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Papillary Immature Metaplasia of the Uterine Cervix: a Report of 5 Cases with an Emphasis on the Differential Diagnosis from Reactive Squamous Metaplasia, High-Grade Squamous Intraepithelial Lesion and Papillary Squamous Cell Carcinoma

Papillary immature metaplasia (PIM) is a distinctive exophytic lesion of the uterine cervix and shares some histologic and cytologic features with ordinary squamous metaplasia (SM), atypical immature squamous metaplasia (AIM), high-grade squamous intraepithelial neoplasia (HSIL) and papillary squamous cell carcinoma (PSC). PIM has been suggested to be a subset of condyloma associated with low-risk type human papilloma virus (HPV), however, the etiologic role of HPV and biologic behavior of the disease are still elusive. We compared the clinical and histopathological findings, immunohistochemical expression of Ki-67 and p53 protein, and HPV typing of 5 cases of PIM with SM (n=9), HSIL (n=6), and PSC (n=4) to know the helpful features for the differential diagnosis. Histologically, all 5 cases showed a papillary proliferation of immature metaplastic cells involving the proximal transformation zone and endocervix. On HPV typing by polymerase chain reaction-restriction fragment length polymorphism, 2 out of 5 PIM were confirmed to have HPV 6 or HPV 11, while 2 out of 4 PSC were proved having HPV 31 and HPV 16 each. Ki-67 labeling index and mitotic index of PIM were significantly lower than those of HSIL or PSC. There were no significant differences of Ki-67 labeling index and mitotic index between PIM and SM. The expression of p53 varied among the groups and thus it was not helpful for the differential diagnosis.

Key Words : *Metaplasia; Cervix Neoplasms; Carcinoma, Papillary; Papillomavirus; Ki-67 Antigen; Protein p53*

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INTRODUCTION

Papillary immature metaplasia (PIM) has been introduced in 1992 as a subset of exophytic cervical precursor lesions (1) and suggested to have a common etiology with condylomata (1, 2). Because the lesion shows filiform papillae, variable cytological atypia, and rare koilocytotic atypia, it can be confused with various benign or malignant epithelial lesions of the uterine cervix, such as ordinary squamous metaplasia (SM), condyloma acuminatum, papillary squamous cell carcinoma (PSC), or high-grade squamous intraepithelial lesions (HSIL), depending on the cytoarchitectural severity. However, because of the rarity of the documented cases, the histopathological characteristics and the clinical behavior of the lesion are not well recognized to the pathologists. Herein, we describe clinical and histopathological findings, the immunohistochemical expression of Ki-67 and p53 protein, human papilloma virus (HPV) status in 5 cases of PIM of the uterine cervix and compared the Ki-67 and p53 expressions with those of SM (n=9), HSIL (n=6), and PSC (n=4) to clarify the clinicopathologic characteristics and to know the helpful histopathologic features for the differential diagnosis, if any.

MATERIALS AND METHODS

Clinicopathologic Analysis

Five cases of PIM were obtained from the consultation files and recent archives of the Department of Diagnostic Pathology, Asan medical Center, University of Ulsan College of Medicine, Seoul, Korea. In all cases, clinical histories, hematoxylin and eosin (H&E)-stained slides, and paraffin blocks were available. Previous cervicovaginal smears were reviewed in 4 cases. Clinical data including age, obstetric and gynecological histories, initial presentation, treatment method,

and follow-up results were obtained from the clinical records. Additional groups composed of typical cases of PSC (n=4), SM (n=9), and HSIL (n=6) of the uterine cervix were randomly selected for the comparison of histologic parameters, such as mitotic index (MI), cytologic atypia, and immunohistochemical findings for Ki-67 and p53 expression. In 2 cases, additional HPV DNA screenings were performed using cervicovaginal smears by HPV hybrid capture method.

