Scholars LITERATURE

La Prensa Medica Argentina

Research Article

DOI: https://doi.org/10.47275/0032-745X-279
Volume 106 Issue 6

Role of p16 INK4A Protein in Evaluation of Benign, Premalignant and Malignant Cervical Lesion in Iraqi Patients (Immunohistochemical Study)

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Abstract

Aim: Present study designed to evaluated immunohistochemical expression of both p16 INK4a and HPV (Human papilloma virus). Different cervical lesion (Benning, Premalignant and malignant) and its relation one to other in development of cervical carcinoma.

Materials and Methods: forty-nine cases of cervical lesion formalin fixed - paraffin embedded tissue specimens of the cases. Stained tissue sections of all the cases were evaluated to confirm diagnosis and assure the presence of representative tissue material. All cases with tissue sections that were appropriate for immunohistochemical evaluation, positive tissue controls were obtained according to primary antibodies manufacturer's data-sheets tissue sections of a uterus adenosequamus as p16 primary antibody, sections of skin wart tissue were used as positive control for HPV marker. A statistical analysis was preformed between the immunohistochemistry for marker each other.

Results: The test results for control group: All patients had with histopathology changes and staining of cytoplasm. Among Benign group; twenty samples (100%) showed benign lesion change. Among premalignant group seven samples (78%) showed CIN-1 change. two samples (22%) showed CIN-2. Among Malignant group; six samples (30%) showed poorly differentiated. Eleven samples (55%) showed moderate differentiated. three samples (15%) showed well differentiated. Also, the intensity of premalignant lesion shows between weak and moderate and no one showing in strong, while for malignant intensity showing two sample in weak and two sample in moderate and two strong intensity.

Keywords: p16 INK4a; Human Papilloma Virus; Cervical Squamous Cell Cancer; Immuno Histochemistry

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Citation: Jumaah AS, Abbod BH, Al Aasam SRM, et al. (2020) Role of p16 INK4A Protein in Evaluation of Benign, Premalignant and Malignant Cervical Lesion in Iraqi Patients (Immunohistochemical Study). Prensa Med Argent, Volume 106:6. 279. DOI: https://doi.org/10.47275/0032-745X-279.

Received: May 10, 2020; **Accepted:** June 03, 2020; **Published:** June 08, 2020

Introduction

Cervical cancer (CC) is the end result of a continual contamination through oncogenic human papillomaviruses (HPV) [1]. High-risk HPV infections associated to CC encompass a quantity of special HPV types, including HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 [2]. Sexually energetic people are at chance of contracting HPV [3], with an international estimated incidence of infection amongst female between 2% and 35% [4]. More than 1/2 a million new cases of Cervical cancer had been recognized and extra than a quarter million female died due to the disorder global in 2012 [5]. p16 is essential cyclin- structured kinase inhibitor (CKI) and a tumor suppressor gene encoded on 9p21 area of the human genome [6,7], at the INK4A/ARF/INK4B locus. p16 locus is a 35kb multi-gene area that encodes three distinct main tumor suppressor genes, p14ARF, p15 and p16 [8]. INK4A/ARF/INK4B gene locus is repressed in an immature and everyday cell by means of histone H3 lysine27 (H3K27) trimethylation and polycomb proteins [9,10] and is lead throughout the length of getting older or by using hyper-proliferative oncogenic stress or stimuli. The INK4A/ARF locus has been speculated to have a world anti-aging impact through favoring cell quiescence and limiting cell proliferation [11]. Papillomaviruses are small, non-enveloped, epitheliotropic, double-stranded DNA viruses that infect mucosal and cutaneous epithelia in a large range of greater vertebrates in a species-specific manner and result in cell proliferation [12]. So, the aim of study was to detected relationship between expression of p16 INK4a in biopsy specimens from uterine cervix and human papilloma virus as accusative agent to development of cervical squamous cell cancer.

Materials and Methods

Sample

A forty-nine instances of cervical injury were gathered from a private research facility in Al-Najaf. Formalin fixed - paraffin implanted

tissue examples of the cases. Stained tissue areas of the considerable number of cases were assessed to affirm conclusion and guarantee the nearness of delegate tissue material. The last example size contained twenty instances of benevolent cervical sore and nine instance of premalignant cervical injury and twenty instances of threatening cervical sore; all cases with tissue segments that were suitable for immunohistochemical assessment.

Study Design Selection and Criteria

The present study included the collection of 49 cases (from January -2017 to July 2019). Age of patient were found to be ranged between (40 - 70 years) diagnosed by histopathologist in AL sadder Hospital in Najaf City. These samples were removed through surgical operations included total abdominal hysterectomy or lesion area. These tissues were immediately fixed in 10% formalin and were processed in paraffin blocks. The cases in the present study divided as following:

First group: including persons with benign cervical tumors.

Second group: including person with premalignant cervical tumors.

Third group: including persons with malignant cervical tumors.

p16 Antigen Immunohistochemistry

Immunohistochemistry for p16-expression was done based on the manufacturer's procedure like provided by abcam Histology kit.

Human Papillomavirus Screening

Mouse and Rabbit Specific HRP/DAB Detection IHC pack (Abcam®, [EP1551Y]; 7ml) was utilized for the location of all the principal antibodies.

