ORIGINAL ARTICLE

High Risk Human Papilloma Virus Genotype Distribution in Cervical Intraepithelial and Invasive Carcinoma

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ABSTRACT

Background: High-risk (HR) Human Papillomavirus (HPV) is an established cause of cervical cancer. HPV genotype detection is significant in preventing cervical cancers through targeted vaccination. Our study aimed to identify HRHPV16/18 and non 16/18 in cervical intraepithelial neoplasia (CIN) and squamous cell carcinoma (SCC).

Methods: A retrospective study was performed at Pathology Department, BMSI, JPMC, Karachi. About 96 cases of CIN and SCC were included. Analysis of HPV genotypes was performed by DNA extraction, PCR amplification and flow-through hybridization technique. The probes used had a cluster of 13 HRHPV into a group of 3 as HPV HR 1, 2 and 3. Chi square/ Fischer Exact test were applied to observe the association of morphological types of the lesion and expression of HPV genotypes.

Results: HPV DNA positivity was 44% in our series. HPV HR 1 was observed in majority of cases (61.9%), followed by HPV 16 in 23.8%, HPV HR 3 in 9.5%, and HPV HR 2 in 4.7% cases respectively. The unique finding was absence of HPV 18 in the series. High grade lesions and invasive cancers showed positivity for HPV HR 1 and HPV 16, while low grade lesions were positive for HPV HR 1, 2 and 3 respectively.

Conclusion: HPV HR1 are major causative agents for low and high grade intraepithelial and invasive SCC, followed by HPV 16. Absence of HPV 18 was the novel finding. Our results differ from studies within and outside the region, suggestive of diversified genetic makeup and impact of detection techniques on results.

Keywords: Human Papilloma Virus; Genotype; Squamous Intraepithelial Lesion; Cervical SCC; Flow-through Hybridization.

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INTRODUCTION

Globally, cervical cancer is the second most common malignancy among females. Majority of deaths from cervical cancer are being reported from developing countries[1,2]. In Pakistan, it is reported to be the second most common gynecologic malignancy, causing about 2876 deaths[3,4]. Cervical Intraepithelial Neoplasia (CIN) represents precursor lesions of cervical squamous cell carcinoma. According to WHO, histologically, cervical intraepithelial lesions are divided into low grade (LSIL) and high grade squamous intraepithelial lesions (HSIL)[5,6]. It is well documented that the progression of intraepithelial squamous lesion to high grade malignancy involves molecular changes. Therefore, invasive carcinoma is preventable if it is detected and treated early[7, 8]. Progression of low grade lesions to high grade is less than 10 %, while high grade lesions have more than 12 % risk of progression to invasive carcinoma. Some studies, however found CIN3 to be more progressive than CIN2 [9,10]. High-risk (HR) Human Papillomavirus (HPV) is an established cause of cervical cancer[11, 12].

Worldwide, About 99.7% invasive cervical cancers show presence of HPV in invasive cervical cancer.

HPV genotypes are divided into high-risk (HR) and low-risk (LR) groups. It has been established that approximately 70% of cervical cancers are due to HPV types16 and 18. Moreover, HPV 16, 18, 45, 33, and 31 are also frequently associated with cervical cancer. Most of the HPV related research concentrates on HPV types 16 and 18. Majority of women population in the world is vaccinated against HPV type 16/18. This leaves them vulnerable to non 16/18 HPV[13-15]. Effective HPV vaccines are available which are extremely useful in prevention of cervical cancer[16]. Flow-through hybridization technique has a proven potential of reducing manpower and duration of HPV genotype testing. It is based on PCR and flow through hybridization, which takes about 3 hours to identify 33 genotypes of HPV[17, 18].

The current study was aimed to determine the frequency of HRHPV-DNA in different grades of cervical dysplasia and invasive cervical cancer. The published literature from the region concentrated majorly on only invasive carcinoma and HPV 16/18 using PCR technique. The current study was designed to identify 16/18 and non 16/18 high risk HPV DNA in invasive cancers as well as intraepithelial lesions of the cervix utilizing Flow through hybridization technology.

