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ORIGINAL ARTICLE

Histopathological characteristics and HPV status in cervical biopsy specimens diagnosed as flat condyloma

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ABSTRACT

Background and Objectives: HPV infections cause a wide spectrum of pathological changes in lower anogenital epithelium. The aim of this study was to investigate the HPV DNA status and histological findings in cervical biopsy specimens diagnosed as flat condyloma.
Materials and Methods: This study included 20 cervical biopsy specimens diagnosed as flat condyloma. The histopathological criteria and presence of HPV DNA were evaluated. HPV genotyping was determined in HPV-positive specimens using BioEdit software and the results were analyzed in SPSS software.
Results: HPV DNA was not found in 30% of specimens and relative frequency of HPV genotypes was: 15% HPV6, 15% HPV11, 5% HPV16, 5% HPV18, 5% HPV53, 5% HPV68, 5% HPV84, 10% HPV45. Relative frequency of histopathological criteria was as below: 100% of specimens had koilocytosis, 100% acanthosis, 15% nuclear immaturity, 100% atypia, 15% mitotic activity, 50% dyskeratosis, 35% parakeratosis and 10% hyperkeratosis.
Conclusion: There were significant differences between HPV positivity and two pathologic criteria; multinucleation and hyperkeratosis (P Value: 0.02). Nuclear immaturity was significantly more prevalent in high risk HPV-positive specimens (P Value: 0.03).

Keywords: Human papillomavirus viruses; Human papillomavirus; Human papillomavirus; Sexually transmitted diseases; Warts

INTRODUCTION

Human papillomavirus (HPV) is the most prevalent sexually transmitted infection (1, 2). Lower genital HPV infections may cause a wide spectrum of clinical manifestations from subclinical infection and benign genital warts (condyloma) to various intraepithelial neoplasms and cancers (3-7).

Human papillomaviruses are categorized in five major genera based on its L1 gene sequences (8, 9). Clinically important types are belonging to Alpha papillomaviruses (10). Human papillomaviruses have been divided into general groups of high and low risk based on their oncogenic potential in particular asso-

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ciation with genital cancer and premalignant lesions (11). About 14 genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 are classified as high-risk, genotypes 26, 30, 34, 53, 67, 69, 70, 73, 82, 85, and 97 as possible oncogenic (probably high risk) and types 6, 11, 28, 32, 44, 43, 44, 54, 55, 57, 61, 62, 71, 72, 74, 81, 83, 84, 86, 87, and 89 as low-risk genotypes (12). High-risk HPV E6 and E7 oncoproteins are involved in development of cancer by inactivating main cell tumor suppressor proteins pRB and p53 (13). Viral integration into the cell genome thought to be the essential event leading to cancer development (14). Although more than 90% of high-risk HPV infections clear within 2 years spontaneously (3), a small percentage of infections persist in the host and can lead to lower anogenital cancers or oropharyngeal and other malignancies (15).

Persistent genital high-risk HPV infection causes 99.7% of cervical cancer (16,17). Two High-risk HPV types 16 and 18 are responsible for 70% of cervical cancers (17) and two low-risk HPV types 6 and 11 associated with more than 90% of genital warts (18). HPV type 11 also is associated with RRP (Recurrent Respiratory Papillomatosis) most commonly in children (19).

The main histopathological characteristics of condyloma include acanthosis, papillomatosis, hyperkeratosis, parakeratosis and koilocytosis and mononuclear inflammatory infiltration in microscopic examination (20-24). Koilocytotic atypia considered as pathognomonic criteria for productive HPV infected squamous epithelium with perinuclear cytoplasmic clear zone and condensed nuclei (25, 26). Cytoplasmic vacuoles and relatively enlarged nucleus is the predominant morphological feature (27). Koilocytosis is the hallmark of a productive HPV infection in mature squamous epithelial cells, although it may not exist (28).

