

Eosinophilic dysplasia of the cervix associated with HPV 6 infection – case report and review of the literature

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SUMMARY

Eosinophilic dysplasia of the cervix is recently described unusual and somewhat obscure dysplastic lesion of squamous epithelium. We present histological features of a lesion in 41 years old woman. It was composed of cells with brightly eosinophilic cytoplasm contoured by a sharp and slightly broader cytoplasmic membrane, lacking maturation, with mild increase in nuclear-cytoplasmic ratio, slight chromatin clumping and uneven mild nuclear clearing. Electronmicroscopic study showed mild crevices of the nuclear membrane in some dysplastic cells. Tissue in situ hybridization study confirmed the presence of HPV 6 in the form of patchy dotted pattern of integrated type. Immunohistochemistry revealed diffuse positive expression of antigen p16, extraordinarily in this case focally sparing basal part of the epithelium. Underestimation of this lesion can be avoided by paying attention to strong eosinophilia of the cytoplasm and sharp cellular contouring of the examined epithelium in routine hematoxylin-eosin staining.

Keywords: Eosinophilic dysplasia – PCR – in situ hybridization – CIN - cervical intraepithelial neoplasia – HPV 6

Eozinofilná dysplázia krčka maternice asociovaná s infekciou HPV 6 – kazuistika a prehľad literatúry

SÚHRN

Eozinofilná dysplázia krčka maternice bola popísaná nedávno ako neobvyklá a trochu nejednoznačná dysplastická lézia dlaždicového epitelu. Popisujeme túto jednotku diagnostikovanú u 41 ročnej ženy. Histologicky pozostáva z buniek s jasne eozinofilnou cytoplazmou, ktoré sú ostro ohraničené výraznou cytoplazmatickou membránou. Epitel je bez vyzrievania, s miernym zvýšením nukleocytoplazmatického pomeru buniek, s miernym zhrudkovaním chromatinu a nerovnomerným miernym prejasňovaním jadier. Elektronová mikroskopia ukazuje mierne zárezy jadrovej membrány v niektorých bunkách. Metóda tkanivovej in situ hybridizácie potvrdzuje prítomnosť HPV 6 ako ložiskovú bodkovitú pozitivitu signálu - integrovaný typ pozitivitu. Imunohistochemicky je prítomná difúzna pozitívna expresia antigénu p16, ktorá v tomto prípade neobvykle ložiskovo vynecháva bazálnu vrstvu epitelu. Poddiagnostikovaní tejto lézie sa možno najúčinnejšie vyhnúť, ak si v bežnom farbení hematoxylin-eozín všimneme výraznú eozinofiliu cytoplazmy a ostré bunkové hranice hodnoteného epitelu.

Kľúčové slová: eozinofilná dysplázia – PCR – in situ hybridizácia – CIN – cervikálna intraepiteliálna neoplázia - HPV 6

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The term "eosinophilic dysplasia of the cervix" (ED) was used for the first time by Ma in 2004 (1). It was considered a variant of cervical squamous dysplasia (cervical intraepithelial neoplasia – CIN) presenting itself in „pure“ form or in association with conventional squamous dysplasia (HSIL or LSIL). The analysis of HPV DNA showed the presence of intermediate- and high-risk HPV types in 90 % of analyzed cases (1). Histomorphology (Fig. 1) of the epithelium with eosinophilic dysplasia includes following features: abundant eosinophilic cytoplasm and sharp opaque cell borders; lack of maturation; mild increase in nuclear-cytoplasmic ratio; slight

chromatin clumping; uneven mild nuclear clearing. This morphology is considered to represent intermediate degree of metaplastic differentiation and cytologic atypia. Significant inter-observer variability was apparent by examination of these lesions, with diagnoses ranging from benign reactive changes (10 %) and undetermined atypia to LSIL (CIN I) and HSIL (CIN III) (1). One of the major textbooks of gynecologic pathology - Gynecologic and Obstetric Pathology by Crum (2) - considers this lesion to be a type of immature flat metaplastic LSIL. It comments further on diagnostic difficulty in distinguishing reactive changes from this type of dysplasia presenting spectrum from mild atypia (LSIL) to high-grade dysplasia.

CASE REPORT

41 year old patient underwent conization due to a suspicious colposcopic finding 7 months prior to conization and positive HPV testing using Hybrid capture 2 method (HC2) 6 months before conization. Reviewing patient's history we came across gynecologic screening slides taken 24, 12, 6 and 2 months before conization.

