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Biomarkers in Condyloma of the Genital Region and Differences Given to Place of Origin

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Abstract

Background: Development of various neoplasia has been associated with Human Papilloma Virus (HPV) infection. Numerous reports suggest linkage of p16, p53 and pRb expression to HPV status in cervical carcinomas. There have been reports on protein expression and DNA content in both cutaneous and mucosal carcinomas, but correlation of these parameters is still not clear. The aim of this study was to determine a possible correlation between HPV type, protein expression and DNA content, in two types of condylomas Condylomata Plana (CP) and Condylomata Acuminata (CA), as well as differences between studied groups in these parameters.

Methods: Sections of formalin fixed paraffin-embedded tumor tissue from 71 cases of CA and 36 cases of CP, were subjected to HPV genotyping using LiPA (Line immuno-Probe Assay) and flow cytometry for DNA content analysis. Also, immunohistochemical staining was performed. Obtained data were analyzed in SPSS using Chi square test.

Results: Statistically significant correlations were found in both condylomas between parameters p53-pRb and in CP between pRb and p16. Statistically significant differences between studied groups of condylomas were found in HPV prevalence, p53 expression and DNA content. Significant correlation between analyzed parameters was observed between p53 and pRb expression in both groups.

Conclusion: CA and CP differ in most parameters, thus suggesting different pathobiology pathways for different sites of neoplasia development, given to place of origin.

Keywords: HPV; Protein expression; Flow cytometry; Condyloma

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Introduction

Condyloma development is associated with Human Papilloma Virus (HPV) infection. Condylomata Acuminata (CA) are benign warts that usually arise on the skin. In most cases they are localized on the external genitals, but can also be found on the cervix, urethra, bladder and rectum. They can spontaneously regress in 3 to 6 months persist for years or develop into a neoplasia. Their growth can be accelerated by immunological changes. Condylomata acuminata are mostly associated with low risk HPV (lrHPV) types [1,2].

Condylomata Plana (CP) usually arise on the mucosa localized on the genital area and have a different shape. Their main causes are high risk HPV (hrHPV) types, namely 16,18,31 and 33, which have a high oncogenic potential that may lead to carcinoma development. Condylomata plana occur often in both men and women [3,4].

HPV is a proven human carcinogen [5]. The roles of proteins p16, p53 and pRb in diseases related to HPV infections have also previously been described [6-9]. The value of flow cytometry analysis of DNA content in HPV related diseases was also previously described [10] and aneuploidy can be useful diagnostic markers and have prognostic significance [11].

As most condylomas are related to HPV infection, the aim of this retrospective study was to determine potential differences in HPV status and HPV related proteins, p16, pRb and p53, as well as DNA content, between two groups of condylomas (CA and CP) which arise in different sites. These findings could lead to better understanding of differences between similar changes due to different sites of origin (mucosa *vs.* skin). Determining a correlation between p16, pRb and p53 expression, HPV status and DNA content in each of the studied groups could help explain the significance of each parameter in cancer development.

Materials and Methods

Case selection

Paraffin embedded tumor tissue blocks were randomly selected from the archives of the Pathology Department, Clinical Hospital Center Zagreb and Zagreb University School of Medicine from the patients diagnosed with condylomata acuminata and condylomata plana, from 1978 to 2007. Tumor samples were histologically re-examined on tissue sections of formalin-fixed (10 %) and paraffin-embedded tissue stained with hematoxylin-eosin. There were in total 71 cases of patients with Condylomata acuminata localized in the genital area, mean age 33.12 years and 36 cases of patients with Condylomata plana localized in the genital area, mean age 27.54 years.

HPV detection

DNA extraction from paraffin-embedded tissue sections (6x10 μm) was performed with commercially available QiAmp DNA Mini Kit (QIAGEN, Hilden, Germany), according to manufacturer's recommendations. Detection of HPV DNA was performed using LiPA (Line immuno Probe Assay) by INNO-LiPA HPV genotyping v2 system (Innogenetics, Gent, Belgium), according to their protocol, which is based on amplification of the L1 region of HPV DNA. Results were visually interpreted. HPV genotypes were divided into groups: hrHPV and lrHPV types.