METHODS

A retrospective study was designed which included diagnosed cases of cervical squamous cell carcinoma, HSIL and LSIL. It was carried out at the department of Pathology, Basic Medical Sciences institute (BMSI), Jinnah Post graduate Medical center Karachi (JPMC). Institutional review board of BMSI, JPMC granted the ethical approval of the study (IRB no. F.1-2/2019/BMSI-E.COMT/072/JPMC). In three-year duration, from January 2015-December 2017, our department received a total of 96 cases including 40 LSIL, 10 HSIL and 46 cases of invasive SCC. The lesions were mostly identified within diagnostic biopsies or hysterectomy specimens on histopathological examination. The most probable cause of low frequency of High grade cases was late presentation of patients to the Gynecology departments due to lack of awareness. Poorly differentiated tumors, cases of adenocarcinomas of cervix as well as metastatic tumors were excluded from the study. Reviewed microscopy was performed using scanner (4x), low power (10x), and high power (40x) lenses. After the morphological review, the selected cases were analyzed using PCR and subsequently flow through hybridization to detect HPV DNA.

Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 22.0. Categorical variables were explained in terms of frequency and percentages. Chi square/ Fischer Exact test were applied to observe the association of morphological types of the lesion and expression of HPV genotypes. A p value of < 0.05 was considered significant.

The DNA extraction from embedded tissue block was carried out by using Epicenter Kit (cat# MCD85201). Polymerase chain reaction (PCR) was carried out ensuring the required amount of extracted tissue DNA with the Amplification Control (signal at B5). Following the protocol, PCR reaction was prepared. It included PCR reaction Mix 18.6µl, 25X Primer Mix 1.0 µl, DNA Taq Polymerase 0.4 µl, DNA Template Up to 5.0 µl, DNase Free Water-Variable. About 25 µL of the final volume was prepared. The Master Mix contained PCR Reaction Mix, 25X Primer Mix and DNA Taq Polymerase. Approximately 20 µL of master mix was combined with DNA template into each PCR tube. DNase Free Water was used to increase the volume, when required. Spinning was done for PCR reaction mixture along with the extracted tissue DNA. A thermal cycler was utilized for amplification of the sample tubes, which were cooled afterwards.

For flow through hybridization, a membrane-based flow-through hybridization technology (FT Pro Flow-through - US Patent number 5741647) was utilized. The probes used for high risk HPV detection, were comprised of HPV 16, HPV 18, Individually and a Cluster of 13 High risk HPV into a group of 3 as HPV HR 1: 31, 33, 45, 52, 58; HPV HR 2: 53, 59, 66, 81; and HPV HR 3: 35, 39, 51, 56. The genotyping of PCR amplified products was performed by flow through hybridization according to instructions provided in the manual[17,18].

RESULTS

Overall HPV DNA positivity was 44% in our series. Out of the total 40 cases of LSIL, only 29% (12) cases showed positive HPV DNA. Similarly, out of 46 cases of SCC, 43.4% (20) cases were positive for HPV. Regarding HSIL, 100% (all 10 cases) positivity was noted (Table 1). HPV type HR 1 type, which was found in majority26 (61.9%) of cases, followed by HPV 16 in 10 (23.8%), HPV HR 3 in 4 (9.5), and HPV HR 2 in 2 (4.7%) case respectively. HPV 18 was negative among all cases (Table 2). Only HPV HR 1 and HPV 16 were positive in high grade lesions and invasive cancers. Low grade lesions, however, showed positivity for HPV HR 1, 2 and 3 respectively. No significant association was observed in morphological subtypes of the lesion and HPV genotypes (p = 0.05).

Table 1: Distribution of HPV DNA Positivity in various

HPV DNA	Morphological Subtypes n(%)			Total n(%)	*p*-Value
	LSIL	HSIL	SCC		
Positive	12 (28.5)	10 (23.8)	20 (47.6)	42 (44)	0.05
Negative	28 (51.8)	—	26 (48)	54 (56)	
Total	40 (41.6)	10 (10.4)	46 (47.9)	96 (100)	

Table 1 shows frequency of HPV DNA Positivity in cases of LSIL (Low-grade squamous intraepithelial lesion), HSIL (High-grade squamous intraepithelial lesion) and SCC (Squamous cell carcinoma) ± Fischer Exact.