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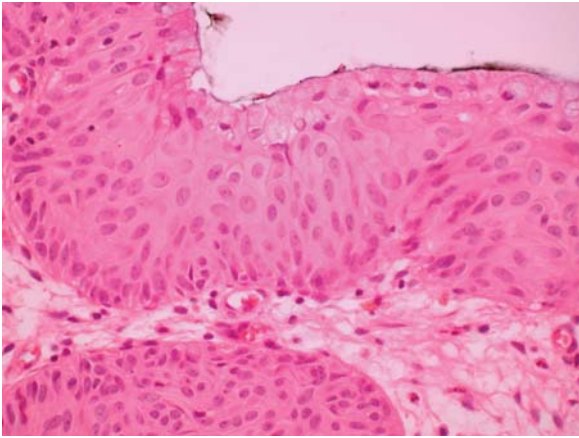


Figure 1. Eosinophilic dysplasia - dysplastic epithelium with predominantly vertical orientation of nuclei and cells, with clearly visible nucleoli and pink sharply contoured cell borders. Mild anisonucleosis is noticeable. Residual superficial endocervical epithelium is also visible. (HE, Original magnification x 400).

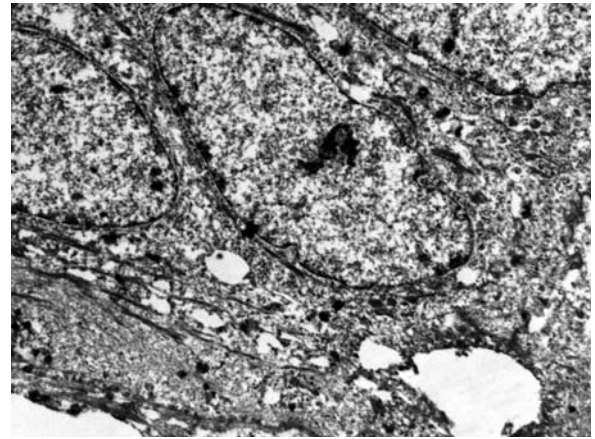


Figure 2. Electronmicroscopic picture of eosinophilic dysplasia with small nuclear membrane crevices of centrally depicted nucleus and partly visible nucleus in the upper right corner of the picture. (Original magnification x 5 600).

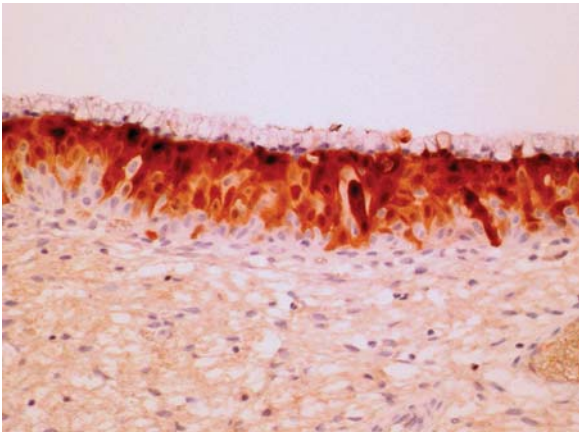


Figure 3. Eosinophilic dysplasia with diffuse strong to intermediate cytoplasmic and nuclear expression of p16 antigen in dysplastic epithelium capped by original cylindrical endocervical cells. Of note is negative basal expression of p16. (Original magnification x 100).

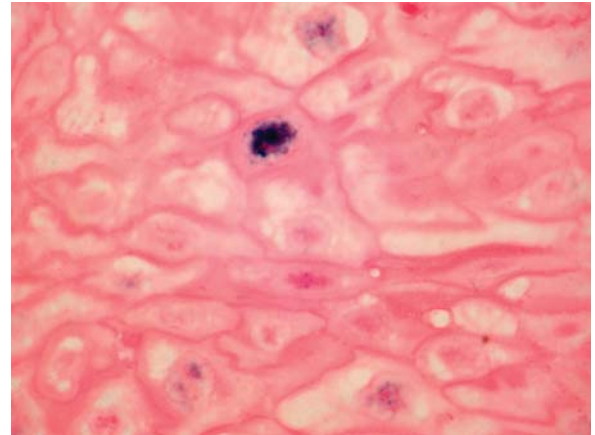


Figure 4. A group of cells within eosinophilic dysplasia of squamous cervical epithelium presenting variable ISH nuclear blue dotted staining pattern of integrated type. (Original magnification x 800).

MATERIAL AND METHODS

Sections from surgical specimen were fixed in 10% buffered formalin, embedded in paraffin and stained with hematoxylin-eosin.

Immunohistochemical studies were performed on representative section of the lesion using the routine avidine-biotin-peroxidase complex technic (BioGenex, San Ramon, CA) along with the appropriate positive and negative controls. We have used Ki-67 (Clone MIB-1, mouse monoclonal, 1:1000, Dako, Denmark) and p16 (clone INK4a, Syntec) for Ventana automated system. HPV detection was performed from liquid based cytology (LBC) material using HC2 during the course of routine gynecologic screening. For the molecular genetic studies, DNA from formalin-fixed, paraffin-embedded tissue was extracted by the Nukleospin Tissue Kit (Macherey Nagel, Amtsergericht Düren, Germany) according to manufacturer's protocol.