Immunohistochemical Staining for Ki-67 Protein and p53 Protein

Immunohistochemical staining to evaluate Ki-67 indices and p53 expression was performed on formalin-fixed, paraffin-embedded tissue sections, using the labeled streptavidin-biotin (LSAB) method. For antigen retrieval, sections were boiled for 1 hr in pH 7.0 citric acid buffer using a steam cooker for 1 hr. The primary antibodies used were rabbit anti-human Ki-67 antigen (1:100, Dako, Carpinteria, U.S.A.) and mouse anti-human p53 antigen (1:1,600, Dako, Carpinteria, U.S.A.). Diaminobenzidine was used as chromogen. The Ki-67 labeling indices and p53 expression were presented with percentages of positively stained nuclei out of 300 nuclei. The counting of the number of positively labeled nuclei was assisted by positioning a 10×10 square grid in the microscopic eyepiece that encompasses 0.0625 mm^2 area and the number was counted in 10 such fields under a $\times 400$ magnification that include the highest mitotic activity. The Ki-67 index and p53 expression were presented as a mean number of positively stained cells under $\times 400$ magnification fields.

HPV Typing by Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP)

Five cases of PIM and 4 PSC were analyzed for HPV nucleic acids using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) with extracted archival DNA as previously described (3). Five consecutive 10-μm thick, formalin-fixed, paraffin sections were dewaxed in xylene followed by ethanol and then air-dried. Tissues were digested in lysis buffer (10 mM Tris, pH 8.5; 10 mM EDTA; 0.5% SDS; 100 mM NaCl) with proteinase K (500 μg/mL, Boehringer Mannheim, Germany). DNA was extracted with phenol-chloroform and precipitated with ethanol. PCR was performed at 94°C for 5 min followed by 30 cycles of 94°C for 30 sec, 55°C for 2 min and 72°C for 2 min with a final extension for 10 min at 72°C The reaction mixture was in a volume of 25 μL containing 1 μg of extracted DNA, 10 pmol of primers, 0.2 mM of dNTP, and 1 unit of *Taq* polymerase (Takara, Japan) in $1 \times$ PCR buffer (10 mM Tris, pH 8.3; 50 mM KCl; 1.5 mM $MgCl_2$). Sequences of PCR primers, HPV pU-1M, pU-2R and pU-31B, were the same as previously described (3). β-Globin was amplified for the internal control and primers were as follows: 5′, GAAGAGCCAAGGACAGGTAC; 3′, CAACTTCATCCACGTTCACC. The PCR products in the range of 228 to 268 bp were analyzed on a 2.5% agarose gel, stained with ethidium bromide, and visualized by UV illumination. The HPV-positive samples were subjected to re-PCR in a 100 μL reaction volume. The PCR products were extracted with phenol-chloroform, precipitated with ethanol, then air-dried, dissolved in 10 μL of distilled water and digested with the restriction enzyme set (Takara, Japan) for the HPV typing according to the manufacturer's instructions. Digested PCR products were analyzed on an 8% polyacrylamide gel with a size marker to identify the type of HPV.

Statistical analysis

Values of Ki-67 labeling index, p53 expression, and mitotic index were expressed as mean±standard deviation. To test for equality of variance, Levene's test was employed. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 9.0 (SPSS, Inc., Chicago, IL, U.S.A.). T-test and ANOVA tests were used to test for significant differences between the groups. A difference was considered significant if $p \leq 0.05$.

RESULTS

Clinicopathologic Features

Ages of the patients ranged from 22 to 38 yr (mean, 32) and obstetric histories were variable from nulligravida to multigravida. In all 5 patients, the lesion was incidentally discovered without clinical symptoms. In 2 patients, abnormal cells were discovered in the routine pap smears and the remaining 3 patients were referred due to abnormal cervicographic findings showing exophytic masses on the endocervical mucosa, which raised a suspicion of invasive carcinoma. Cytologic diagnoses before the biopsy or conization were reported as normal in 2 cases, atypical squamous cells of undetermined significance (ASCUS) in 1, and HSIL in 2 cases. PIM was associated with HSIL in the adjacent mucosa in one patient, who showed ASCUS on pap smear.