Table 2: Distribution of High-risk HPV types in various cervical lesions.

Morphological subtypes	HPV DNA in Selected Cases n(%)						*p*-Value
	HPV 16	HPV 18	HPV HR 1	HPV HR 2	HPV HR 3	Total	
LSIL	—	—	6 (14%)	2 (4.7 %)	4 (9.5 %)	12 (28.5 %)	0.05
HSIL	4 (9.5 %)	—	6 (14.2 %)	—	—	10 (23 .8%)	
SCC	6 (14.2 %)	—	14 (33.3 %)	—	—	20 (47.6 %)	
Total	10 (23.8 %)	—	26 (61.9 %)	2 (4.7 %)	4 (9.5 %)	42 (100 %)	

Table 2 shows frequency of HPV Genotpes16, 18, HR1, HR2, and HR3, (high risk types 1, 2, and 3) in LSIL (Low-grade squamous intraepithelial lesion), HSIL (High-grade squamous intraepithelial lesion) and SCC (Squamous cell carcinoma) ± Fischer Exact test.

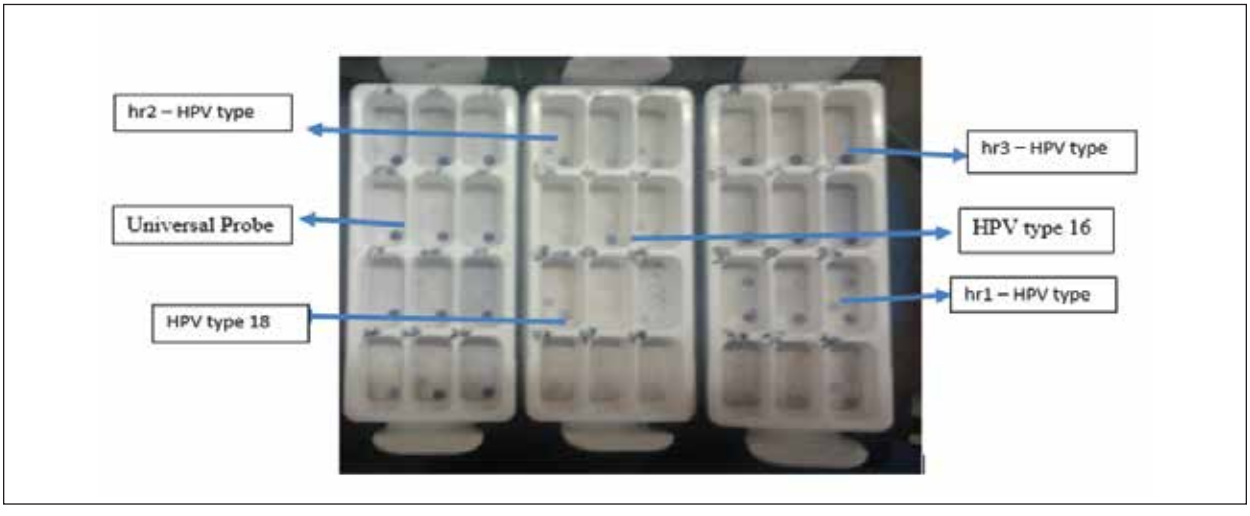

Figure 1: Photo showing gene flow hybridization results on a membrane.