The HPV DNA detection was performed using a set of several PCRs with different primers to cover a wide detection range of predominantly high- and low-risk HPV types. The following primer's systems were used: CPSGB (3), GP5+/GP6+ (4) and type specific primers for HPV 16, 18, 31, 33, 35, 45 (5,6). Furthermore, INNO-

LiPA HPV Genotyping kit Extra (Innogenetic NV, Belgium) was run in order to reveal possible multiple HPV type infections. All PCR were run on the cyclor GeneAmp PCR System 9700 (PE/ Applied Biosystem, Forster City, CA). Amplicons were analyzed in 2% agarose gel with ethidiumbromide. Positive PCR samples were genotyped by hybridization to type specific probes, or sequenced and compared to BLAST databases. Positive and negative controls were included in every single run. In situ hybridizations with INFORM HPV II Family 6 Probe targeting types 6 and 11, and with INFORM HPV III Family 16 Probe targeting types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 66 (Roche Molecular Diagnostics, Pleasanton, CA, USA) were performed on Ventana automated system. All available PAP-smears were reviewed.

RESULTS

Transformation zone of the specimen presented in 5 blocks stratified epithelium of squamous metaplastic type with vertical cell orientation in lower layers and prevailing horizontal orientation in the upper third of the epithelium (Fig. 1). Cells were characterized by

abundant eosinophilic cytoplasm, often sharp pink contoured cell borders and mild anisonucleosis with mild chromatin clumping and uneven nuclear clearing, with multiple (1 to 3) clearly visible nucleoli. Original endocervical epithelium was focally found as the most superficial cellular layer. Electronmicroscopic study revealed that some nuclei had smooth oblique nuclear membrane whereas some other presented mild nuclear membrane crevices (Fig. 2). Neighboring small areas of low grade dysplasia with koilocytes were also found. There was diffuse positive expression of antigen p16, focally sparing the basal part of the epithelium (Fig. 3). Further, prevailing basal positive nuclear expression of antigen MIB1 was noticed, focally reaching 2/3 of the thickness of the epithelial layer. In contrast to the published data (1), PCR HPV DNA analysis detected low-risk HPV type 6, which was not documented before. Multiple HPV types infection was ruled out. Tissue in situ hybridization study (ISH) confirmed the presence of HPV 6 in the form of patchy dotted pattern resembling integrated form of HR-HPV infection (Fig. 4). Patients PAP-smears taken 24, 12, 6 and 2 months before conization were reported three times as NILM and lastly as LSIL. Dysplastic cells presented delicate low grade dysplastic changes without any specific feature. Interestingly, HPV detection by HC2 provided positive result 6 months before conization.

DISCUSSION

Eosinophilic dysplasia of the cervix (ED) is recently described entity set apart from the spectrum of unusual and diagnostically difficult cases of regenerative or metaplastic changes in squamous cervical epithelium. Original description (1) puts the lesion into the spectrum of HSIL (CIN II) lesions strongly associated with intermediate- and high-risk HPV types, although simultaneously pointing out histological similarity with HPV 6 and 11 associated lesions. Kitahara et al. (7) have also recently identified five cases of intermediate type of cervical dysplasia calling it "deceiving dysplasia" as-

sociated with non-16, non-18 HPV infection detected by The Invader assay without further HPV typization. Recent edition of authoritative gynecologic pathology textbook (2) connects this lesion with LSIL (CIN I) in the background of immature metaplasia with secondary HPV infection admitting diagnostic difficulties contemplating possible spectrum of lesions LSIL-HSIL. Eye catching similarity of the ED cells to the cells of glassy cell carcinoma noticed by Luevano (8) is of interest, but probably of no clinical relevance as replied by the authors of the original article (9) since there is no report of carcinoma associated with ED so far. Preceding HC2 high-risk HPV testing positivity might be due to the well described but scarcely discussed phenomenon of the high load of low-risk HPV DNA that may cause positive results of this test (10-13). We propose to assign ED to LSIL (CIN I) or HSIL (CIN II and CIN III) category based on the histological appearance of the lesion and the association with low- or high-risk HPV type.

In conclusion, we present a case of rarely mentioned eosinophilic dysplasia of the cervix documenting that it may also be associated with low-risk HPV (i.e. HPV 6) infection, in addition to the previously documented association with high- and intermediate-risk HPV types. Cytologically, this lesion has presented as LSIL in PAP smear. This type of dysplasia is histologically characterized by cells with brightly eosinophilic cytoplasm, contoured by sharp and slightly broader cytoplasmic membrane. This feature might be of a great value to differentiate ED from other cases in the spectrum of metaplastic lesions, thus avoiding pitfalls of diagnosis of a reactive change. Immunohistochemistry of p16 and MIB1 and in particular tissue HPV DNA testing is of a great importance for such cases.

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