Histologically, PIM involved endocervical mucosa along with transformation zone in all 5 cases. Exophytic masses were composed of filiform papillae reminiscent of condyloma acuminatum, but lined by thick proliferating immature metaplastic cells (Fig. 1), on top of which mucinous columnar cells were retained (Fig. 1B). Core of the papillae was narrow and short and not as prominent as in PSC. Polarity of the cell layers was lost from the basal to the superficial layer, but cytologic atypia was not as severe as in HSIL. The cells had minimal to mild nuclear hyperchromasia, minimal irregularity of nuclear membrane, occasional binucleation,

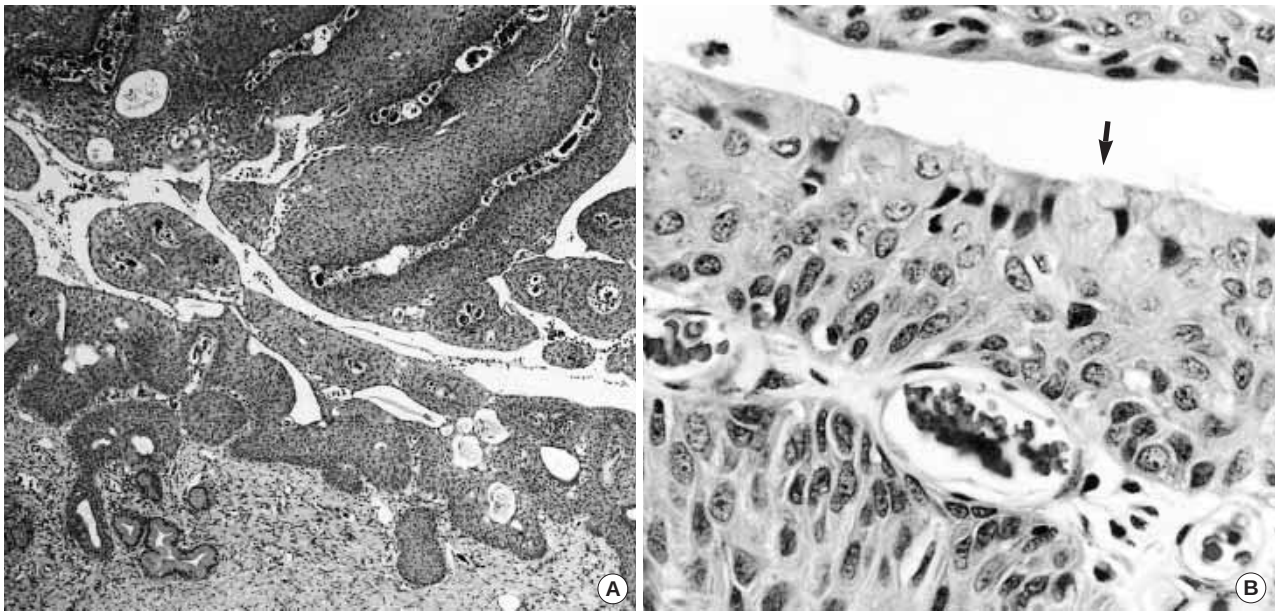

Fig. 1. Papillary immature metaplasia shows characteristic filiform papillae with an involvement of endocervix (A, H&E, ×40). Immature squamous epithelium retains the mucous cells (arrow) on the surface of the papillae (B, H&E, ×200).

