Pak. J. Biotechnol. Vol 15 (2) 561-566 (2018) *ISSN Print: 1812-1837*
www.pjbt.org *ISSN Online: 2312-7791*

QUALITATIVE DETECTION OF HIGH RISK HPV AND GENOTYPING ASSOCIATED CERVICAL ABNORMALITIES

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Article received 20.4.2018, Revised 31.5.2018, Accepted 8.6.2018

ABSTRACT

Introduction: Change in cervix epithelial tissue as abnormal cells as dyskaryosis or dysplasia can be due to inflammation or infection. Human papilloma virus (HPV) is the main viral infection of the female reproductive system. The current study is aimed to detect the HPV genotypes that associate with cervical abnormalities.

Materials and methods: Ninety subjects included 70 Iraqi patients with cervical abnormalities and 20 apparently healthy which were included in the work. Pap smears were taken from the cervix of the subjects, DNA was extracted, and HPV genotyping was done to all samples.

Results: In general, our results showed that 42.86% of women had positive HPV infection and 57.14% negative HPV. The seventy cervical intraepithelial neoplasia biopsy that were obtained from patients classified histopathologically as: squamous cell Atypia 30 out of 70 (33.33 %), low grade squamous intraepithelial lesions (CIN I) constituted 18 out of 70 (20.00%) where as the high grade (CINII &III) constituted 16 out of total 70 (11.11% and 6.67 %) respectively and squamous cell carcinoma 6 out of total 70 (6.67 %). According to cytology patients samples were classified as: 10 (11.11%) with non-specific cervicitis, 18 (20.00%) cases of LSIL, 16(17.78%) cases of HSIL, 20 (22.22%) cases of ASCUS and squamous cells Carcinoma 6 (6.67 %).

Conclusion: The results showed that 30 of patients were positive to HPV. The common HPV genotype was HPV-16, HPV-59 and HPV-56 followed by HPV types 66, 51, 58 and 68. Also multiple infections of viruses were observed in patients. It was found that variation in the results showed high significant on each histological grads examination.

Keywords: HPV, Cervix, Genotypes, Histopathology, Cytology

INTRODUCTION

The medical importance of the cervical dysplasia because its potential association to progress to cervical cancer (Massad *et al*., 2013). Rt-PCR was also advance technique that explore the Knowledge about diseases (Ibraheam *et al.,* 2016; Azziz *et al.,* 2017). The screening reports that describe the changes in cervix showed that these changes could be due to infection especially with virus (Jeronimo *et al.,* 2016). Human papilloma virus-HPV is the main virus reported to be associated with cervical abnormalities. It has a 7900bp as a double-stranded circular DNA with eight overlap-ping open reading frames some of them v-oncogenes (King *et al*., 2015) that somehow can drive cell proliferation processes (Huh *et al*., 2015). Human papilloma viruses (HPVs) are a member of the family of Papillomaviridae that contain about 200 HPV types classified into low-risk and high-risk types (Bernard *et al.,* 2010). They can cause numerous benign and malignant lesions at different anatomical locations (Lee *et al*., 2017). The high-risk HPV can induce squamous and ade-nosquamous cervical cancers as well to genital warts or low grade dysplastic lesions (Pyeon *et al.,* 2009). High-risk HPV genotypes16, 18, 31 the most cause of 70% of cervical cancers (Schiffman *et al.,* 2007; zur Hausen 2009; Cuzick *et al.,* 2014). This could be due to E6/E7 oncogenes that exist in the HPV genome (Maufort *et al.,* 2007; Darragh *et al.,* 2012) which reduce p53 and pRb expression, which in turn stimulates cellular pro-liferation (Gao and Zheng, 2010). The current study was designed to detect HPV virus and their geno-types and their association to grades of cervical abnormalities.

MATERIALS AND METHODS

This study was done from April 2017 to February 2018 on a total number of 90 subjects included 70 Iraqi patients with cervical abnormalities which were referred to the surgical pathology department of the teaching laboratories in the Medical City Teaching Hospital and Al-Elweya Teaching Hospi-tal and 20 apparently healthy women. The patients and healthy were aged between 25-55 years. Pap smears which were collected from the cervix of these patients, were immediately dipped in absolute methanol for a mini-mum period of 20 minutes then stained by Papanicolaou stain. That was followed by microscopical examination of the specimens by a competent histopathologist and cytopathologist who categorized the findings. The same specimens were then subjected for HPV detection.