Protein detection

Immuno-enzymatic staining for detecting protein expression was performed on 4 μm thick paraffin sections immobilized on slides using Dako Cytomation Autostainer Instrument (Dako, Denmark) according to the protocol recommended by the manufacturer of each visualization kit: for p53 EnVision kit (DAKO, Denmark), for pRB LSAB+System-HRP (Dako, Denmark) and for p16 CINTec p16INK4A Histology Kit (Dako, Glostrup, Denmark). Counterstaining was performed with Hematoxylin (Dako). Nuclear and cytoplasmic staining of >30% of cells was considered positive.

Flow cytometry for DNA analysis

The samples (3x40 μm tissue sections) were prepared using the Hedley method [12]. Cellular DNA content was analyzed the following day on FACS Calibur Flow Cytometer (Becton Dickinson, San Jose, CA, SAD), at wavelength of 488 nm and using red filter. Program CellQuest was used for collection of 20,000 nuclei per sample and DNA histograms were analyzed in ModFit LT V3.0 program (Verity Software House Inc., Topshame, ME and Becton Dickinson, SAD). Samples with DNA Index (DI) equal to 1 were considered diploid, those with 1<DI<2 were aneuploid and those with DI=2 were tetraploid.

Statistical analysis

All statistical analyses were performed with the statistical package SPSS for Windows 9.0. The Chi square test was used to compare studied groups. P values less than 0.05 ($p<0.05$) were considered statistically significant.

Results

HPV detection

Four (4) out of 71 (5.6 %) CA cases were HPV negative, 46 (64.8 %) cases were infected with lrHPV and 21 (29.6 %) cases were infected with hrHPV (Figure 1).

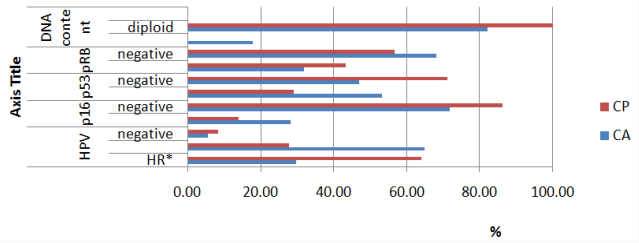

Figure 1: Comparison of analyzed parameters in CA and CP.
CA : Condyloma Accuminata; CP : Condyloma Plana; LR: Low Risk; HR: High Risk

Table 1: Statistical comparison between study groups.

Diagnosis	HPV	p16	p53	pRB	DNA content
Condylomata plana *vs* Condylomata accuminata	0.001	0.099	0.027	0.273	0.046

Table 2: Correlation of analyzed parameters in study groups.

Studied Parameters	Condyloma accuminata	Condyloma plana
HPV-p53	0.407	0.362
HPV-pRb	0.609	0.159
HPV-p16	0.563	0.872
HPV-ploidy	0.393	0.781
p53-pRb	0.001	0.044
P53-p16	0.877	0.269
p53-ploidy	0.657	0.123
pRb-p16	0.083	0.042
pRb-ploidy	0.121	0.547
p16-ploidy	0.367	0.736

From 36 cases of CP, there were 3 (8.3 %) HPV negative cases, 10 (27.8 %) were lrHPV and 23 (63.9 %) were hrHPV.

Cases of Condylomata acuminata show a higher frequency of lrHPV compared to cases of Condylomata plana with statistical significance p=0.001 (Table 1).

Protein detection

p53: From 71 cases of CA there were 7 (9.9 %) cases that could not be analyzed, and from the remaining 64 cases, in 30 (46.9 %) cases p53 expression was not detected, where in 34 (53.1 %) cases p53 expression was detected.

There were 5 out of 36 (13.9 %) cases of CP that could not be analyzed, and from the remaining 31 cases, in 22 (71 %) cases p53 expression was not detected where in 9 (29 %) cases p53 expression was detected.

