



Cytopathologic features of human papillomavirus-independent, gastric-type endocervical adenocarcinoma

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Background: Gastric-type endocervical adenocarcinoma (GEA) is unrelated to human papillomavirus (HPV) infection and is clinically aggressive compared with HPV-associated usual-type endocervical adenocarcinoma (UEA). The cytological diagnosis falls short of a definitive diagnosis of GEA and is often categorized as atypical glandular cells (AGCs). To improve cytologic recognition, cytological findings of HPV-independent GEA were analyzed and the results compared with HPV-associated UEA. **Methods:** Cervical Papanicolaou (Pap) smears from eight patients with a histopathologic diagnosis of GEA and 12 control cases of UEA were reviewed. All slides were conventionally prepared and/or liquid-based prepared (ThinPrep) and stained following the Pap method. A mucinous background, architectural, nuclear, and cytoplasmic features were analyzed and compared with UEA. **Results:** Preoperative cytologic diagnoses of the eight GEA cases were AGCs, favor neoplastic in three cases, adenocarcinoma in situ in one case, and adenocarcinoma in four cases. Cytologically, monolayered honeycomb-like sheets ($p = .002$) of atypical endocervical cells with vacuolar granular cytoplasm ($p = .001$) were extensive in GEA, and three-dimensional clusters ($p = .010$) were extensive in UEA. Although the differences were not statistically significant, background mucin ($p = .058$), vesicular nuclei ($p = .057$), and golden-brown intracytoplasmic mucin ($p = .089$) were also discriminatory findings for GEA versus UEA. **Conclusions:** Although GEA is difficult to diagnose on cytologic screening, GEA can be recognized based on cytologic features of monolayered honeycomb sheets of atypical endocervical cells with abundant vacuolar cytoplasm and some golden-brown intracytoplasmic mucin. UEA cases are characterized by three-dimensional clusters.

Key Words: Cervix uteri; Gastric type adenocarcinoma; Cytology; Papanicolaou smear

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Gastric-type endocervical adenocarcinoma (GEA) is a variant of endocervical adenocarcinoma showing gastric differentiation unrelated to high-risk human papillomavirus (HPV) infection [1]. Morphologically, GEA ranges from extremely well differentiated adenocarcinoma (previously termed “minimal deviation adenocarcinoma”, MDA), with a deep haphazard claw-like gland distribution and limited desmoplasia, to poorly differentiated glands, clusters, and single cells [1].

GEA is defined as a mucinous carcinoma showing clear and/or pale eosinophilic voluminous cytoplasm with distinct cell borders [2,3]. The cytoplasm contains neutral mucins that stain pale pinkish-red on Alcian blue/periodic acid-Schiff (PAS) special staining (in contrast to the dark purple of acid mucins of the normal endocervix) [2,4]. The immunohistochemical gastric (pyloric) markers, HIK1083 and MUC6, are frequently positive [5].

Compared with the HPV-associated usual-type endocervical

adenocarcinoma (UEA), GEA has a higher prevalence of destructive invasion, extrauterine spread, and advanced stage at presentation. Patients with GEA had a significantly decreased 5-year disease-specific survival rate compared with the non-gastric type (30% vs. 77%; $p < .001$), and the gastric-type morphology was associated with a significant risk of disease recurrence ($p = .001$) [2].

Cervical cytology has been less effective in preventing adenocarcinoma than in preventing squamous cell carcinoma, although screening detects adenocarcinoma earlier than diagnosis in the absence of screening [6]. Early diagnosis of GEA is difficult due to problematic challenging cytologic recognition and limited utility of HPV testing.

Increased awareness of this aggressive tumor entity is crucial due to difficulties in initial diagnosis of GEA compared with HPV-associated UEA. Therefore, to improve the cytologic recognition of GEA, the cytological findings of both HPV-indepen-

dent GEA and HPV-associated UEA were analyzed in the present study.

MATERIALS AND METHODS

Case selection and HPV testing

After obtaining Institutional Review Board approval (CNUH-IRB 2021-12-001) of the Chungnam National University Hospital (CNUH) (Daejeon, Republic of Korea), the pathology database was searched for GEA and UEA diagnoses in cytological and/or surgical pathology specimens at CNUH from July 2016 to June 2021. Clinicopathologic findings and follow-up results were obtained from the electronic medical records (Table 1).