Genomic DNA isolation: Total DNA was isolated from the Pap smear samples for molecular studies utilizing genomic *HPV* HCR genotype-titre-RT PCR kit (AmpliSens® /Russia) with Transport Medium with Mucolytic Agent.

Molecular detections of human papilloma virus (HPV): The molecular detections part of this study

was focused on the analysis of extracted DNA form women using AmpliSens® *HPV* HCR genotype-titre-FRT PCR kit variant FRT-100 (AmpliSens® /Russia). The procedure depends on multiplex PCR of DNA fragments of most *HPV* genotypes and β-globin gene DNA fragment in one tube as an internal endogenous control as described by Theodora *et al.*, (2013).

Statistical Analysis: Chi-square test, statistical analysis system- SAS (2012) program and Least significant difference-LSD test (ANOVA) were used to analysis results.

RESULTS AND DISCUSSION

Analysis of risk factor cervical epithelial cell abnormality: Many risk factors were suggested to have a role in cervical epithelial cell abnormality (Table-1). The results revealed that Contraceptives, Obesity, Abortion and infection with HPV were the mean risk factors followed by chronic disease and family history. Almost all cervical cancer cases are result of infection with HPV high-risk types. HPV commonly spreads through sexual contact. It can spread without sex, by skin-to-skin contact (Frumovitz, 2013; Wright, 2014). Drugs suppression of immune response is another cause that places women at high risk for HPV infection (ACS, 2014). Patients with family history to cervical cancer have 2-3 times risk of developing cervical cancer than women without family history (Frumovitz, 2013). Oral contraceptives are another risk factor. Long-term use (>-5 years) increases risk of cervical epithelial cell abnormalities including cervical cancer (ACS, 2014). Smoking and Obesity were also increase risk of squamous cell abnormalities by exposing body to chemicals and also by weakening immune system (Clinical Summary, 2014).

Table-1: Distribution of sample study according to difference factors

Factors	Result	No.	Parentage (%)	P-value
HPV infection	Yes	30	42.9	0.0095 **
	No	40	57.1	
Contraceptives	Yes	41	58.57	0.0094 **
	No	29	41.43	
Smoking	Yes	1	1.43	0.0001 **
	No	69	98.57	
Chronic diseases	Yes	19	27.14	0.0001 **
	No	51	72.86	
Abortion	Yes	33	47.14	0.088 NS
	No	37	52.86	
Obesity	Yes	26	37.14	0.00034 **
	No	44	62.86	
Family history	Yes	13	18.57	0.0001 **
	No	57	81.43	
** (P<0.01), NS: Non-Significant.				

Histopathological and cytological classification of patient's pap smears: Cervix is usually affected with various pathological and cytological conditions (Reichert, 2012). The cervical intraepithelial neoplasia classified as CIN 1, 2, or 3 where CIN3 represent acute dysplasia and carcinoma in situ. While CIN2 is a moderate dysplasia and CIN1 is a mild dysplasia (Kyrgiou *et al*., 2006).

The seventy cervical intraepithelial neoplasia biopsies of the current study were classified as squamous cell Atypia 30 out of 70 (33.33%), low grade squamous intraepithelial lesions (CIN I) constituted 18 out of 70 (20.00%) where as the high grade (CINII &III) constituted 16 out of total 70 (11.11% and 6.67%) and squamous cell carcinoma 6 out of total 70 (6.67%). The distribution of samples according to the histology examination is shown in table-2.

Table-2: Distribution of sample study according to Histology

Histology	Number	Percentage (%)
Atypia	30	33.33
CIN I	18	20.00
CIN II	10	11.11
CIN III	6	6.67
Carcinoma	6	6.67
Normal	20	22.22
Total	90	100%
Chi-square value	---	9.361 **
P-value	---	0.00491
** (P<0.01).		

CIN – cervical intraepithelial neoplasia.

