THE PROGNOSTIC SIGNIFICANCE OF P16, Ki-67, P63, AND CK17 EXPRESSION DETERMINED BY IMMUNOHISTOCHEMICAL STAINING IN CERVICAL INTRAEPITHELIAL NEOPLASIA 1

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Objective
To evaluate the prognostic significance of p16, Ki-67, p63, and cytokeratin (CK) 17 expression determined by immunohistochemical staining in cervical intraepithelial neoplasia (CIN) 1.

Methods
Biopsy tissue samples from 33 patients diagnosed with CIN 1 were stained immunohistochemically for p16, Ki-67, p63, and CK17. The staining results were correlated with the clinical course of the disease.

Results
Seventeen of 18 (94.4%) p16-negative patients experienced regression, and only 1 patient (5.6%) developed persistent disease. Fifteen of the 16 (93.8%) Ki-67-negative patients experienced regression, and 1 patient (6.3%) developed persistent disease. Negative p16 and Ki-67 expression correlated significantly with disease regression (P=0.004 and P=0.017, respectively). Fourteen of 15 (93.3%) patients negative for both p16 and Ki-67 experienced regression, and 1 patient negative for both p16 and Ki-67 (6.7%) developed persistent disease. The expression levels of p63 and CK17 were not significantly associated with disease regression or persistence (P=0.149 and P=0.642, respectively). Ten of the 13 (76.9%) p16-positive patients had a high-risk HPV infection. High-risk HPV infection was significantly associated with p16 expression (P=0.049).

Conclusion
CIN 1 with p16- or Ki-67-negative immunohistochemical staining was associated with spontaneous disease regression. The p63 and CK17 expression patterns were not related to the behavior of CIN 1.

Keywords: Cervical intraepithelial neoplasia 1, p16, Ki-67, p63, Cytokeratin 17

Cervical intraepithelial neoplasia (CIN) is a precursor to invasive squamous cell carcinoma. CIN is classified as CIN 1, CIN 2, and CIN 3; CIN 2 and CIN 3 have a high progression rate to a higher grade of CIN or invasive squamous cell carcinoma [1]. CIN 2 frequently progresses to CIN 3 or invasive cancer, and CIN 3 to invasive cancer, but the progression rate from CIN 1 to a higher-grade lesion or to cancer is low (e.g., 9% from CIN 1 to CIN 3 and 1% to invasive cancer) [1-3]. The 2006 American Society for Colposcopy and Cervical Pathology guidelines recommend observation for the management of CIN 1 [4]. However, as a few reported cases of CIN 1 have progressed to higher-grade lesions, frequent

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Su Mi Kim, et al. Predicting the prognosis of CIN 1

Cytological follow-up and biopsies are often performed in women with CIN 1. This can lead to overtreatment and unnecessary controls during the follow-up of patients whose CIN does not progress (most cases), creating an inefficient burden for the healthcare system. The ability to predict the behavior of CIN 1 would be valuable in clinical practice because it would allow individualized management of cervical lesions according to the risk of progression.

Recently developed biomarkers may be useful for evaluating the biological potential of early CIN lesions. p16 is a tumor suppressor protein whose main biological function involves regulation of cell cycle progression at the G1/S boundary [5]. The value of p16 as a diagnostic marker for cervical dysplasia and cervical carcinoma has been demonstrated. p63 is a homologue of the tumor suppressor gene p53 and is expressed in the embryonic, adult murine, and human basal squamous epithelium. Previous studies have shown that p63 has potential as a marker for grading CIN. Ki-67 is a nuclear proliferation-associated antigen and a well-known cell proliferation marker. Cytokeratin (CK) is a cytoskeletal intermediate filament protein. The CK isotype depends on the cell type and the localization of CK in the cytoplasm. In this study, we used immunohistochemical staining to study the expression of p16, Ki-67, p63, and CK17, and we investigated the prognostic potential of the staining pattern to predict the progression of CIN 1.

Materials and Methods

1. Patients

CIN 1 patients who underwent a colposcopy-directed punch biopsy at Daejeon St. Mary’s Hospital between 2000 and 2009 were recruited retrospectively and their medical records were reviewed. Eighty-seven patients were enrolled, of whom 54 were excluded for the following reasons: 26 patients were lost to follow-up, 15 patients underwent conization and hysterectomy instead of observation after their punch biopsy, and 13 patients had an insufficient biopsy sample to perform immunohistochemical staining or no CIN 1 tissue when reviewed by the pathologists. We performed immunostaining on the tissue samples from the remaining 33 CIN 1 patients. This study was approved by the hospital’s institutional review board.

We retrieved the samples from the formalin-fixed, paraffin-embedded archives of the Department of Pathology at Daejeon St. Mary’s Hospital. Tests for high-risk human papillomavirus (HPV) infection were performed through vaginal swab at the time of the biopsy.

An oligonucleotide microarray DNA chip (MyGene Inc., Seoul, Korea) or an HPV hybrid capture II kit (Digene/Abbott, Gaithersburg, MD, USA) was used to detect high-risk HPV. Follow-up tests were performed between 6 months and 18 months after the initial punch biopsy. Patients who had an abnormal colposcopic finding received a punch biopsy or conization of the cervix in addition to a Pap smear. We defined disease as persistent if a sample showed CIN 1 or a higher-grade lesion at the follow-up punch biopsy or conization of the cervix.

Spontaneous regression of disease was defined as a normal colposcopic finding and within the normal limits or reactive changes Pap smear. All patients who had CIN 1 or a higher-grade lesion at the follow-up test received conization of the cervix.

2. Immunohistochemical staining

All tissue samples were examined pathologically by 2 experts. Four-micrometer-thick sections were cut from the paraffin blocks, and these sections were mounted on positively charged glass slides for immunohistochemistry. The paraffin sections were deparaffinized, rehydrated, and subjected to antigen retrieval using a vapor lock. The primary antibodies were as follows: p16 (1:100; Dako, Glostrup, Denmark), Ki-67 (1:100; Dako), p63 (1:100; Dako), and CK17 (1:50; Epitomics, Burlingame, CA, USA). Immunostaining for p16, Ki-67, p63, and CK17 was performed.

3. Evaluation of p16, Ki-67, p63, and CK17 expression

The immunoreactivity of p16 and p63 was judged as positive when more than 50% and 10% of the tumor cell nuclei showed a strong intensity, respectively. We considered that the expression of CK17 had been lost when the cells that were reactive for CK17 represented less than 10% of the cells evaluated in a tumor. The Ki-67 proliferative index was defined as the percentage of Ki-67-positive cells in a total of 1,000 dysplastic cells counted. Ki-67 was considered positive when the Ki-67 proliferative index was more than 10%.

4. Statistical analysis

The associations between the CIN 1 behavior and the p16, Ki-67, p63, and CK17 expression levels were evaluated using Fisher’s exact test. Pearson’s chi-square test and Fisher’s exact test were used to analyze the associations between the p16, Ki-67, p63, and CK17 immunohistochemical expression levels and the presence of a high-risk HPV infection. SPSS ver. 12.0 (SPSS Inc., Chicago, IL, USA) was used to analyze the data. A P-value<0.05 was regarded as significant.