A total of 10 GEA cases were included in the study (Table 2). Diagnosis of GEA was based on the morphologic criteria of GEA

[1,2] (Fig. 1A-F) and negative HPV test results. For comparison, 12 control cases of UEA from the same pathology file were also reviewed. These cases were histopathologically diagnosed based on the morphologic criteria of UEA (Fig. 2A), a positive high-risk HPV test, and block-type positivity for p16 (Fig. 2B). Immunostained slides were retrieved from the pathology archives and the authors reviewed the slides or pathology reports.

A total of 19 patients (9 GEA and 10 UEA) had HPV based on PANA RealTyper HPV kit (PANA RealTyper, PANAGENE, Daejeon, Korea) results. The remaining one GEA case and two UEA cases had in situ hybridization test results for high-risk HPV using paraffin blocks.

Cytologic examination

Among 10 GEA patients, two patients (cases Nos. 6 and 10) had no Papanicolaou (Pap) test results. Pathologists with expertise in cytopathology and gynecologic pathology (M.K.Y., G.E.B., and K.S.S.) reviewed preoperative cervical smears from the eight GEA patients and 12 UEA patients. All cytologic slides were prepared using the conventional method and/or the liquid-based preparation (LBP; ThinPrep Pap test, Hologic, Bedford, MA, USA), and Pap staining was performed. Both conventional smear and LBP were performed in five GEA cases and nine UEA cases. Only LBP was performed in three GEA cases and one UEA case. Only a conventional smear was performed in two UEA cases. All cytologic results were reported according to The Bethesda System terminology [7].

Among cytologic features, a mucinous background, architectural (monolayered honeycomb-like sheets, 3-dimensional clusters, picket fence-like feathering), nuclear (vesicular nuclei, hyperchromasia, prominent nucleoli, grooves, intranuclear cytoplasmic pseudoinclusions [INCI]), and cytoplasmic features (finely vacuolated cytoplasm, cytoplasmic golden-brown mucin, intracytoplasmic neutrophils) were analyzed.

Cytologic findings of the 20 cases (eight GEA and 12 UEA) were scored. A mucinous background and architectural findings were scored as absent, focal (rare or mild degree; 1–2 fields, × 100/slide) or extensive (moderate to high degree; > 3 fields, × 100/slide). Nuclear and cytoplasmic findings were scored as absent, focal (rare or mild degree; < 30% of tumor cells/slide) or extensive (moderate to high degree; > 30% of tumor cells/slide). Cytologic scores were analyzed.

Statistical analysis

All statistical analyses were performed using Statistical Package for Social Sciences Statistics for Windows (SPSS ver. 26.0,

Table 1. Clinicopathologic findings of GEA and UEA

Clinicopathologic findings	GEA (n=10)	UEA (n=12)
Age (yr)		
Mean ± SD	61.7 ± 11.9	45.3 ± 10.9
Range	48–80	31–68
Type of surgical treatment		
Radical hysterectomy	7 (70.0)	8 (66.7)
Total hysterectomy	0	3 (25.0)
Trachelectomy	1 (10.0)	0
Conization	2 (20.0)	1 (8.3)
FIGO stage		
I	1 (10.0)	9 (75.0)
II	4 (40.0)	1 (8.3)
III	3 (30.0)	2 (16.7)
IV	2 (20.0)	0
Human papillomavirus status		
High-risk HPV	0	12 (100)
Not detected	10 (100)	0
p16 expression		
Block positive	1 (10.0)	12 (100)
Patchy positive/negative	9 (90.0)	0
p53 expression		
Diffuse strong positive	5 (50.0)	0
Complete loss (null)	1 (10.0)	0
Patchy positive	4 (40.0)	12 (100)
Follow-up results		
No evidence of disease	2 (20.0)	9 (75.0)
Alive with disease	3 (30.0)	0 (0.0)
Died of disease	4 (40.0)	0 (0.0)
Died of other disease	0	1 ^a (8.3)
Not available	1 (10.0)	2 (16.7)

Values are presented as number (%) unless otherwise indicated.

GEA, gastric-type endocervical adenocarcinoma; UEA, usual-type endocervical adenocarcinoma; SD, standard deviation; FIGO, International Federation of Gynecology and Obstetrics; HPV, human papillomavirus.

^aDied of stomach cancer.