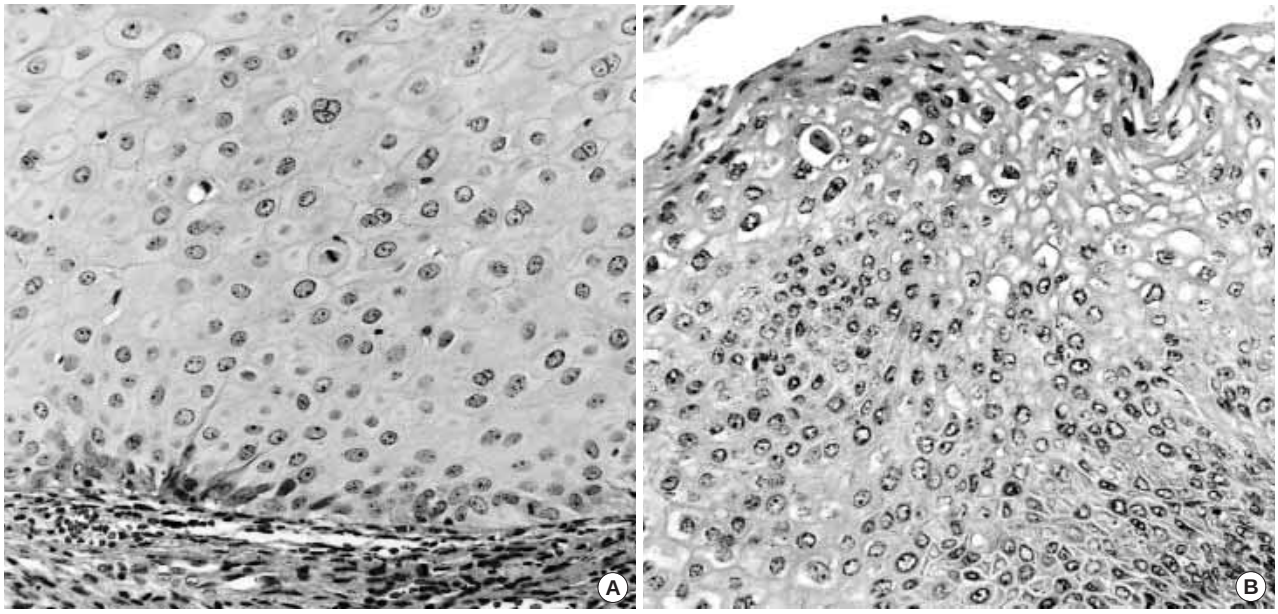

Fig. 2. Papillary immature metaplasia. Epithelial cells show mild nulcear atypia, infrequent mitotic figures, and occasional binucleation (A, H&E, ×100). Koilocytotic atypia is more frequently identified in the cases that were associated with HPV 6 or HPV 11 than in the remainder (B, H&E, ×100).

and rare koilocytotic atypia (Fig. 2A). Binucleation and koilocytoic atypia were more frequently identified in the cases that were associated with HPV 6 or HPV 11 than in the remainders (Fig. 2B). MI ranged from 0 to 15/10 high power field (HPF) (mean 11.2±9.2) in PIM, and the mitotic figures were mostly identified in the lower portion, but abnormal ones were not observed. Papillary endocervicitis was present in the adjacent endocervical mucosa in all cases.

In contrast, PSC showed a narrower and longer fibrovascular cores with more diffuse and marked cytologic atypia and pleomorphism (Fig. 3). MI was 22±4.7 and there was a statistically significant difference between PIM and PSC or HSIL. Abnormal mitotic figures were frequent. Mucous cells were not observed on the surface of the epithelium.