The distribution of samples according to the cytology examination is shown in table-3. Out of 70 cas es, 10 (11.11%) of them were reported as Non- specific cervicitis and cases were found to be abnormal pap smears as follow: 18 (20.00%) cases of LSIL, 16 (17.78%) cases of HSIL, 20 (22.22 %) cases of ASCUS and squamous cells carcinoma 6 (6.67 %).

Table-3: Distribution of sample study according to Cytology.

Cytology	Number	Percentage (%)
Non- specific cervicitis	10	11.11
ASC-US	20	22.22
LSIL	18	20.00
HSIL	16	17.78
Carcinoma	6	6.67
Apparently healthy control	20	22.22
Total	90	100%
Chi-square value	---	8.416 **
P-value ** (P<0.01).	---	0.0058

The results agreed with those reported by Bayan *et al*., (2017) who reported that ASC-US is the high diagnostic category which describe the cellular abnormalities (Solomon and Nayar, 2004).

The results agreed with those reported by Zhang (2015) who found that the cervical inflammation associated with cervical lesions (Anorlu *et al.,* 2003). Other epidemiologic evidence showed that women with chronic cervicitis have a risk to have carcinoma unless chronic cervicitis cured as early as possible. Also, inflammation recurs and persists, resulting in atypical squamous epithelial hyperplasia that leads to progression to carcinoma (Ye *et al*., 2015). Moreover, chronic cervicitis decreases tissue immunity which activate HPV infection (Sjams, 2016). It is well known that early detection of precancerous lesions of cervix can be done by cytological examination of cervix by Pap smears. These precancerous lesions are likely to progress to invasive cancers unless treated early. It is proven that the cytological screening programs conducted in well developed countries played a good role in reducing mortality and morbidity of cancer cervix (Zaal *et al*., 2012; Tjalma *et al.,* 2013; Ceccato *et al.,* 2015).

Detection of HPV by RT-PCR: Our study showed that patients with abnormal cervix positive to virus HPV high risk and negative to virus HPV virus as 42.86 % and 57.14% (Table- 4**).** These results suggest that HPV infection could be a good screening marker. Persistent infection with high-risk HPV is the main cause of CIN3 and cancer (Anacker and Moody, 2017; Bravo and Felez-Sanchez, 2015). The high percentage of patients positive to high-risk HPV reflect a high risk of HPV in our general population which could be due to the low health care in the country and low culture about sexual health care. In addition, other factors and agents might multifactorially or cofactorially play a role in initiation and promotion in cervical carcinogenesis.

Table-4: Distribution of patient study according to test of HPV RT-PCR

Results	Number	Percentage (%)
Positive (+ve)	**30**	**42.86**
Negative (-ve)	**40**	**57.14**
Total	**70**	**100%**
P-value	**---**	**0.0095 ****
**** (P<0.01).**		

Detection of genotypes of high risk HPV in patients with cervical abnormalities: The HPV DNA amplified from 70 specimens of cervical abnormalities showed that the genotype 16 is the common high-risk HPV types among them (26.67 %) (Table-5 and figure-1). The HPV 59 and 56 were also showed a risk with a total rate of 13.33 %, and 13.33%, respectively followed by HPV types 66, 51, 58 and 68 with the total rate of 10 %,10%,10% and 6.67% respectively. The less frequent genotypes were HPV-18, 39 and 45 (3.33%) each.

Table-5: HPV genotypes in patients with cervical abnormalities.

Genotype of HPV	Positive	
	Number	Percentage %
16	**8**	**26.67**
18	**1**	**3.33**
59	**4**	**13.33**
39	**1**	**3.33**
66	**3**	**10.00**
56	**4**	**13.33**
51	**3**	**10.00**
68	**2**	**6.67**
45	**1**	**3.33**
58	**3**	**10.00**
Total	**30**	**100%**
Chi-square value	**---**	**9.026 ****
P-value	**---**	**0.0002**
**** (P<0.01).**		

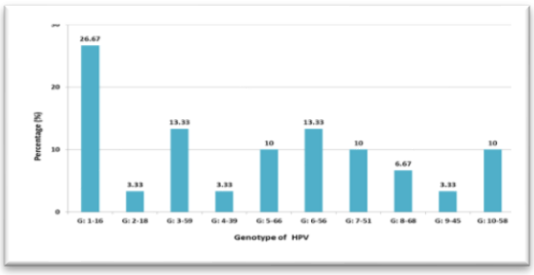

Figure-1: Frequency of HPV-HR genotypes.