Table 2. Clinical and immunohistochemical findings of 10 cases of gastric-type endocervical adenocarcinoma

Case No.	Age (yr)	Preparation type		Cytologic diagnosis	Type of surgery	FIGO stage	HPV	p16	p53	MUC-6	Adjuvant treatment	Follow-up (mo)	Outcome
		CS	LBP										
1	48	+	+	AIS	Trachelectomy	IVA	-	-	+	+	CC	20	DOD
2	80	+	+	Adenocarcinoma	RH	IIB	-	-	Wild	- ^a	RT	38	DOD
3	52	+	+	AGC	RH	IIIC1	-	-	Wild	+	CCRT	48	AWD
4	58	+	+	Adenocarcinoma	RH	IVA	- ^b	-	+	+	CC	23	DOD
5	54	+	+	Adenocarcinoma	RH	IIA1	-	+	+	+	NA	FU loss	NA
6	54	NA	NA	NA	Conization	IIIC1	-	-	Wild	+	CCRT	17	DOD
7	78	NA	+	Adenocarcinoma	Conization	IIA2	-	-	+	- ^a	RT	21	NED
8	63	NA	+	AGC	RH	IB1	-	-	Null	+	CCRT	21	NED
9	76	NA	+	AGC	RH	IIB	-	-	+	+	RT	10	AWD
10	54	NA	NA	NA	RH	IIIC1	-	-	Wild	+	CCRT	10	AWD

CS, conventional smear; LBP, liquid-based preparation; FIGO, International Federation of Gynecology and Obstetrics; HPV, human papillomavirus; AIS, adenocarcinoma in situ; CC, combined chemotherapy; DOD, died of disease; RH, radical hysterectomy; RT, radiotherapy; AGC, atypical glandular cells, favor neoplastic; CCRT, combined chemotherapy and radiotherapy; AWD, alive with disease; NA, not available; FU, follow-up; NED, no evidence of disease.

^aPale pinkish-red cytoplasmic neutral mucin on Alcian blue/PAS special staining; ^bHigh risk HPV DNA in situ hybridization; +, p16, block-type positivity; +, p53, overexpression.

IBM Corp., Armonk, NY, USA) software. Groups were compared using Pearson's χ^2 test for categorical variables and $p < .05$ were considered statistically significant.

RESULTS

Clinical features

The mean age of the 10 GEA patients and 12 UEA patients was 61.7 ± 11.9 years and 45.3 ± 10.9 years, respectively ($p = .003$). Among the 10 patients with histologically diagnosed GEA, a radial hysterectomy was performed in seven, a trachelectomy in one, and conization in two patients. Among the 12 UEA patients, eight (8/12, 66.7%) underwent radical hysterectomy, three (3/12, 25.0%) total hysterectomy, and one (1/12, 8.3%) conization. Based on Revised International Federation of Gynecology and Obstetrics 2018 staging [8], nine GEA patients (9/10, 90.0%) were diagnosed with stage II or higher and nine UEA patients (9/12, 75.0%) with stage I tumor. Follow-up information was available in 9 GEA and 10 UEA patients with follow-up times ranging from 10–45 months (mean, 22.3 months) and 15–49 months (mean, 24.9 months), respectively. One GEA patient and two UEA patients were lost to follow-up. The three GEA patients who underwent radical hysterectomy and 1 GEA patient who had conization received chemoradiation therapy. Four GEA patients died of the disease 17–38 months post-operatively. Nine UEA patients (9/12, 75.0%) had no evidence of disease and 1 UEA patient died of stomach cancer 11 months after conization (Tables 1, 2).

HPV testing

HPV tests were performed on cytologic or surgical specimens from patients. Among 10 GEA patients, high-risk HPV was not detected in nine subjects. High-risk HPV DNA detection based on in situ hybridization was negative in one patient (case No. 4). Among 12 UEA cases, HPV 18 was positive in nine patients and HPV 16 in one patient. High-risk HPV DNA was detected based on in situ hybridization using a paraffin block in the remaining two UEA cases (Tables 1, 2). Detection of high-risk HPV based on in situ hybridization showed punctate nuclear signals in tumor nuclei indicating the presence of high-risk HPV DNA.

