Variations of Histone Acetyltransferase 300 in Patients with Human Papillomavirus Type 6-Associated Anogenital Warts

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ABSTRACT

Background and objectives: Pathogenesis of human papillomaviruses (HPVs) is controlled by viral and host factors, among which human histone acetyltransferase p300 (EP300) plays an important role. This study aimed to examine single nucleotide polymorphisms (SNPs) at the EP300 binding site in patients with HPV-associated anogenital wart.

Methods: After DNA extraction, polymerase chain reaction was performed to determine HPV genotypes. Human p300 was amplified to detect SNPs using Sanger sequencing.

Results: Overall, 35.3% of HPV-positive patients had Ile997Val substitution at the EP300 binding site. Another SNP containing A to G point mutation leading to Glu983Gly was also detected. In addition, Ile997Val substitution of EP300 was frequently observed in the patients.

Conclusion: Our findings suggest that the EP300 genotype Ile/Val can be involved in HPV-6 pathogenesis. In addition, we introduced a new genotype (Glu983Gly) at the EP300 bromodomain site, which requires further investigation.

Keywords: Human Papilloma Virus; Anogenital warts; Histone acetyltransferase p300; Polymorphism; EP300; Single Nucleotide Polymorphism
INTRODUCTION

Human Papillomavirus (HPV) is the leading cause of cervical cancer, the second most common cancer in women worldwide (1). The virus is also involved in the progression of anogenital warts and tumors (2,3). Currently, more than 200 HPV genotypes have been identified and only 1/5th of them are associated with anogenital infections. Genital genotypes of HPV are divided into high-risk (HR) and low-risk (LR) groups in terms of their carcinogenic potential (4,5). The genotype of HR HPVs include types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82, while the HPV genotypes 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, 89 are considered as low risk (6–8). HPV-16, -18 and -51 are the predominant oncogenic genotypes that contribute to the most global cases of cervical cancer (9,10).

Low-risk HPV types commonly occur in benign anogenital warts (11). Although most genital warts are caused by HPV-6 or -11 infections, HR genotypes may often be found in patients with genital warts (12-14). Pathogenesis of HR and LR HPVs is primarily concerned with the control of host factors and their interactions with viral counterparts. These factors are currently being investigated for their potential as a biomarker of HPV pathogenesis. Genetic alterations of gene products targeted by HPV oncoproteins can more effectively promote the development of cancer than virus-host interactions alone (15).

Histone acetyltransferase (HAT) p300 (EP300) is a human protein, which facilitates the interaction of HPV oncoproteins with other host factors. The coding region of EP300 is located on chromosome 22 (22q13.2) (16) and its product plays an important role in the cell cycle process and as a tumor suppressor (17,18). Somatic mutations of EP300 have been identified as cancer facilitators (19–21). The genotypes Ile997Val and Val/Val EP300 increase the risk of hepatocellular carcinoma. It has been shown that there may be an association between Val/Val genotype in EP300 and hepatocellular carcinoma risk (OR, 3.03; 95% CI, 1.08–8.47; P = 0.028) (22).

It is known that EP300 also plays an important role in the maintenance of p53 stability (23,24) and functions as an intrinsic HAT, which is also a transcriptional coactivator involved in tumorigenesis (25,26). Codon 997 maps the EP300 bromodomain responsible for HAT activity closely upstream and in a region of interaction with junction mediating and regulatory protein, p53 cofactor, a cofactor known to increase apoptosis and p53-dependent transcription (27). By targeting EP300, HPV E6 protein may potentially abrogate P53 maintenance. By interacting with CREB-binding protein/p300, HPV E6 prevents p53 acetylation and maintenance that could reduce its affinity to DNA promoters (28). This could be the only possible explanation for the significant reduction of p53 activity in HR HPV infections (18). Therefore, mutation at p300 region and particularly its active site, could determine the severity of HPV infection. Thus, single nucleotide polymorphism (SNP) at p300 could serve as a potential marker for estimating the effect of certain HPV types in cancer progression. There is no knowledge of EP300 variations in patients with LR HPV-associated anogenital warts. In the present study, we aimed to investigate SNP(s) of EP300 at the C-terminal bromodomain among patients with HPV-6-associated anogenital warts.

MATERIALS AND METHODS

This cross-sectional study was performed from February 2018 to July 2018 on anogenital wart biopsy samples of 18 patients (14 men and 4 women) at Skin Clinics in Gorgan, Iran. The study was approved by the Ethics Committee of the Golestan University of Medical Sciences (Gorgan, Iran) with the approval code IR.GOUMS.REC.1396.120. A written informed consent was obtained from all subjects. The patients were able to leave the study due to their own concerns. After the initial diagnosis of genital warts was made by a dermatologist, a surgeon collected one representative biopsy sample from each patient. Characteristics such as marital status, smoking, condom use, educational level and medical history were collected using a questionnaire.