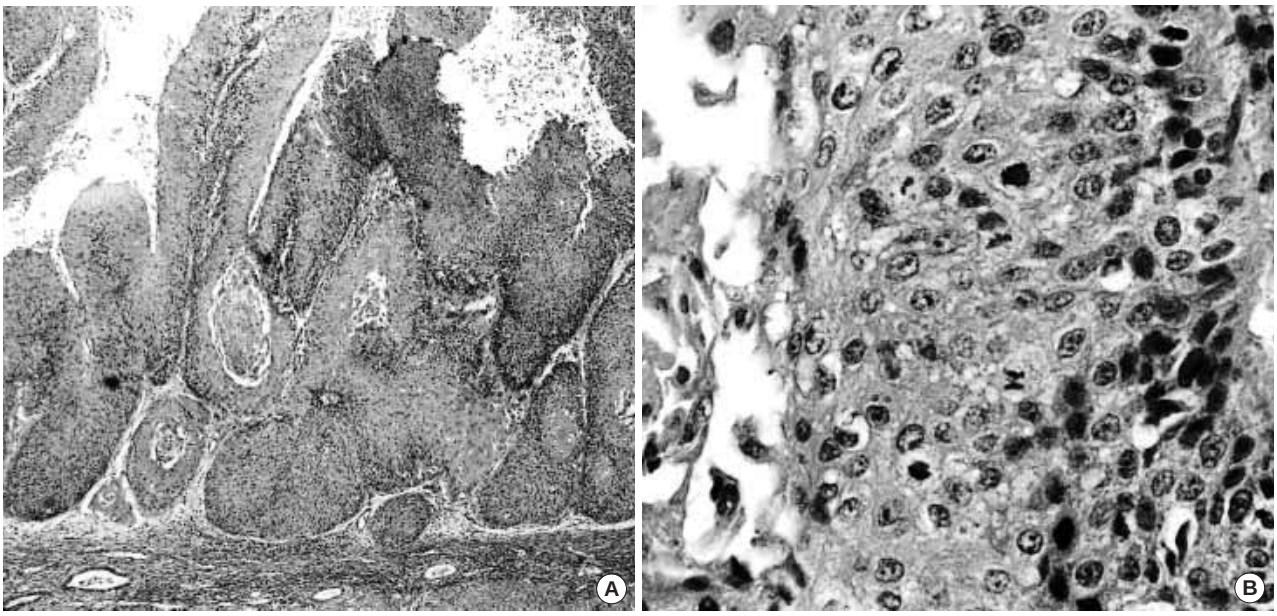

Fig. 3. Papillary squamous cell carcinoma (case 9). Papillae are more confluent with narrow and long fibrovascular cores. Keratin pearl formation are frequently observed (A, H&E, ×40). Under a higher magnification, the epithelium shows marked hypercellularity, nuclear irregularity, pleomorphism, and frequent mitotic figures (B, H&E, ×200).