Histopathological and immunohistochemical findings

Histologically, the GEA case (case No. 4) consisted of irregular angulated glands invading the cervical stroma (Fig. 1A). Nine GEA cases exhibited a negative or patchy immunohistochemical reaction for p16 (Fig. 1B) but one case showed block-type positivity for p16. All UEA cases showed block-type positivity for p16. Aberrant p53 (overexpression or null pattern) expression was observed in six GEA cases (60%): overexpression in five cases (Fig. 1C) and a null pattern in one case. All UEA cases showed patchy positive reactions for p53. Eight GEA cases showed diffuse or focal positive reactions for MUC6 (Fig. 1D) but two cases were negative (cases Nos. 2 and 7). The 2 MUC6 negative cases (Fig. 1E) showed pale pinkish-red cytoplasmic neutral mucin on Alcian blue/PAS special staining (Tables 1, 2, Fig. 1F).

Cytologic features

Preoperative cytologic diagnoses of the eight GEA cases were

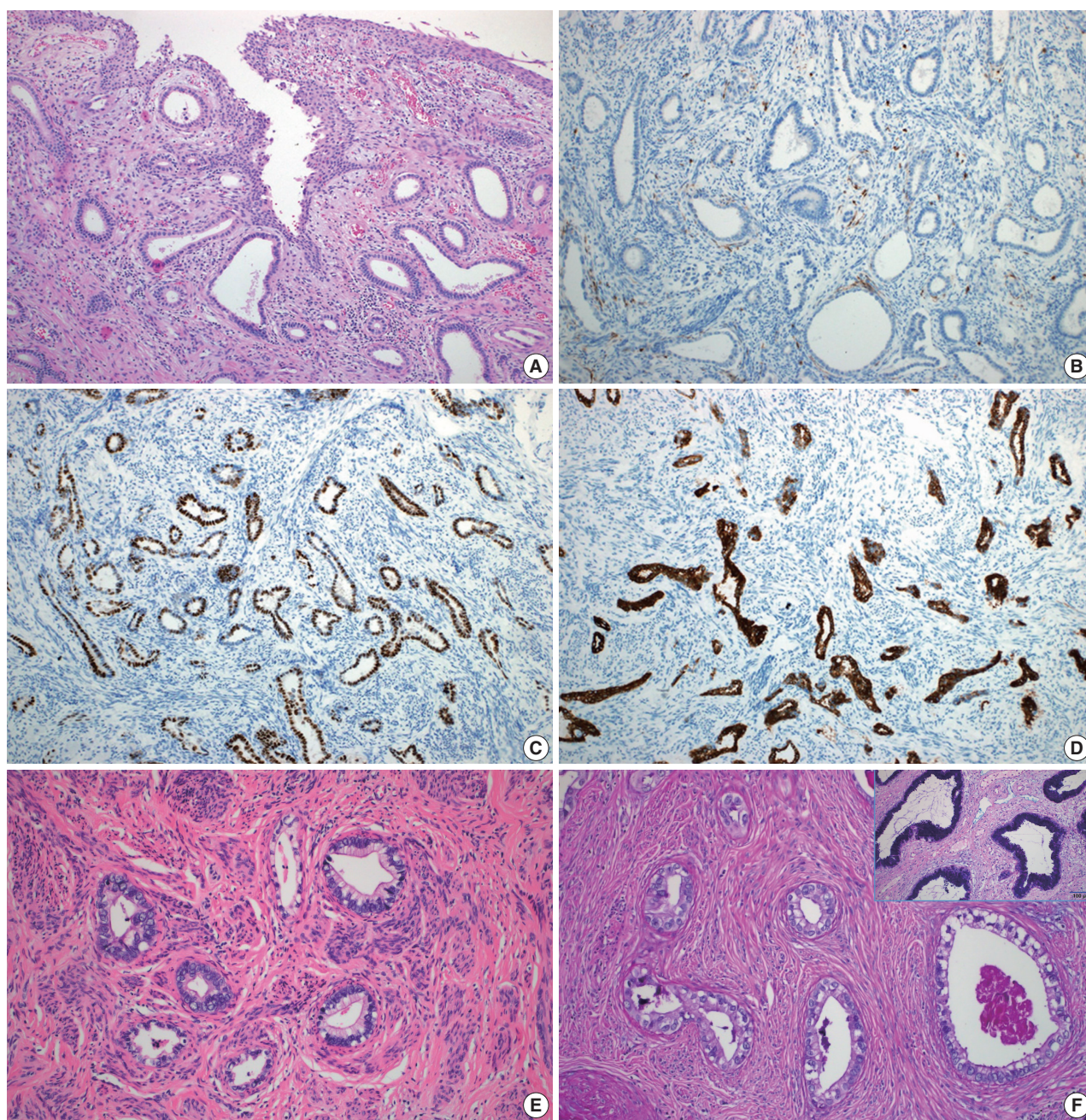


Fig. 1. Human papillomavirus-independent gastric-type endocervical adenocarcinoma. Irregular angulated glands invading the cervical stroma (A). Tumor cells show negative immunohistochemical reactions for p16 (B), aberrant nuclear overexpression for p53 (C), and positivity for MUC6 (D). Atypical glands with voluminous clear cytoplasm and distinct cell borders (E). The cytoplasm contains neutral mucins that stain pale pinkish-red on Alcian blue/periodic acid-Schiff special staining in contrast to the dark purple of acid mucins of the normal endocervix (inset) (F).