Histologically cervical condylomatus lesions have one of three patterns; flat condyloma, papillomatus condyloma and endophytic type. Flat condyloma is the most frequent type and 78.9% of condylomatus lesions of cervix are as flat condyloma (24). Flat condyloma is a flat lesion in which there is productive viral infection display cytoplasmic cavitation and nuclear atypia, have been designed in the past as koilocytic atypia, koilocytosis (29, 30), mild dysplasia or CIN I. Now, based on the current knowledge of HPV biology and pathogenesis of cervical cancer precursors, the two-tiered LAST terminilogy for lower anogenital intraepithelial lesions has been widely accepted; low grade SIL and high grade SIL. Low grade SIL corresponds to CIN I and all lesions with HPV cytopathic effect without dysplasia. Traditionally cervical intraepithelial lesions have been classified by a three-tiered classification system (CIN I,II,III) and pathologists favor considering two additional terminology for HPV related intraepithelial lesions (29), koilocytic atypia/ flat condyloma corresponding to viral cytopathic effects without dysplasia (5, 7, 12).

There are limited studies about relationship between histopathological changes of flat condyloma and frequency of HPV genotypes in lesions with pathologic diagnosis as flat condyloma. The objective of this descriptive study was to investigate HPV status, HPV genotyping and any relationship between detection of human papillomavirus DNA and detailed histopathological findings in cervical biopsies diagnosed as flat condyloma.

MATERIALS AND METHODS

A descriptive study is designed to explore the association between histological findings and frequency of HPV detection and it's genotypes in lesions diagnosed as flat condyloma. A total of 20 formalin-fixed paraffin-embedded (FFPE) specimens were collected during this study as archived samples.

Specimens and sampling. In this study, the presence of HPV and frequency of HPV genotypes were detected using PCR followed by DNA sequencing in cervical biopsy specimens who were performed in colposcopy clinic of Bu Ali hospital (under supervision of Islamic Azad University of Medical Sciences) in recent years. Participants included women with cervical biopsies were diagnosed as flat condyloma. At first histopatholoigcal diagnosis of flat condyloma reconfirmed by two pathologists. All formalin-fixed paraffin-embedded (FFPE) tissue specimens with poor DNA quality were excluded from this study. Finally, a total of 20 specimens entered into the study. Patient's medical information was available in pathologic request sheaths. There were no history of cancer in any patient and none of the participants had been vaccinated against HPV.

Ethical approval. Ethical approval for this study

was obtained from the Ethics Committee of Islamic Azad University of Medical Sciences, College of Medicine (number: IR.1389.4620). All experiments were performed in accordance with relevant guidelines and regulations. Informed consent form had been signed by all participants at the time of admission to the colposcopy clinic and they permitted any unnamed use of the data and specimens for research purposes.

Histopathological analysis. Two 5-10 µm-thick FFPE sections were used for histopathological analysis and DNA extraction. All the hematoxylin-eosin (HE) stained slides were reviewed by two pathologists and histopathological criteria of flat condyloma were assessed; including koilocytosis, multinucleated squamous cells, papillomatosis, acanthosis, cellular immaturity, nuclear changes, mitotic activity, dyskeratosis, parakeratosis, and hyperkeratosis. Another specimen stored at -80ºC for DNA isolation and HPV-DNA assay.

DNA extraction. DNA extraction from each tissue specimen was performed according to standard protocol. Thick FFPE sections were deparaffinized using xylol and alcohol, then pellets were suspended and digested in 400 µl digestion buffer (Tris-Cl100mM, pH=7.5, Tween20=0.05%) and 20 µl proteinase K (20 mg/ml proteinase K, Roche Diagnostics GmbH, Mannheim, Germany). The integrity of extracted DNA was assessed by PCR amplification of a β-globin gene (268 bp fragment) (31) and each DNA was analyzed by electrophoresis on a 1% agarose gel.

HPV DNA detection and genotyping. HPV detection was carried out in a nested PCR system, using MY09/MY11 and GP5+/GP6+ primers. Primer sequences and PCR conditions are listed in the Table 1.

HeLa (with 10-50 copies of HPV 18) and CaSki (with 600 copies of HPV 16) cells were obtained from a cell bank (Pasteur Institute of Iran) and used as positive controls.

