aisah, M.A. and Kwan, S.W., : (2003) An immunohistochemical study of retinoblastoma gene product in normal, premalignant and malignant tissues of the uterine cervix. Malaysian Tournal of Medical Sciences, ,9 (2), 52 – 59.
40. Shere CJ (1996).Cancer cell cycles. Science; 274(5293): 1672-1677.
41. Foster , R. S. J.r .(1996). The biologic and clinical significance of lymphatic metastases in breast cancer .J. Surgoncolclin N .Am 5:79-104.
42. Goran, L . ; Hanna, O .; Niels, H . ;Goran, R . ; Stefan, E .; Angelike, M. andArun, S. (2001). Down regulation of the potential suppressor gene . IGFBP – rPI in human breast cancer is associated with inactivation of the retinoblastoma protein ,cyclin E, Overexpression and Increased proliferation in estrogen receptor negative Tumor . J. home .No(7): 3497 – 3505 .
43. Wang, H.L. and Lu, D.W., : (2004).DetecƟon of human papillomavirus DNA and expression of p16, Rb, and p53 protein in small cell carcinomas of the uterine cervix. The American Journal of Surgical Pathology, ,28 (7), 901 908.
44. Zur Hausen H (2002) Papillomaviruses and cancer: from basic studies to clinical application. Nature Reviews Cancer 2: 342–350. doi: 10.1038/nrc798
45. Liu , Y. ; Heyman, M . ; Wang , Y., and Dekker . (1994).Retinoblastoma gene in primary ovarian cancer cells . I'm cancer ; 58:663-7.
46. Lie, X. N. ; Cai, H. B.; Liu, X. M.; Huang, Y.; Zhou, Q.; Pan, F. L.; Wang, Z. YH.;; Li,. Z. Y.; Wang, X. Y., and Dai, Y. (2010). Trends in cervical cancer in young women in Hubei, China. J. International Gynecology Cancer. 20(7):1240-1243.
47. Kalof AN, C.K. (2007).Our approach to squamous intraepithelial lesions of the uterine cervix.*J ClinPathol*60(5), 449-455
48. Magaldi TG, A.L., Bellone S (2012). Primary human cervical carcinoma cells require human papillomavirus E6 and E7 expression for ongoing proliferation. *Virology* 422, 114–124.

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