as follows: atypical glandular cells (AGCs), favor neoplastic in three cases, adenocarcinoma in situ (AIS) in one case, and adenocarcinoma in four cases.

Cytologic findings of GEA and UEA cases are summarized in Table 3.

Mucinous background

Background extracellular mucin (Fig. 3A) was identified in six GEA and 10 UEA cases. This finding was more extensive in GEA (4/8) than UEA patients (1/12). However, the difference was not statistically significant ($p = .058$).

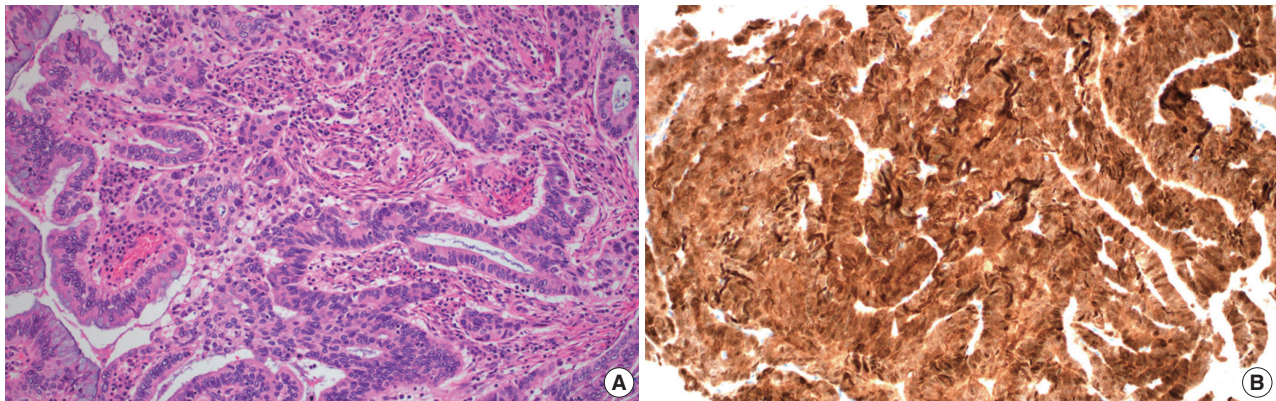


Fig. 2. Human papillomavirus–associated usual-type endocervical adenocarcinoma. Irregular confluent glands composed of mucin-depleted cells with pseudostratified, hyperchromatic nuclei (A). Tumor cells show a block-type immunohistochemical reaction for p16 (B).

Architectural findings

Monolayered honeycomb sheets of atypical endocervical cells (Fig. 3B) were identified in eight cases and were extensive in 87.5% (7/8) of GEA patients; however, these were identified in only 50.0% (6/12) of UEA patients and were extensive in only one UEA case (8.3%) ($p = .002$). Three-dimensional clusters of hyperchromatic nuclei were identified in 8 GEA and 12 UEA cases (Fig. 3C) and were more extensive in UEA (11/12) than in GEA patients (3/8; $p = .010$). Feathering clusters were also observed in 5/8 GEA cases and 9/12 UEA cases but were extensive in only 4/12 UEA cases ($p = .189$) (Fig. 3D).