The initial PCR reaction was carried out in 25-µl reaction volume containing 100 ng of DNA template in a 10 µl, 2.5 µL 10× PCR buffer, 2 µL $MgCl_2$ (50 Mm), 1 µL dNTPs (100µM), 3 µL of MY09 Primer (10 pmol/µl), 3µL MY11 Primer (10 pmol/µl) and 0.5 µL of taq DNA polymerase (5 U/µL) and 3 µl DW. Then nested PCR was performed in 60 µl reaction volume containing 10 µL of the first PCR reaction, 6 µL 10× PCR buffer, 3 µL $MgCl_2$ (50Mm), 1 µL dNTPs (100µM), 6 µL of GP5+ Primer (10 pmol/µl), 6 µL GP6+ Primer (10 pmol/µl) and 0.5 µL of taq DNA polymerase (5 U/µL) and 18.5 µl DW.

The HPV genotypes were identified by sequencing. The positive PCR products were extracted from the agaros gel using a Qiagen PCR purification kit and sent to Macrogen Inc (Seoul, Korea) for sequencing. Finally sequencing results were aligned using BioEdit software (BioEdit, v.7, CA).

Statistical analysis. HPV typing data and histopathology results were analyzed using SPSS version 16.0 (SPSS, Inc, Chicago, IL, USA) and related analysis were conducted by Chi-square test.

RESULTS

A total of 20 archived specimens with confirmed diagnosis of flat condyloma were included in the study. Fig. 1 displays distribution of the HPV genotypes in the specimens. Based on the results, 6 cases (30%) were negative for HPV DNA testing, 7 cases (35%) were positive for low-risk HPV types and 7 cases (35%) were positive for high-risk HPV genotypes.

Histopathological examination revealed that all cases (100%) displayed prominent koilocytosis and ac-

Table 1. List of primer sets and PCR conditions

Primer Sequence (5' → 3')		PCR product (bp)	PCR conditions
GP5	TTTGTTACTGTGGTAGATACTAC	150 bp	95°C 15 min, 94°C 1 min, 55°C 1 min, 72°C 1 min for 40 cycles and 72°C 5 min
GP6	GAAAAATAAACTGTAAATCATATTC		
MY9	CGTCCMARRGGWACTGATC	450 bp	94°C for 5 min, 2 min at 40°C and 2 min at 72°C (in the first round) 43 cycles of amplification: 94°C for 1 min, 40°C for 1 min and 72°C for 5 min (in the second round)
MY11	GCMCAGGGWCTATAAYAATGG		

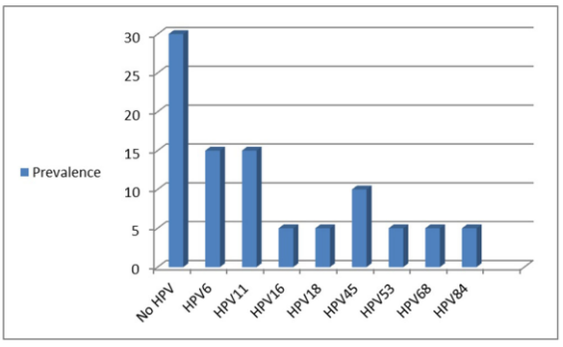

Fig. 1. Prevalence of HPV positive and HPV genotypes in cervical biopsy specimens diagnosed as flat condyloma

anthosis, 11 cases (55%) had multinucleate cells and 11 cases (55%) had papillomatosis morphology. Also, mitotic figures were visible only in 5 cases (25%). Other histopathological findings include dyskeratosis in 10 cases (50%), parakeratosis in 7 cases (35%) and hyperkeratosis in 2 specimens (10%). The koilocytosis, binucleation nuclear atypia, including enlargement, variation in size and shape, hyperchromasia, and coarse chromain with rare mitotic figures are shown in Fig. 2. Correspondingly, the frequencies of histologic characteristics based on the HPV genotypes