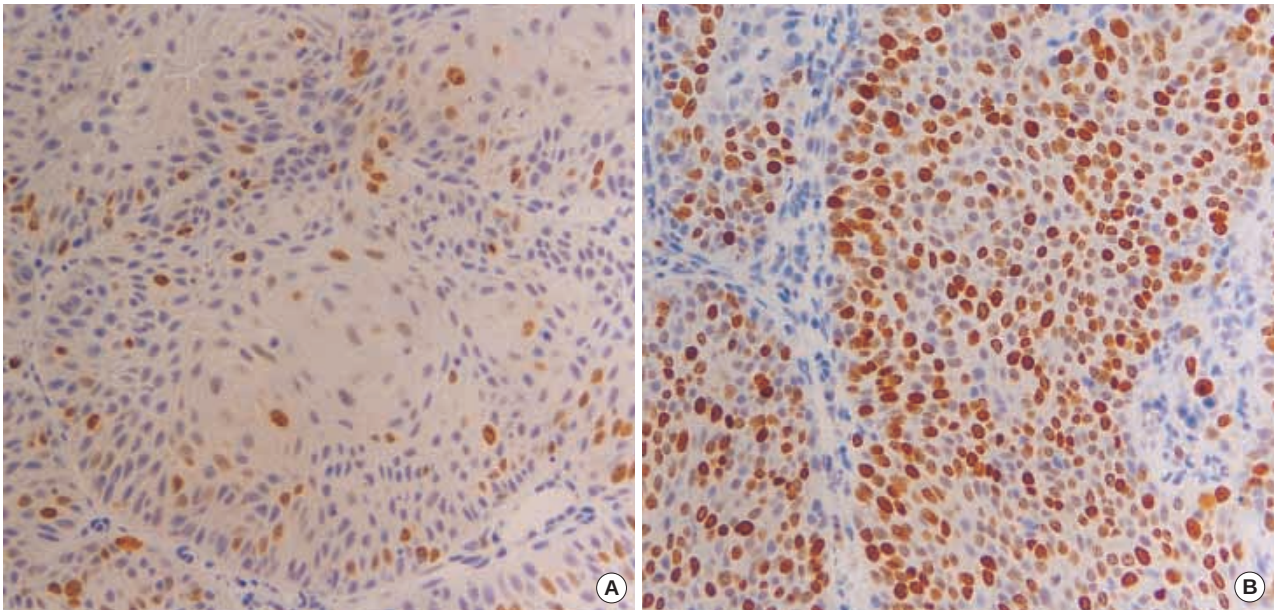

Fig. 4. Ki-67 labeling index. In papillary immature metaplasia (A, immunohistochemical stain, ×100), positive cells are extended to the upper layers, whereas positive immunoreactivity is observed throughout the epithelium in papillary squamous cell carcinoma (B, immunohistochemical stain, ×100).

Ki-67 and p53 Protein Expression

In the normal cervix, Ki-67 immunoreactivity was mostly seen in the parabasal zone, whereas PIM showed scattered immunoreactivity extending to the upper part of the epithelium (Fig. 4A). In PSC and HSIL, there were numerous immunoreactive nuclei throughout the entire epithelium (Fig. 4B). Ki-67 labeling indices were 11.2%±9.2 in PIM, 14.6%±8.6 in SM, 61.48%±22.7 in HSIL, and 52.8±24.2 in PSC (Table 2). There were significant differences of Ki-67 labeling indices between the PIM and HSIL (p=0.035) and between the PIM and PSC (p=0.005). p53 expression in each

Table 1. Clinical features of papillary immature metaplasia of the uterine cervix

Case	Age (yr)	Initial presentation	Cytologic diagnosis	Diagnosis	HPV typing*	HPV typing†	Treatment
1	38	abnl pap	ASCUS	PIM, HSIL	HPV 6	HPV 18	conization
2	36	abnl cervicography	Normal	PIM	neg	ND	conization
3	38	abnl pap	HISL	PIM	neg	ND	conization
4	22	abnl cervicography	HSIL	PIM	neg	low and high risk type‡	conization
5	30	abnl cervicography	Normal	PIM	HPV 11	ND	conization

abnl: abnormal, ASCUS: atypical squamous cells of undetermined significance, HSIL: high-grade squamous intraepithelial lesion, PIM: papillary immature metaplasia, HPV: human papilloma virus, HPV typing*: HPV typing using tissue sections, HPV typing†: HPV typing using cervicovaginal smear, neg: negative, ND: not done. ‡: typing was not performed

Table 2. Ki-67 labeling index, p53 expression, and mitotic index in various cervical lesions

	Ki-67 labeling index	P53 expression	MI
PIM	11.2±9.2	18.6±21.7	4.4±2.6
SM	14.6± 8.6	5.4±5.4	5±5.1
PSC	52.8±24.2	10.0±10.9	22±4.7
HSIL	61.48±22.7	1.5±1.5	16.8±4.1

LI: Labeling index expressed as a percentage of positively stained nuclei. MI: Mitotic index representing the number of mitoses per 0.625 mm^2 area (ten square grid areas under ×400 magnification field). PIM: papillary immature metaplasia. SM: ordinary squamous metaplasia. PSC: papillary squamous cell carcinoma. HSIL: high grade squamous intraepithelial lesion

group varied widely and there was no significant difference among the groups.