Nucleus

Vesicular tumor cell nuclei were extensive in all GEA patients (8/8) (Fig. 4A) but were also identified in 11/12 UEA patients and were extensive in six subjects ($p = .057$). Nuclear hyperchromasia was present in all UEA patients (12/12) and extensive in three patients. Nuclear hyperchromasia was also present in six GEA cases (6/8) but not extensive in any GEA case ($p = .082$). Nuclear grooves were present in all GEA patients (8/8) and extensive in two patients (Fig. 4A). Among UEA cases, nuclear grooves were also present in 9/12 patients and extensive in one patient ($p = .230$). Prominent nucleoli were present in 8/8 GEA patients and extensive in five patients. Among UEA cases, nucleoli were present in 9/12 cases and extensive in three patients ($p = .146$). INCI were found in one GEA case and one UEA case ($p = .761$). Among nuclear features, vesicular nuclei tended to be extensive in GEA cases but without statistical significance. Nuclear hyperchromasia was more extensive in UEA cases but also without statistical significance.

Cytoplasm

Vacuolar granular cytoplasm was extensive in 8/8 GEA patients (Fig. 4B). Vacuolar cytoplasm was also identified in 10/12 UEA cases but extensive in only two patients ($p = .0001$). Cytoplasmic golden-brown mucin was identified in 5/8 GEA patients but extensive in only one patient (Fig. 4C). Among UEA cases, cytoplasmic golden-brown mucin was identified in only 2/12 cases and not extensive in any patient ($p = .089$). Intracytoplasmic neutrophils were identified in 8/8 GEA patients and prominent in six patients (Fig. 4D). Among UEA cases, intracytoplasmic neutrophils were also identified in 11 patients (11/12) and extensive in six patients ($p = .461$). Among cytoplasmic features, vacuolar granular cytoplasm was extensive in GEA cases (Table 3).

Both conventional smear and LBP were performed in only 5 GEA cases. The cytologic findings of GEA in LBP compared with conventional smears included cleaner background with reduced background mucin and smaller cellular sheets or clusters. On conventional smears, two-toned mucin (golden-brown gastric-type and pink normal endocervical) was identified in GEA cases (Fig. 4B).

DISCUSSION

According to a new World Health Organization classification, adenocarcinomas of the uterine cervix are divided into HPV-associated and HPV-independent types with different implications for prognosis [1]. The HPV-independent category includes gastric, clear cell, mesonephric, and endometrioid types [1]. GEA, representing the most common HPV-independent adenocarcinoma, is an aggressive tumor type with a poor prognosis regardless of the degree of differentiation [9]. GEA should not be graded but should be considered high-grade regardless of morphology [10].

Table 3. Cytologic findings of gastric-type adenocarcinoma (GEA) and usual-type endocervical adenocarcinoma (UEA)

Cytologic finding	GEA (n=8)	UEA (n=12)	p-value
Mucinous background			.058
Absent	2 (25.0)	2 (16.7)	
Focal	2 (25.0)	9 (75.0)	
Extensive	4 (50.0)	1 (8.3)	
Architecture			
Monolayered honeycomb-like sheet			.002
Absent	0	6 (50.0)	
Focal	1 (12.5)	5 (41.7)	
Extensive	7 (87.5)	1 (8.3)	
3-dimensional clusters			.010
Absent	0	0	
Focal	5 (62.5)	1 (8.3)	
Extensive	3 (37.5)	11 (91.7)	
Feathering			.189
Absent	3 (37.5)	3 (25.0)	
Focal	5 (62.5)	5 (41.7)	
Extensive	0	4 (33.3)	
Nuclei			
Vesicular nuclei			.057
Absent	0 (0.0)	1 (8.3)	
Focal	0 (0.0)	5 (41.7)	
Extensive	8 (100.0)	6 (50.0)	
Hyperchromasia			.082
Absent	2 (25.0)	0	
Focal	6 (75.0)	9 (75.0)	
Extensive	0 (0.0)	3 (25.0)	
Nuclear groove			.230
Absent	0 (0.0)	3 (25.0)	
Focal	6 (75.0)	8 (66.7)	
Extensive	2 (25.0)	1 (8.3)	
Nucleoli			.146
Absent	0 (0.0)	3 (25.0)	
Focal	3 (37.5)	6 (50.0)	
Extensive	5 (62.5)	3 (25.0)	
Intranuclear pseudoinclusion			.761
Absent	7 (87.5)	11 (91.7)	
Focal	1 (12.5)	1 (8.3)	
Extensive	0	0	
Cytoplasm			
Vacuolar/granular			.001
Absent	0	2 (16.7)	
Focal	0	8 (66.7)	
Extensive	8 (100)	2 (16.7)	
Golden-brown mucin			.089
Absent	3 (37.5)	10 (83.3)	
Focal	4 (50.0)	2 (16.7)	
Extensive	1 (12.5)	0	
Neutrophils			.461
Absent	0	1 (8.3)	
Focal	2 (25.0)	5 (41.7)	
Extensive	6 (75.0)	6 (50.0)	

Due to widespread HPV vaccination, the proportion of HPV-independent GEA may increase, rendering recognition of GEA at an early stage even more important [11]. In the present study, cytological findings of HPV-independent GEA were analyzed and compared with HPV-associated UEA. Monolayered sheets (p = .002) of atypical endocervical cells with vacuolar granular cytoplasm (p = .001) were extensive in GEA; however, three-dimensional clusters (p = .010) were characteristic of UEA.