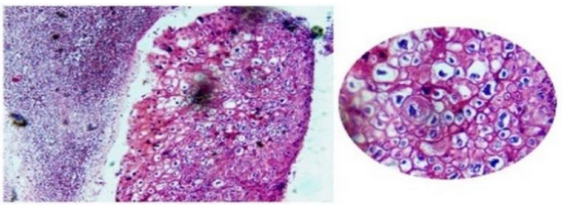

Fig. 2. The image shows flat condyloma. At these magnification acanthosis, koilocytosis, binucleation nuclear atypia, including enlargement, variation in size and shape, hyperchromasia, and coarse chromain with rare mitotic figures are seen.

are described in Table 2.

Association of HPV positivity with histologic characteristics in flat condyloma cases is also given in the Table 3. The results of this study showed that there was no statistically significant difference between HPV DNA positive and negative cases of flat condyloma (P Value=0.074) and also between HPV genotypes and flat condyloma features (P Value=0.478). There were significant differences between HPV positivity and multinucleated squamous cells (91% vs 9%, P Value: 0.02) and hyperkeratosis was significantly more prevalent in HPV negative specimens (0 of 14 vs 2 of 6 HPV negative specimens, P Value: 0.02). There was no significant relationship between HPV positivity and other histopathological findings.

According to Table 4, comparison of histopathological characteristics between the specimens with high risk and low risk HPV genotypes showed that there was significant difference in the frequency of positive HPV types in terms of nuclear immaturity variable (P Value: 0.03), while there are no significant differences in the prevalence of high risk HPV in all other histopathological characteristics.

Table 2. Histologic characteristics based on HPV genotypes in 20 cervical biopsy specimens diagnosed as flat condyloma

Histologic features/ HPV Genotype	Hyperkeratosis N (%)		Parakeratosis N (%)		Dyskeratosis N (%)		Mitotic activity N (%)		Acanthosis N (%)		Nuclear immaturity N (%)		Papillomatosis N (%)		Multinucleated squamous cells N (%)		Koilocytosis N (%)	
	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
HPV Neg (n=6)	4 (66)	2 (33)	5 (83)	1 (16)	2 (33)	4 (66)	6 (100)	0	6 (100)	0	6 (100)	0	3 (50)	3 (50)	5 (83)	1 (16)	0	6 (100)
HPV6 (n=3)	3 (100)	0	1 (33)	2 (66)	2 (66)	1 (33)	1 (33)	2 (66)	0	3 (100)	3 (100)	0	2 (66)	1 (33)	1 (33)	2 (66)	0	3 (100)
HPV11 (n=3)	3 (100)	0	2 (66)	1 (33)	2 (66)	1 (33)	2 (66)	1 (33)	0	3 (100)	3 (100)	0	0	3 (100)	1 (33)	2 (66)	0	3 (100)
HPV16 (n=1)	1 (100)	0	0	1 (100)	0	1 (100)	1 (100)	0	0	1 (100)	1 (100)	0	1 (100)	0	0	1 (100)	0	1 (100)
HPV18 (n=1)	1 (100)	0	0	1 (100)	0	1 (100)	0	1 (100)	0	1 (100)	1 (100)	0	0	1 (100)	0	1 (100)	0	1 (100)
HPV31 (n=1)	1 (100)	0	1 (100)	0	0	1 (100)	1 (100)	0	0	1 (100)	0	1 (100)	1 (100)	0	1 (100)	0	0	1 (100)
HPV45 (n=2)	2 (100)	0	2 (100)	0	1 (50)	1 (50)	2 (100)	0	0	2 (100)	0	2 (100)	2 (100)	0	1 (50)	1 (50)	0	2 (100)
HPV53 (n=1)	1 (100)	0	0	1 (100)	1 (100)	0	0	1 (100)	0	1 (100)	1 (100)	0	0	1 (100)	0	1 (100)	0	1 (100)
HPV68 (n=1)	1 (100)	0	1 (100)	0	1 (100)	0	1 (100)	0	0	1 (100)	1 (100)	0	0	1 (100)	0	1 (100)	0	1 (100)
HPV84 (n=1)	1 (100)	0	1 (100)	0	1 (100)	0	1 (100)	0	0	1 (100)	1 (100)	0	0	1 (100)	0	1 (100)	0	1 (100)

