Prevalence of human papilloma virus positivity and cervical cytology. Is there a new HPV gene?

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Abstract

Background: Human Papillomavirus (HPV) infection has been incriminated in cervical cancer. Twenty of more than 100 types were classified as high-risk (HR), and associated with cervical cancer and precancerous lesions. The great question that this article wants to answer is, is there any new genome responsible for the cervical cytological changes other than the known high risk human papilloma virus gene. The second aim of this study is to raise awareness about HPV and cervical cancer and to draw attention to the need for more studies about this subject.

Materials and method: A cross sectional study with a convenience sample of 188 females was used. Information was obtained by interviewing the patients. Pap smear was done for all patients. HPV screening, and genotyping test was done for 151 patients.

Results: Positive HPV Pap smear (Positive HPV Pap) was found in 31 (16.5%) patients, while (Positive HPV test) was found among 4 cases (2.7%). Negative HPV test/Positive HPV Pap was found among 31 (20.5%). Three HPV genes were detected HPV (16, 18, 65), 2 (50%), 1 (25%), 1 (25%), respectively. The mean age for those who had Negative HPV test/Positive HPV Pap (33.5±8.3), was significantly lower than those had Positive HPV test/Negative HPV Pap (38.8 ±11.1), and those (Negative HPV/Positive HPV Pap) was found among 31 (20.5%). Three HPV genes were detected HPV 16, 18, 65, 2 (50%), 1 (25%), 1 (25%), respectively. The mean age for those who had (Negative HPV/ Positive HPV Pap) (33.5±8.3), was significantly lower than those who had Positive HPV/Negative HPV Pap (38.8 ±11.1), and those with (Negative HPV/Negative Pap) (41.2±118).

Conclusion: Heterogeneity was common among Iraqi patients, decreasing with increasing age. Results suggest presence of a new genotype.

Key words: cervical cancer, human papilloma virus, HPV genotype, HPV test and cytological changes.

Introduction

About 80% of cervical CA is occurring in developing countries, and age standardized mortality rate of cervical cancer in Iraq is 1.3 per 100,000/per year. HPV is blamed as the main causation of cervical cancer. About 11.4 million females above the age of 15 years in Iraq, with age standardized incidence of cervical cancer and is 1.9 per 100,0001). Current recommendation is performing of HR-HPV testing with routine cytology screening in women 30 years and older (2). HPV is more prevalent and transient among females aged < 30 years with low incidence of invasive cervical cancer. HPV prevalence decreased with increasing age(3,4).

More than 100 HPV types have been described, and 40 can infect the ano-genital tract(5). Genital HPV types are categorized according to their association with cervical cancer (6). Productive HPV infection may cause mild cellular changes called Low grade squamous intraepithelial lesion (LSIL), or loss of normal cell cycle control, epithelium and genetic instability, resulting in high grade squamous intraepithelial lesions (HSIL)(7) About 20 are classified as high risk (HR) types and are associated with cervical cancer and precancerous lesions, as well as low-grade cervical pathology.

Worldwide, HPV types 16 and 18 cause 70% of cervical cancers; HPV types 31, 33, 35, 45, 52 and 58 account for an additional approximately 20% of cases, although there is substantial geographical variation in the relative frequency of different HR types(8)

Cells exhibit dysplasia (multiple cytological changes) before development of cervical cancer. This dysplasia has multiple names based on the diagnostic methods: SIL (squamous intraepithelial lesion) and CIN (cervical squamous intraepithelial neoplasia)(9).

Squamous intraepithelial lesion (SIL) is a term that represents dysplasia of cervical cells when diagnosed by Pap smear (cervical cytology); it is classified on the basis of dysplasia severity to : mild dysplasia termed as (low-grade intraepithelial lesion (LSIL), and moderate or severe dysplasia termed as (high-grade intraepithelial lesion (HSIL); both of which may or may not progress to cancer. One of the findings of a cervical biopsy is CIN which are abnormal cervical cells. CIN may change to cancer and extends to surrounding normal tissue and is graded on the basis of a scale of 1-3, established on degree of microscopic cellular abnormalities' appearance and degree that cervical tissue is influenced.

CIN1, mildly abnormal cells, frequently resolve spontaneously without therapy. Sometimes it becomes cancer and extends to surrounding normal tissue. CIN1 is sometimes known as mild dysplasia or low-grade dysplasia.