A genomic DNA extraction kit (Macherey-Nagel, Germany) was used for DNA extraction from anogenital warts sections. Multiplex polymerase chain reaction (PCR) was performed to investigate presence of HPV genotypes 54, 18, 16 and 6.
For this purpose, four pair of primers was designed covering coding sequences of HPV L1 (Table 1). PCR reaction was performed as follows: initial denaturation at 94 ° C for five minutes followed by 35 cycles at 94 °C for 30 seconds, 58 °C for 30 seconds and 72 °C for 40 seconds. PCR products were electrophoresed on 1% agarose gel containing SYBR safe stain (Sinaclon Inc., Iran).

Exon 14 of the human p300 gene corresponding to the bromodomain was amplified for the detection of SNPs. The amplification process was performed using Platinum ® Taq DNA Polymerase (Thermo Fisher Scientific, USA) under the following conditions: initial denaturation at 94 °C for 5 minutes, followed by 35 cycles at 94 °C for 30 seconds, annealing at 55 °C for 30 seconds and extension at 72 °C for 20 seconds. Later, the products were sent for Sanger sequencing (Macrogen, Inc., South Korea). The DNA sequencing data were analyzed for heterogeneous regions by examining chromatogram. Finally, SNPs were detected by sequence alignment of p300 data against NCBI’s human reference genome database.

Descriptive analyses were performed using SPSS 16.0 software package. Data were analyzed using the cross-tabulation and Chi-square tests. A p-value of less than 0.05 was considered as significant.

RESULTS

In the present study, 18 HPV-positive patients with a mean age of 32.11±7.08 years (range: 24 to 48 years) were enrolled. Based on the results, 31 patients (77.5%) with genital warts were infected with HPV type 6. We found no significant association between type of HPV infection and the demographic variables. Among the positive HPV-6 patients, six cases (35.3%) had the A64395 to G transition leading to substitution of Ile997Val.

In addition, five men (50%) had at least one substitution in EP300 protein. Substitutions E983 G and genotype I/I were only observed in men. Patients P14 and P17 were positive for HPV-16 and did not have any change in the p300 region (Table 2). Furthermore, we found another SNP containing A to G transition at nucleotide 64355 leading to Glu983Gly in EP300 of two HPV-6 positive patients (11.76%).

The sequencing data were submitted to the GenBank database with MN844891-MN844908 accession numbers. The results of this study revealed the existence of different forms of EP300 among women with anogenital HPV infection.

Table 1. Sequences of the primers used in the multiplex-PCR experiment

<table>
<thead>
<tr>
<th>HPV genotypes</th>
<th>Primers (5’ to 3’)</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV-54</td>
<td>F: TTGCATCCACGCAGGATAGC</td>
<td>386</td>
</tr>
<tr>
<td></td>
<td>R: ACGTAGGCCAGCCTGTAGTA</td>
<td></td>
</tr>
<tr>
<td>HPV-18</td>
<td>F: CGTGGTCAGCCCTTAGGTGT</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>R: GAAACATTAGACGGCGGC</td>
<td></td>
</tr>
<tr>
<td>HPV-16</td>
<td>F: CGTGGTCAGCCCTTAGGTGT</td>
<td>643</td>
</tr>
<tr>
<td></td>
<td>R: TGCCATTATGTCGAGGCCTGT</td>
<td></td>
</tr>
<tr>
<td>HPV-6</td>
<td>F: AAATGTTGTCACCGGAGTC</td>
<td>204</td>
</tr>
<tr>
<td></td>
<td>R: AGACGATGTCAGCGACGGCAC</td>
<td></td>
</tr>
<tr>
<td>p300</td>
<td>F: TTGGCTGAGAAGCAGCCTTC</td>
<td>193</td>
</tr>
<tr>
<td></td>
<td>R: CTTGGATGTTCTCCCTCTC</td>
<td></td>
</tr>
</tbody>
</table>
In the present study, there was no relationship between smoking and HPV infection. In line with our findings, previous studies reported no link between demographic characteristics and HPV infection in patients with anogenital warts (35,36). The pathogenicity of certain LR HPVs in anogenital tissues depends on both viral and cellular factors (37). The major HR-HPV oncoproteins are E6 and E7, which are known to interact with several cell proteins (14). It has been recently found that a HAT, the transcriptional coactivator p300, is essential for several biological functions such as proliferation, differentiation and apoptosis. The protein p300 was first identified by its specific interaction with the Adenovirus early region 1A (E1A).

**DISCUSSION**

Genital warts are benign growth of epithelial cells caused by the sexually transmitted HPV (29-31). Genotyping of genital HPV has great clinical significance in terms of treatment, follow-up and prevention (32). Low-risk HPVs are often related to anogenital warts or Condylomata acuminate (33). HPV-6 is detected in more than 90% of the clinical samples of genital warts (34). In our study, HPV-6 was present in 77.5% of patients with genital warts. In a study on tissue specimens of 100 HPV positive women with genital warts in Iran, the prevalence of HPV-6 and HPV-11 was 49% and 67%, respectively. Similar to our findings, the mentioned study also found no significant correlation between HPV genotype and marital status (13).