Cases of Condylomata acuminata show a higher frequency of p53 expression compared to cases of Condylomata plana with statistical significance p=0.027.

PRb: From 71 cases of CA there were 2 (2.8 %) cases that could not be analyzed, and from the remaining 69 cases, in 47 (68.1 %) cases pRb expression was not detected, where in 22 (31.9 %) cases pRb expression was detected.

Out of 36 CP cases, there were 6 (16.7 %) cases that could not be analyzed, and from the remaining 30 cases, in 17 (56.7 %) cases pRb expression was not detected, where in 13 (43.3 %) cases pRb

expression was detected.

We observed that there was no statistically significant difference in pRb expression between cases of Condylomata acuminata and cases of Condylomata plana (p=0.273) (Table 1).

p16: p16 expression was not detected in 51 (71.8 %) cases of CA, where in 20 (28.2 %) cases p16 expression was detected (Figure 1).

p16 expression was not detected in 31 (86.1 %) cases of CP, where in 5 (13.9 %) cases p16 expression was detected (Figure 1).

We observed that there was no statistically significant difference in p16 expression between cases of Condylomata acuminata and cases of Condylomata plana (p=0.099) (Table 1).

DNA content

From 71 cases of CA, there were 32 (45.1 %) cases that could not be analyzed, and from the remaining 39 cases, 7 (17.9 %) were aneuploid and 32 (82.1 %) were diploid (Figure 1).

Out of 36 CP cases, there were 17 (47.2 %) cases that could not be analyzed, and from the remaining 19 cases, all were diploid (Figure 1).

Cases of Condylomata acuminata show a higher frequency of aneuploidy compared to cases of Condylomata plana with statistical significance p=0.046 (Table 1).

Correlation of HPV genotype, protein expression and DNA content

Statistically significant correlation in CA was found only between expression of p53 and pRb (p=0.001) (Table 2).

In CP statistically significant correlation was found between p16 expression and pRb expression (p=0.042) and between p53 and pRb expression (p=0.044) (Table 2).

Discussion

HPV prevalence in condylomas is as expected and previously reported, it is known that CA are associated with low-risk HPV, and CP with high-risk HPV [1,2,13].

Though we have found a higher prevalence of p53 expression in CA than previously reported [13-15], this is not contradictory. In most cases of CP p53 expression has not been detected, which is also in concordance with previous studies [16]. Statistically significant differences between CP and CA were found for p53 expression, where there were more positive cases of p53 expression in CA. We can presume that p53 expression is associated with morphological changes arising on the skin, but further investigation has to be done.

In both CP and CA, in most cases pRb expression was not detected, though there were more positive cases of pRb expression in CP. Nevertheless, statistically significant differences between CP and CA were not found for pRb expression.

It is expected to find higher levels of p16 in higher levels of HPV related malignancies [17-20]. p16 expression was not detected in neither CP nor CA. This finding was expected due to above mentioned studies.

Although some previously reported data show that all CA cases were diploid [21], we have found aneuploidy in CA. Also, our results show more diploid cases in CA than other previously reported data [22], which show an equal distribution of diploidy and aneuploidy. In addition, it has been reported that aneuploid cases are not correlated with HPV, which is consistent with our results [22]. All analyzed cases in CP show diploidy.

Although we found more hrHPV in CA than in CP and low p16 and pRb expression was found in both CP and CA, no statistically significant correlation was observed between HPV and p16 or between HPV and pRb in studied groups. Interestingly, we found a positive correlation between pRb and p16 expression in CP (p=0.042).

We also observed a statistically significant correlation between p53 and pRb expression in both groups of condylomas (CA p=0.001; CP p=0.044).

Conclusion

Results show statistically significant differences in most parameters between CP and CA, which indicate differences in mechanisms of origination of similar changes.

Also, statistically significant correlation of studied parameters was found between p53 expression and pRb expression in CA and CP, as well as between pRb and p16 expression in CP.

Although we found differences between studied groups, further investigation is needed for better understanding of diverse pathobiology pathways for condylomas.

Compliance with Ethical Standards

This study is part of a project which has been approved by the local Ethical committee.

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