CIN 2, moderately abnormal cells located on the cervical surface is not cancer, but may change to cancer and extend to surrounding normal tissue, without treatment. CIN treatment may include: cryotherapy, laser therapy, loop electrosurgical procedure (LEEP), or cone biopsy to remove or destroy the abnormal tissue. CIN 2 is sometimes known as moderate dysplasia or high-grade dysplasia.

CIN 3, severely abnormal cells are present on the cervical surface when cervical biopsy is done. Without treatment, these severely abnormal cells may become cancer and extend to surrounding normal tissue. CIN 3 treatment is composed of cryotherapy, laser therapy, loop electrosurgical procedure (LEEP), or cone biopsy to remove or destroy the abnormal tissue. CIN 3 is sometimes known as severe dysplasia high-grade dysplasia (9).

LSIL is equal to the CIN1, while the diagnosis of HSIL, may reveal when diagnosed histologically, CIN2, and CIN3(10).

In Iraq very little data is available about HPV prevalence and cervical cytological changes. This study noticed that most of the patients with positive cytological changes have negative HPV test.

The 1st aim of this research is to study the correlation between HPV testing with cytological changes stratified by age. The great question that this article wants to answer is, is there any new genome responsible for the cervical cytological changes other than the known high risk human papilloma virus genes.

The second aim of this study is to raise awareness about HPV and cervical cancer to draw attention to it and for motivation and to make more studies about this subject.

Materials and methods

A cross sectional study was done of 188 females who attended the women health center at Al-Elwyia Teaching Obstetrics Hospital, from 1st January-30th December 2018. All patients attending the center during the period were included. Information regarding residence, parity, smoking, and occupation was obtained by interviewing the patient. Pap smear was done for all patients, using the conventional method; cytological study was done by local cytopathology service. HPV screening and genotyping test was done only for 151 patients. HPV screening and genotyping was done in Central Public Health Laboratory/ Molecular Biology Department using the PCR genotyping by chip technology. Genotypes detected by the test were, High risk human papilloma virus (HR-HPV) genotypes 16, 18, 31, 33, 35, 39, 45, 52, 56, 58, 59, 66 in the cervical swabs and low risk human papilloma virus (LR-HPV) genotypes 6, 11, 40, 42, 43, 44/45. Data entry and analysis was done using SPSS 25. The student t-test to compare means, and chi-square test was used to compare frequencies.

Results

Positive HPV Pap was found in 31 (16.5%) females and all of them (100%) had Negative HPV test. Another 4 patients (2.5%) had Negative HPV Pap but surprisingly had Positive HPV test; this relation was statistically significant (P value 0.0001), as shown in Table 1.

Table 1: The correlation between HPV test and cytological changes

		HPV test			Total
		Positive	Negative	Not done	
HPV Pap	Yes	0	31	0	31
		0%	100%	0.0%	16.5%
	No	4	116	37	157
		2.5%	73.9%	23.6%	83.5%
Total		4	147	37	188
		2.10%	78.20%	19.7%	100%

Likelihood Ratio=16.89, df=2, P value= 0.0001

The mean age for those who had Positive HPV test/ Negative HPV Pap was (38.8 ±11.1), those who had Negative HPV/Positive HPV Pap was (33.5±8.3), and those with Negative HPV/Negative Pap was (41.2±118). This relation was statistically significant, as shown in Table 2.

Table 2: The mean age according to the HPV changes in pap cytology and HR-HPV test

Age	N	Mean	SD	Minimum	Maximum
HPV test+/Pap –ve	4	38.8	11.2	23.00	48.00
HPV test-ve/pap +ve	31	33.5	8.3	20.00	49.00
HPV test -ve/pap-ve	114	41.2	11.8	20.00	70.00
Total	149	39.5	11.5	20.00	70.00

F=5.85, df=2, P=0.004 (significant) (One way ANOVA test)

From 151 patients those who had Positive HPV test/ Negative HPV Pap was 4 (2.7%), Negative HPV test/ Positive HPV Pap 31(20.5%), and Negative HPV/Negative Pap 116 (76.8%). The four Positive HPV test cases had the HPV genes HPV 16, 18, 56, 2 (50%), 1(25%), and 1(25%), respectively.

Positive HR-HPV was found among 1 (1.1%), 1 (2%), 1 (16.7%), 1 (20%), of ASCUS, Low-grade squamous intra-epithelial lesion (LSIL), High-grade squamous intra-epithelial lesions (HSIL), and cervical cell carcinoma, respectively. Heterogeneity found among 10 (11.2%), 18 (35.3%), 1 (16.7%), and 2 (40%) of ASCUS, Low-grade squamous intra-epithelial lesion (LSIL), High-grade squamous intra-epithelial lesions (HSIL), and cervical cell carcinoma, respectively. This relation was statistically significant, as shown in Table 3.