DISCUSSION

To the best of our knowledge, this is a unique analysis from this region on HPV genotyping, including invasive cervical cancers as well as cervical intraepithelial lesion cases. Overall HPV DNA positivity was identified in 44% cases. Siddiqua et al. and Loya et al. observed an overwhelming 94.81% and 87.5% HPV DNA prevalence, respectively[19,20]. However, another study from low-income suburb of Karachi showed a low prevalence of HPV DNA (27.3%) supporting our findings[21]. This variation may be attributed to degree of involvement of cervix and aggressiveness of the disease. It may also emphasize that prevalence of HPV infection in cervical lesions is comparatively low in Karachi. The distribution of HPV DNA in our series was highest in invasive squamous cell carcinoma (SCC) (47.6%), followed by LSIL (28.5%) and HSIL (23.8%) respectively. Siddiqua et al. supported our findings by recording HPV frequency in 57.1% SCC, 14.2% HSIL and 4.2% LSIL cases respectively. Our result also corresponded to a study conducted in China on intraepithelial cervical lesions[19,22]. Differences in HPV positivity may vary according to geographical location, and severity of the lesion.

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In the current study, however, HPV- HR1 (Human Papilloma Virus – high risk type 1) including types 31, 33, 45, 52, 58 were found to be most frequently (61.9%) involved in intraepithelial as well as invasive lesions. Our findings are in agreement with a Chinese meta-analysis[1]. HPV 16 was the second most frequent (23.8%) subtype in our series. Tanzi et al. reported 18.8% of HPV16 in Italian population. According to our result, 14.2% HPV16 were identified in ICC and 9.5% in HSIL, but in LSIL cases, it was completely absent. This finding is also strengthened by a study of Siddiqua et al., which also could not detect HPV DNA in LSIL cases[19, 23, 24]. Most of the HPV studies from China and Pakistan concluded that HPV 16 was the most common genotype in the local population[1,19,20,21,23]. Except one, all other studies used PCR technique to detect HPV DNA.

HPV HR 3: 35, 39, 51, 56 and HPV HR 2: 53, 59, 66, 81 were recorded in 9.5% and 4.7% case respectively. The important finding with both these high risk HPV DNA groups was that they were expressed only in low grade morphology. Raza et al. in their series from Karachi supports our finding by observing 0.2% frequency of HPV 35, 51 and 56 and 0.1% frequency of HPV type 59 and 66 each, respectively. Similarly, Loya and colleagues also recorded a very low (0.8%) prevalence of HPV type 35 and 39 each in invasive SCC cases[20, 21].

The unique and interesting finding of the current study was absence of HPV18 among all cases. Interestingly, different studies from within China and Pakistan had reported it to be the second common genotype after HPV 16[1, 8, 19, 21, 23] . All of these were PCR based detections with varying primers, emphasizing once again on the significance of detection techniques used for HPV sub-typing.

For low grade lesions, the overall frequency of HPV positivity was less (28.5%) in comparison to SCC (47.6%), which is a promising feature. It reflects a slower rate of progression of low grade lesions into high grade. Another distinguishing finding of the present study was that HPV 16 and 18 were completely absent in CIN 1. This observation is supported by a meta-analysis from Clifford et al[25].

The impact of our results could be strengthened by studies using larger sample size. Effective screening program, better public health measures along with public awareness campaigns are the need of the day. HPV diagnostic tools should be approachable by patients and may be included in national health program. Vaccines against prevalent high risk groups need to be developed and administered. The limitation of our study was financial constraints to carry it out on a larger scale.

CONCLUSION

HPV HR1 (31, 33, 45, 52, 58) are major causative agents for low grade, high grade cervical intraepithelial lesions and SCC, in the local population followed by HPV 16. Frequency of HPV HR 2 and HPV HR 3 was low and seen only in low grade lesions. HPV 18 was found to be negative in all cases. Genotyping for HRHPV varies within a population. It may prove useful in designing preventive HPV vaccines, which include prevalent HRHPV including type 16/18.

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CONFLICT OF INTEREST

There is no conflict of interest among the authors of the study.

ETHICS APPROVAL

The ethics approval was obtained from BMSI on 20-08-20119 with the reference code as mentioned F.1-2/2019/BMSI-E.COMT/072/JPMC.

PATIENT CONSENT

Informed consents were obtained from all the patients.

AUTHORS' CONTRIBUTION

AZ conceived the idea and did manuscript writing. NJ did editing and performed critical review. SIK did final approval. RG performed statistics and critical review. JI did literature review and MU collected data.

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