Results

Histopathology Differentiation of Tumors

Regarding histopathology differentiation the test results for control group: All patients had with histopathology changes and staining of cytoplasm. Among Benign group; twenty samples (100%) showed benign lesion change. Among premalignant group seven samples (78%) showed CIN-1 change. two samples (22%) showed CIN-2. Among Malignant group; six samples (30%) showed poorly differentiated. Eleven samples (55%) showed moderate differentiated. three samples (15%) showed well differentiated as shown in Table 1.

Relation between p16 and HPV

This table can be see the relation between p16 and HPV, regarding benign lesion there is only one sample is positive p16 staining while all sample are negative HPV, that's mean there is another pathway are triggering p16 mutation. While regarding premalignant CIN-1 there is three sample are showing positive for both p16 and HPV staining while four sample are negative for both p16 and HPV staining and this case may be liable in future for third development for more aggressive carcinoma. Regarding CIN-2 we have two sample, one sample is positive for both p16 and HPV staining, awhile one sample positive in p16 and negative HPV staining, which may indicate maybe there is another way of mutation.

Table 1: Histopathology differentiation of tumors in all groups of study.

Histopathology		Number of cases	Total
benign lesion		20	20
Premalignant	7	7	9
	2	2	
Malignant	Well Differentiated	3	20
	Moderate Differentiated	14	
	Poorly Differentiated	4	
C.S. (*) P Value	-	C.C=0.087	-
		P=0.717	
		(NS)	

While regarding malignant sample we have 20 sample as three sample well differentiation only one of them are positive for both p16 and HPV staining and other two sample are negative for both p16 and HPV staining, that indicate there is another pathway for p16 mutation; thirteen sample of malignant as moderate differentiation, four sample as positive in both p16 and HPV staining and 5 of their sample are positive p16 while negative HPV, and four sample of moderate differentiation are negative in both p16 and HPV staining, that's indicate there is another pathway for p16 mutation.; And four sample of poorly differentiation one sample is positive for both p16 and HPV staining and one sample is positive p16 while negative HPV staining and two sample are negative in both p16 and HPV that's indicated there is another pathway for p16 mutation shown in Table 2.

Relation of HPV positive cases in relation to p16 immunohistochemical staining intensity.

Regarding intensity; the intensity of premalignant lesion shows between weak and moderate and no one showing in strong, while for malignant intensity showing two sample in weak and two sample in moderate and two strong intensity as shown in Table 3.

Table 2: Relation between p16 and HPV result in immunohistochemistry staining.

		HPV P16	+ve	-ve	Total	
Benign Lesion		+ve	0	1	20	
		-ve	0	19		
Premalignant	CIN-1	+ve	3	0	7	9
		-ve	0	4		
	CIN-2	+ve	1	1	2	
		-ve	0	0		
Malignant	Well Differentiated	+ve	1	0	3	20
		-ve	0	2		
	Moderate Differentiated	+ve	4	5	13	
		-ve	0	4		
	Poorly Differentiated	+ve	1	1	4	
		-ve	0	2		
C.S. (*) P Value	-	-	C.C=0.261	C.C.=0.252	-	
			P=0.064	P=0.073		
			(NS)	(NS)		

Table 3: Relation of HPV positive cases in relation to p16 immunohistochemical staining intensity.

P16 intensity		Weak	Moderate	Strong	Total
HPV +ve cases					
Premalignant	CIN-1	3	0	0	4
	CIN-2	0	1	0	
Malignant	Well Differentiated	0	0	1	6
	Moderate Differentiated	2	2	0	
	Poorly Differentiated	0	0	1	
C.S. (*) P Value	-	C.C.=0.283	C.C.=0.313	C.C.=0.000	-
		P=0.069	P=0.056	P=1.000	
		(NS)	(NS)	(NS)	

Discussion

Histopathological assessment of biopsies from female with abnormal Pap test still the "gold standard" for the identification and cervical neoplasia grading. Diagnosis Diversity has been certified among observers and depends on abnormality grade. Despite researches of cervical cancer precursors, the inter-observer difference in interpretation of cervical specimen's histopathology remain form a dilemma [13,14]. Al-Khishali T, et al. (2016) referred that the commonness of p16 articulation in a sum of 60 female, separated into three groups [15]. Twenty females, with searching cervices and normal Pap spreads (bunch I), and 20 female (bunch II) with undesirable searching cervices with low and excessive estimation dysplasia. Gathering III (20) female with squamous cell carcinoma. Results obtained exhibited that none of cervical examples, evaluate with the aid of immunohistochemistry method, delivered p16 energy. Though, the group II samples starting from LSIL (CIN1) to HGSIL (CIN2, CIN3) show a consistent huge increment of overexpression of protein. Being just 1 (5%) show low fine p16 response, 9 (45%) tested respectably advantageous reactivity, and 10 (50%) with excessive positive reactivity. Gathering III exhibited excessive and strong overexpression of p16 with stromal attack. In some other researches, the identical group stated that p16 immunostaining approves for a unique diagnosis of small CIN or lesions of cervical cancer [14]. In distinction to our findings, Tringler B, et al. (2004) determined and stated that staining of p16INK4a used to be detected in samples of cervical [16]. These results are constant with Ming Gou and colleagues. In addition, they demonstrated an elevated in p16 protein expression in accordance to malignancy diploma of lesions, displaying to be a high-quality marker unique for pre-malignant and malignant lesions [17,18]. These oppositions in the consequences between various groups, like the current study, could be referred to the arbitrary cutoffs used via specific investigators, in addition to the laboratory and personnel constraints that had been confronted for the duration of this study.

Conflict of Interest

The authors declare that they have no potential conflicts of interest to disclose.

Funding: not applicable.

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