HPV DNA Analysis

The PCR was performed under the conditions previously described. PCR with HPV pU-31B/HPVpU-2R primers yielded 228 bp PCR products in 2 out of 5 cases of PIM. Typing of the products was analyzed by restriction enzyme digestion with *Afa* I, *Bgl* II, *Ava* I, *Ava* II, and *Acc* I (Fig. 5). Two cases with 228 bp PCR products each contained an *Afa* I site, but gave different digestion patterns. The digestion patterns by the size of the DNA fragments were in agreement with HPV 6 and HPV 11, respectively. PCR with pU-1M/pU-2R primers yielded approximately 232 bp and 238 bp PCR products in 2 out of 4 PSC. Restriction enzyme digestion showed an *Afa* I and *Ava* II site, respectively, and the digestion patterns and the size of the DNA fragments were in agreement with HPV 31 and HPV 16, respectively. In one case, foci of HSIL coexisted in the adjacent mucosa of PIM, however, HPV typing could not be performed separately in the foci of HSIL because of the insufficient tissue.

Follow-Up Results

All 5 patients who had PIM were treated with conization and the resection margins were clear. Follow-up periods after conization ranged from 6 to 18 months (mean, 11 months) and cervicovaginal smears were normal in all 5 cases. Exophytic mass or evidence of recurrence was not identified in cervicography that were performed in 2 cases.

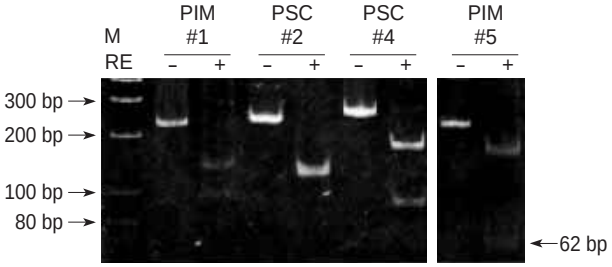

Fig. 5. Detection and typing of HPV DNA by PCR-RFLP. PIM#1 (RE-) and PIM#5 (RE-) each show 228 bp products by PCR with pU-3B/HPVpU-2R primers, but gave different digestion patterns by *Afa* I; 132/96 bp length fragments for HPV 6 and 166/62 bp fragments for HPV 11. PSC#2 (RE-) and PSC#4 (RE-) show 232 and 238 bp products, respectively, by PCR with pU-1M/pU-2R primers. Restriction enzyme digestion yielded 117/115 bp length fragments by *Afa* I and 157/81 bp fragments by *Ava* II, which correspond to HPV 31 and 16, respectively.
M: size marker, RE: restriction enzyme, PIM: papillary immature metaplasia, PSC: papillary squamous carcinoma, #: case number.

DISCUSSION

PIM is a recently described lesion of the uterine cervix that involves the proximal part of the transformation zone and endocervix (1, 2, 4). Histologically, it is characterized by slender filiform papillae, proliferation of immature metaplastic cells with mild to moderate variation of nuclear size, mild nuclear atypia, uniformly distributed chromatin, low MI, rare koilocytotic atypia, and a tendency to extend into the endocervical canal (1, 2, 5, 6). It's papillary structure with the cytologic features may cause a diagnostic confusion with reactive squamous metaplasia, low or high-grade precursor lesions, and carcinomas (5). In SM, nuclear atypia with prominent chromocenter and papillary proliferations of PIM are

usually not identified. For the differential diagnosis from condyloma acuminatum, the frequent involvement of endocervix and preservation of mucinous columnar cells on the superficial epithelium are the helpful features. Viral cytopathic effect or koilocytosis is less frequently identified in PIM than in condyloma acuminatum. At the periphery of the PIM, there were nonpapillary or endophytic epithelial proliferation composed of immature squamous epithelium that resembled atypical immature metaplasia (AIM). AIM is a heterogenous, but yet poorly characterized nonpapillary cervical lesion with uncertain biological and clinical significance. HPV-negativity or the association with HPV of intermediate- or high-risk type has been described (7, 8). There is a histological and cytological overlapping, except papillary configuration, between AIM and PIM, such as infrequent koilocytosis, lack of maturation in the metaplastic squamous epithelium, paucity of mitotic figures, lack of coarsely clumped chromatin or nuclear membrane irregularities, and frequent involvement of endocervix. Therefore, it was thought that at least some of the AIM might share a common etiology and histological changes with PIM. Differentiation from PSC is rarely difficult, but some cases of PSC showing mild nuclear atypia and rare mitotic figures may cause a diagnostic problem. The features suggesting PSC are high cellularity with marked nuclear crowding, inconspicuous cell border, frequent karyorrhexis, focal confluence of papillae, anisokaryosis with enlarged nuclei, dyskeratosis, keratin pearl formation, and high MI. Both lesions may have nucleoli, but those in PSC are not as small and inconspicuous as in PIM (2). In our study, mitotic index was a helpful and objective differential parameter. Likewise, PIM can be differentiated from HSIL with its lack of hyperchromatism, pleomorphism, nuclear crowding, abnormal mitotic figures, and the presence of filiform papillae.