Our results also showed (Table-6) that HPV genotype 16 could be play a role in cervical cancer development since it exists in CIN I, CIN II and carcinoma. In Iraq, several studies declared an association of HPV with cervical carcinoma. The first was done by Mohammed Ali (2001) who found that 25% of cervical carcinoma was positive for HPV. Al-Azzawi (2006) and Al-Shwaikh (2006) were also found that 28.4% and 33% of patients with cervical neoplasia were HPV-positive, respectively.

Table-6: Distribution of sample study according to grade histology with HPV positive and frequency genotype.

Grade of Histo	Genotype HPV (+)	No.	%	HPV (-)	%
Carcinoma	16,18	6	10.00	-	-
CIN I I I	45,16,58	5	8.33	4	25.00
CIN I I	16, 66, 51,	11	18.33	4	25.00
CIN I	, 66, 39,58,59,68	18	30.00	10	58.82
Atypia	,68 ,51,56,66,59,58	20	33.33	12	57.14
Total	---	60	100%	30	100%
Chi-Square (χ^2)	---	---	9.502 **		
** (P<0.01).					

Previous studies reported that some genotypes of high risk HPV are strongly associated with high grade cervical lesions ≥CIN 2\3 (Pista *et al.*, 2013). Such association was also documented by a research study on Portugal women that showed 12 HPV types were associated with high grade cervical lesion, of which especially HPV-16, -18, -31, -33, -35, -45, -51, -52, and -58 (Monsonego *et al.*, 2012). Similarly, HPV-16 was identifying to asso-ciate with ≥CIN 3 cervical lesion and 60.6% of high grade cervical lesions seen to be associated with HPV-31, -33, -45, -52, or -58 in French women (Jun *et al*., 2015). Another study which conducted to evaluate the HPV types in Asian patients with invasive cervical cancer showed that HPV-16, -18, -52, -45, -58, -33, or -31 were strongly associated with cervical cancer (Lisa *et al*., 2018). HPV-16 was also detected as a main high risk in cervical dysplasia and cervical cancer (Bose *et al*., 2005; Mayeaux, 2008; Stewart and Wild, 2014; Mileta *et al*., 2017). We found that this test compared to Pap testing has greater sensitivity for the detection of CIN which lower the incidence of grade 2 or 3 CIN or cancer. The incidence of HPV infection is increased in patients with neoplasm than those with cervicitis (Naucler *et al*., 2007 Mayrand *et al.*, 2007). Current screening algorithms of both co-testing and HPV primary screening guidelines do not reflect the difference in risk for ≥CIN 2 among women with various non-16/18 HR-HPV genotypes (Yeoun Eun *et al*., 2016). However, a number of studies revealed that, in Asia (unlike America, Australia, Europe, and Africa), a higher percentage of HPV-52 and HPV-58 is associated with cervical cancer and HPV-52 and HPV-58 are comparable to HPV-16 and HPV-18 in prevalence for invasive cervical cancer (Clifford *et al*., 2003; Muñoz *et al*., 2004). This is consistent with several investigations that studied Korean women (Hwang *et al*., 2004). Other studies showed that HPV-16, -18, -52, -45, -58, -35, -33, and -31 were strongly associated with cervical cancer and pre-cancer lesions in Asian women, especially Korean women (Quek *et al*., 2013).

Conclusion: The results showed that 30 of patients were positive to HPV. The most common genotype was HPV-16, HPV-59 and HPV-56 followed by HPV types 66, 51, 58 and 68. Also multiple infections of HPV-HR were observed in infected women. It was found that variation in the results showed high significant on each histological grad examination.

ACKNOWLEGEMENT

We are grateful to Drs. and laboratory assistants of the surgical pathology department in the teaching laboratories of the Medical City Teaching Hospital and Al-Elweya Teaching Hospital-Baghdad-Iraq. We thank also the institute of Genetic Engineering and Biotechnology-University of Baghdad for their assistance in the molecular experiment part.

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