Kawakami et al. [12] reviewed the cytologic features of 14 GEA cases compared with 20 UEA control cases based on conventional Pap smear preparations. The characteristic cytologic findings of GEA included monolayered and honeycomb sheets, vacuolar and/or foamy cytoplasm, intracytoplasmic neutrophil entrapment, and vesicular nuclei with prominent nucleoli. Using LBP, tall columnar epithelial cells with pale, foamy or vacuolated cytoplasm were the most common cytologic findings of GEA, followed by well-defined cytoplasmic borders [11]. Recently, Schwock et al. [13] reported cytomorphologic features of GEA based on LBP. The most discriminatory findings for GEA versus UEA were microvesicular cytoplasm (100% vs. 17%), honeycomb-like sheets (87% vs. 8%), prominent nucleoli (93% vs. 25%), and anisonucleosis (93% vs. 50%). UEA is cytologically characterized by syncytial aggregates and 3-dimensional clusters of malignant cells with enlarged pleomorphic nuclei, coarse chromatin, macronucleoli, and a finely vacuolated cytoplasm [7].

In the present study, monolayered and honeycomb sheets of atypical endocervical cells were characteristic findings of GEA cases compared with UEA cases (p = .002). Three-dimensional clusters were significantly more extensive in UEA (11/12) than in GEA patients (3/8, p = .010).

According to Kawakami et al. [12], a mucinous background was significantly more common in GEA than in UEA cases (p = .024) using the conventional method. In contrast, the so-called “background” extracellular mucin may not be easily detectable in LBP versus direct smears due to preparation-related, technical causes [13]. Background mucin may clump and cling to tumor cells in LBP instead of the diffuse distribution observed in direct smears [14]. In general, the diagnostic usefulness of extracellular mucin is doubtful [13]. In the present study, a background extracellular mucin was identified in 6 GEA and 10 UEA cases. This finding was more extensive in GEA (4/8) than in UEA patients (1/12); however, the difference was not statistically significant (p = .058). The cytologic findings of GEA in LBP compared with conventional smears include cleaner background with reduced background mucin and smaller cellular sheets or clusters. Recently, the diagnostic characteristics to detect GEA