Table 3: The correlation between HR-HPV test and Cytological findings

	Correlation			Total
	HPV+/Pap -ve	HPV-ve/pap +ve	HPV -ve/pap-ve	
ASCUS	1	10	78	89
	1.10%	11.20%	87.60%	100.00%
Low-grade squamous intra-epithelial lesion (LSIL)	1	18	32	51
	2.00%	35.30%	62.70%	100.00%
High-grade squamous intra-epithelial lesions (HSIL)	1	1	4	6
	16.70%	16.70%	66.70%	100.00%
Carcinoma	1	2	2	5
	20.00%	40.00%	40.00%	100.00%
Total	4	31	116	151
	2.60%	20.50%	76.80%	100.00%

Likelihood Ratio= 18.96, P =0.004

Discussion

Surprisingly we found that only 4 cases (2.7%) had Positive HPV test of the total number of patients, and this was lower than what was found previously in Iraq, (26.7%)(11), (12.4%)(12), (23%)(13). This is also lower than what reported in other countries; in China (36%)(14) Cameroon (39%)(15), and Gabon (41.5%)(15). This raises the question, is there a new HPV gene that produces the cytological changes without producing HPV test positivity. The results of this study revealed Positive HPV Pap among (16.5%) of patients. This is the 1st and pioneer research that study the correlation between HPV test and HPV Pap cytological changes. The detected 4 genome were as follows; 2 patients had HPV genotype 16, (25%) HPV genotype 18, and (25%) HPV genotype 56. This low number of patients with HPV genome, is still lower than previous Iraqi research on low HPV with consideration of small sample size (11-13). Concerning HPV genotype, this study revealed that 16, 18 HPV genotype was responsible for 3 cases (1.98%) of positive cytological changes that do not have HPV specific changes . Here we should mention that globally 16, 18 HPV genotype is responsible for more than 70% of the cervical cancers, (51.9%) of (HSIL), (25.8%) of (LSIL), and (4.1%) of the normal cytovlogy(15).

Negative HPV test/Positive HPV Pap was found among 31(20.5%). This heterogeneity was significantly related to HPV cytological abnormality. Heterogeneity was 35.3% among Low-grade squamous intra-epithelial lesion (LSIL). This result also is lower than results of Iraqi previous studies (71.42%)(11), (80%)(12). Heterogeneity was also found among (16.7%), and (40%) of High-grade squamous intra-epithelial lesions (HSIL), and cervical cell carcinoma, respectively. This is higher than what was reported previously in Iraq CIN2 (15%) (12), (25%) (11), and cervical carcinoma (5%)(12), (0%)(11).

Positive HPV test was found among (1.1%), (2%), (16.7%), (20%), of ASCUS, Low-grade squamous intraepithelial lesion (LSIL), High-grade squamous intraepithelial lesions (HSIL), and cervical cell carcinoma, respectively. This was lower than found by previous studies in Iraq; non-cancerous (9.7%), CIN1 (28.57%)(11),(18.75%)(12),CIN2 (75%)(11), (33.4%)(12), CIN3 (100%)(11), cervical carcinoma (100%)(11,12). The prevalence of HPV in this study was lower than reported in the world CIN1 (77.5%)(16), CIN2 (89.7%)(16), CIN3 (95.1%)(16_, cervical cancer (88.5%)(17), (95%)(18). The percentages of HPV prevalence increased with the grade of the lesion; this finding is concordance with previous studies(11,12,16).
HPV test is more sensitive and specific for CIN2 and CIN3, than cytological changes, and the low prevalence of the positive test also supports the opinion of presence of HPV genotypes responsible for the cervical changes other than the High and low risk HPV genes(19). The mean age for those who had Positive HPV/Positive HPV Pap (33.5±8.3), was significantly lower than those who had Positive HPV/ Negative HPV Pap (38.8 ±11.1), and those Negative HPV/ Negative HPV Pap (41.2±118). The above results of lower HPV prevalence, heterogeneity, and difference in age distribution, may indicate presence of HPV genotypes other than those tested which were the cause of cervical cytological abnormality. As cancer rates are increasing in Iraq, with advanced stage at diagnosis, and absence of population based screening programs for preventable cancers (20,21), this study indicates the urgent need for more sophisticated study of the HPV genotypes in Iraq other than the HR-HPV and LR-HPV genotypes to make a database for preventive and screening programs.

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