Table 3. Association of HPV positivity with histologic characteristics in 20 specimens with diagnosis of flat condyloma

Histologic features/ HPV positivity	Nuclear immaturity n=3	Papillomatosis n=11	Multinucleated squamous cells n=11	Mitotic activity n=5	Parakeratosis n=7	Dyskeratosis n=10	Hyperkeratosis n=2
HPV Pos (n=14)	3 (100%)	8 (73%)	10 (91%)	5 (100%)	6 (86%)	6 (60%)	0
HPV Neg (n=6)	0 (0%)	3 (27%)	1 (9%)	0 (0%)	1 (14%)	4 (60%)	2 (100)
P Value	0.2	0.7	*0.02	0.09	0.26	0.3	*0.02

Table 4. Association histologic characteristics with HPV genotypes in in specimens with diagnosis of flat condyloma

Histologic features/ HPV Type	Nuclear immaturity n=3 Number (%)	Papillomatosis n=8	Multinucleated squamous cells n=10	Mitotic activity n=5	Parakeratosis n=6	Dyskeratosis n=6	Hyperkeratosis n=0
Low risk HPV (n=7) (6, 11, 84)	0 (0%)	5 (62%)	5 (50%)	3 (60%)	3 (50%)	2 (33%)	0
High Risk HPV (n=7) (16, 18, 31, 45, 53, 68)	3 (100%)	3 (38%)	5 (50%)	2 (40%)	3 (50%)	4 (67%)	0
P Value	*0.03	0.53	0.07	0.19	0.18	0.3	0,07

DISCUSSION

In this study HPV DNA test positivity and histolopathological findings were investigated in cases diagnosed as flat condyloma. Based on the results, HPV DNA was detected in 14 out of 20 cases (70%). In some other studies, HPV (mainly 6, 11, 16, and 18 genotypes) prevalence in flat condyloma patients is varied from 56 to 88% (32-36). Our results and previous studies have been shown that the histological criteria for diagnosis of flat condyloma are not specific for HPV infection. All of the specimens included in our study had main two features of koilocytosis and acanthosis which are diagnostic criteria for flat condyloma, but 6 out of 20 cases were subsequently had negative PCR test for HPV DNA. Beznos and colleagues have previously shown a significant association between the nucleus multiplication feature and the presence of HPV genome in their study (33).

In the present study, there was significant difference between the presence of HPV DNA with multinucleated cells feature and hyperkeratosis. Morphological study confirmed that 10 out of 14 HPV DNA positive cases had displayed cellular multinucleation and all HPV-positive specimens have not shown evidence of hyperkeratosis. Because of this correlation, multinucleated cells feature seems to increase the possibility of HPV infection, but cannot indicate the presence of high-risk HPV. Beznos and colleagues have previously shown a significant association between the nucleus multiplication feature and the presence of HPV genome in their study (33).

In the present study, the clinical specimens were not evaluated for the severity of Koilocytosis and atypical severity. Perhaps if these variables were investigated, the relationship between the severity of cytological atypia and the degree of koilocytosis would be related to each other or to the HPV positivity. In a previous study conducted by Perez and colleagues have reported that in the presence of severe atypia there is no or mild Koilocytosis (32).

The limitations of our study are relatively small size of the sample, it's retrospective design and insufficient data about clinical characteristics of the patients, cervical cytology and indications of colposcopy.

CONCLUSION

The histological findings in flat condyloma seem to be nonspecific for diagnosis of neither HPV infection nor HPV types and histopathological features only may suggest HPV status.

Due to the significant prevalence of high-risk HPV types in flat condyloma and potential carcinogenicity, definitive diagnosis of HPV infection and its genotype will be noteworthy in follow up of the

patients. The patients may need counseling for preventive measures such as safe sexual behavior, more frequent screening and HPV vaccination, especially in developing countries without national vaccination programs.

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