PIM has been reported to be associated with benign type HPV, such as type 6/11, in 70-83% (1, 2, 6). In our study, low-risk type HPV DNA was detected in only 2 cases (40 %) of PIM, and high-risk HPV was not detected in any case. Weak or negative HPV DNA might be attributed to the location of the PIM, since most of the reported cases of PIM involved the proximal portion of the transformation zone or endocervix, where replication and viral assembly were reduced in the immature epithelium (2). It might also be explained as a result of an old infection in which HPV no longer replicates in the host cells.

Although it is difficult to correlate the degree of cytologic atypia, such as binucleation, increased mitosis, or hyperchromasia, with presence or absence of HPV association, HPV usually produces cytologic atypia and increased proliferative activity (9). Three cases in our study that were not associated with HPV DNA showed only minimal cytologic atypia compared to HPV-associated PIM. HPV in the immature metaplastic cells or in the reserve cells of the endocervix, such as in PIM or AIM, is likely to produce less conspicuous cytologic atypia in contrast to HPV in mature squamous epithelium (1, 2, 6, 7, 10, 11).

Although viral data in some literatures indicate that PIM is a variant of low-grade HPV-associated lesion (2), biologic and clinical significances are still unclear. HPV type might depend on the associated high-grade precursor lesion in the adjacent mucosa. The cases that are associated with a high-grade precursor lesion in the adjacent mucosa may result in having high-risk type HPV or both low- and high-risk type HPV, if cytologic smears are used for HPV typing. However, with use of tissue section that includes only PIM, but not HSIL lesion of the adjacent mucosa, low-risk HPV DNA can be identified in the PIM lesion. Three out of 5 in our cases were negative for HPV DNA, but 1 of them had been detected as having both low- and high-risk HPV in the cervical smear before the biopsy was taken.

Ki-67 labeling index reflected the difference in the growth rate between PIM and PSC more obviously, and it was helpful for the differential diagnosis. Ki-67 immunoreactivity was only seen in the parabasal zone in the normal cervix and it is increasingly observed with the increasing grade of the squamous intraepithelial lesions (11). In PSC and high grade cervical intraepithelial neoplasm (CIN), there were numerous immunoreactive nuclei scattered throughout the entire epithelium. In PIM, Ki-67 labeling index has been reported variable but usually as comparable or slightly higher than that of low grade squamous intraepithelial lesions (1, 10) as was shown in the present study.

On the other hand, p53 expression was highly variable among reactive lesions, high-grade precursor lesion, and malignant tumors and thus was not helpful in the differential diagnosis. Recently, it has been proposed that HPV infection has a pathogenetic pathway for vulvar squamous lesion different from the alteration of *p53* gene (12). Our results showing a marked variation of p53 expression may suggest that the alteration of *p53* gene does not play an important role in the pathogenesis of PIM. In conclusion, PIM is presumably a low-grade intraepithelial lesion of the cervix associated with low-risk HPV, which clinically and histologically mimics various low-grade or high-grade precursor lesions and PSC. The histologic features including Ki-67 labeling index and mitotic figures are helpful not to overdiagnose PIM as PSC or HSIL, however, their biologic significance remains to be carefully monitored.

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