---

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Age (years)</th>
<th>Gender</th>
<th>Marital status</th>
<th>History of smoking</th>
<th>HPV type</th>
<th>SNPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>29</td>
<td>Male</td>
<td>Married</td>
<td>No</td>
<td>HPV-6</td>
<td>E983G</td>
</tr>
<tr>
<td>P2</td>
<td>25</td>
<td>Male</td>
<td>Single</td>
<td>Yes</td>
<td>HPV-6</td>
<td>F997V</td>
</tr>
<tr>
<td>P3</td>
<td>46</td>
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<td>Married</td>
<td>Yes</td>
<td>HPV-6</td>
<td>E983G</td>
</tr>
<tr>
<td>P4</td>
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<td>Male</td>
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<td>HPV-6</td>
<td>F997V</td>
</tr>
<tr>
<td>P6</td>
<td>48</td>
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<td>Single</td>
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<td>HPV-6</td>
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</tr>
<tr>
<td>P7</td>
<td>25</td>
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<td>Single</td>
<td>Yes</td>
<td>HPV-6 and -16</td>
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<td>P8</td>
<td>26</td>
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<td>I997V and I/I</td>
</tr>
<tr>
<td>P9</td>
<td>27</td>
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<td>Married</td>
<td>No</td>
<td>HPV-6</td>
<td>None</td>
</tr>
<tr>
<td>P10</td>
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<td>Male</td>
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<td>Yes</td>
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<td>I997V and I/I</td>
</tr>
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<td>P11</td>
<td>41</td>
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<td>Married</td>
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<td>P12</td>
<td>36</td>
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<td>Married</td>
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<td>F997V</td>
</tr>
<tr>
<td>P13</td>
<td>32</td>
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<td>Married</td>
<td>No</td>
<td>HPV-6</td>
<td>None</td>
</tr>
<tr>
<td>P14</td>
<td>26</td>
<td>Female</td>
<td>Married</td>
<td>NA</td>
<td>HPV-6</td>
<td>None</td>
</tr>
<tr>
<td>P16</td>
<td>24</td>
<td>Female</td>
<td>Married</td>
<td>No</td>
<td>HPV-6</td>
<td>F997V</td>
</tr>
<tr>
<td>P17</td>
<td>32</td>
<td>Female</td>
<td>Married</td>
<td>No</td>
<td>HPV-16</td>
<td>None</td>
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<tr>
<td>P18</td>
<td>38</td>
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<td>Married</td>
<td>No</td>
<td>HPV-6</td>
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<tr>
<td>P30</td>
<td>27</td>
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<td>P31</td>
<td>28</td>
<td>Male</td>
<td>Single</td>
<td>Yes</td>
<td>HPV-6</td>
<td>None</td>
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</table>

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It was suggested that E1A-mediated perturbation/reorchestration of the normal function of the cyclic AMP response element-binding protein -p300- APC/C is essential for induction of cellular transformation (38,39). It was subsequently shown that many regulatory proteins of other oncogenic viruses also target p300 to control viral and cellular gene expression. In addition, some important p300-HPV interactions have been reported (39,40). It has been shown that p300 could be important in controlling the expression of E6 and E7 (41-43). In addition, EP300 tends to bind to HPV-16 P97 promoter and regulate the expression of E6 and E7 when HPV is present in episomal or integrated forms (44). Loss of episomes will result in activation of the P97 promoter, allowing high expression of E6 and E7. Thus, collective detection of HR-HPV genotypes could be a robust tool for revealing any association with the outcome of cervical cancer (45-47).

In The present study, EP300's polymorphisms were examined at exon 14 to identify changes in the protein's binding site, which is involved in the HPV oncoproteins interactions with the host p53. It is well-established that p300 may include A/G transition in its coding sequence, which changes the amino acid codon 997 from Ile to Val at bromodomain (27). In our study, 35.3% of positive HPV-6 patients had the A64395 to G transition leading to substitution of Ile997Val. In addition, half of men had at least one substitution in EP300 protein. Moreover, substitutions E983 G and genotype I/I were only observed in men. It should be emphasized that the effect of p300 may be related to the HPV genotype (48,49). Our findings revealed another SNP containing A to G transition at nucleotide 64355 leading to Glu983Gly in EP300 of two HPV-6 positive patients. The role of such SNPs in p300 could affect the pathogenesis and carcinogenesis of LR and HR HPVs. The role of certain polymorphisms such as Val / Val, Ile / Val and Glu/ Gly in the severity of HPV infection remains unclear. We suggest conducting a study on a large population of patients with HPV-associated anogenital infections. In addition, the variations in EP300 and its association with cancer development should be investigated.

CONCLUSION

Polymorphisms at the binding site(s) of EP300 may affect the HPV pathogenesis. Our findings indicate that the EP300 Ile997Val genotype is relatively abundant in HPV-6-associated anogenital warts.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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