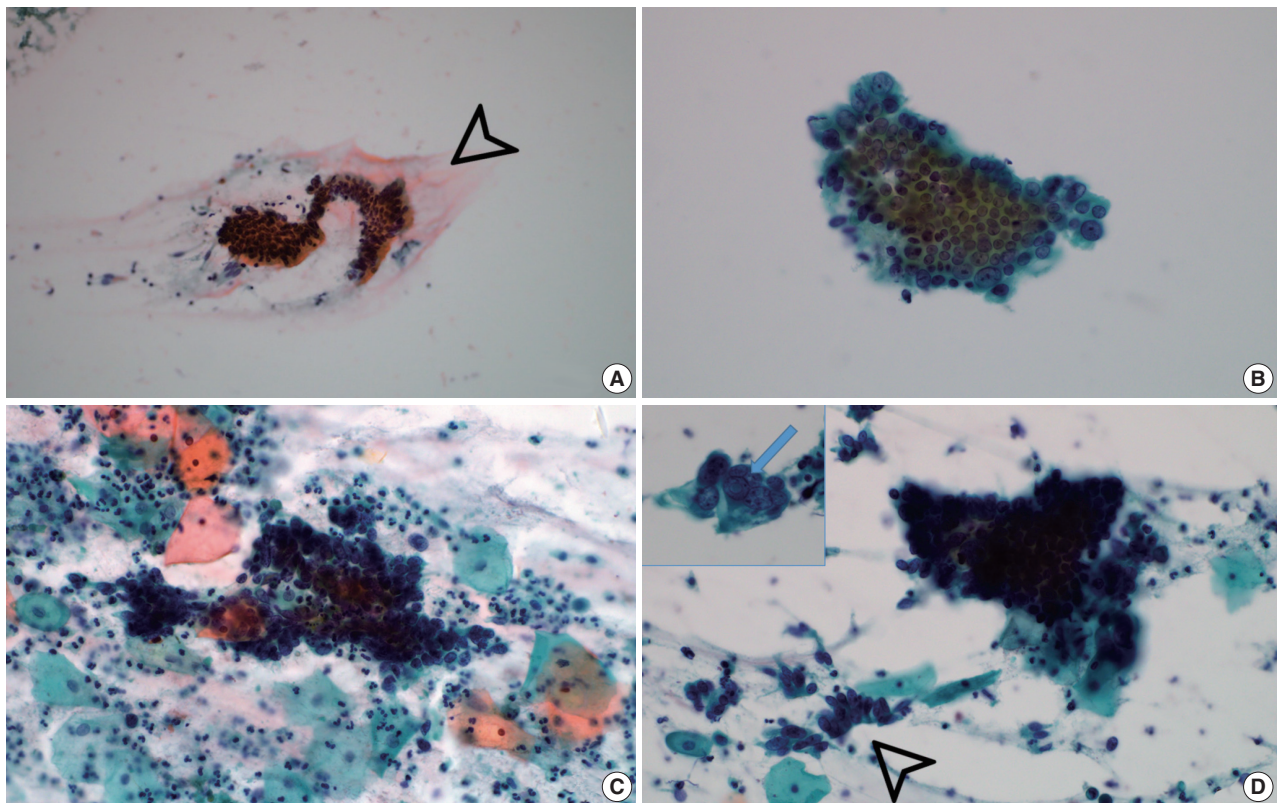


Fig. 3. Human papillomavirus-independent gastric-type endocervical adenocarcinoma. Monolayered honeycomb-like sheets of atypical endocervical cells in a mucinous background (open arrowhead) (A, conventional smear; B, liquid-based preparation). Usual-type endocervical adenocarcinoma. Three-dimensional clusters of hyperchromatic nuclei (C, conventional smear), feathering clusters (empty arrow), and occasional intranuclear cytoplasmic pseudoinclusions (inset, arrow) (D, conventional smear).

using the conventional approach, namely distinct cell borders and prominent nucleoli, were reportedly not useful for excluding UEA in LBP samples. The conventional direct smear provides diagnostic indicators of GEA compared with LBP [15].

Golden-brown mucin on Pap smears appears to represent a gastric phenotype of endocervical glandular cells that is a unique characteristic shared by MDA and pyloric gland metaplasia. Golden-brown mucin is also present in the context of benign gastric-type glandular proliferations [16]. On conventional Pap smears, identification of two-toned mucin (yellow gastric-type and pink normal endocervical) has been proposed a diagnostic indicator for lesions with gastric differentiation. However, recognizing yellow mucin on LBP is difficult because the mucin color becomes paler [17]. Golden-brown mucin may not be a particularly sensitive feature for GEA detection, at least in LBP [11]. In the present study, two-toned mucin was identified on conventional smears of GEA cases.

Golden-brown intracytoplasmic mucin was occasionally observed in GEA patients (6/14, 42.9%) but observed in only 1

UEA patient (1/20, 5%; $p = .034$) [12]. Golden-brown mucin (20%), INCIs (20%), and goblet/Paneth-like cells (20%), although uncommon, represented unique features identified only in GEA [13]. In the present study, cytoplasmic golden-brown mucin was identified in 5/8 GEA cases and only 2/12 UEA cases; however, the difference was not statistically significant ($p = .089$).

Intracytoplasmic neutrophil entrapment/phagocytosis was identified in 93% of GEA and 70% of UEA cases (which included endometrial cytology in 12/20 cases). Marked intracytoplasmic neutrophil entrapment was more common in GEA (7/14, 50%) than in UEA cases (2/20, 10%; $p = .038$) [12]. In contrast, neutrophil entrapment/phagocytosis was identified in a minority (33%) of both GEA and UEA cases [13]. In the present study, intracytoplasmic neutrophils among GEA and UEA cases were not statistically significant ($p = .461$), indicating this feature is of minimal utility for distinguishing between GEA and UEA.

Lobular endocervical glandular hyperplasia (LEGH) is a cervical lesion with pyloric gland metaplasia. Abundant yellow mu-

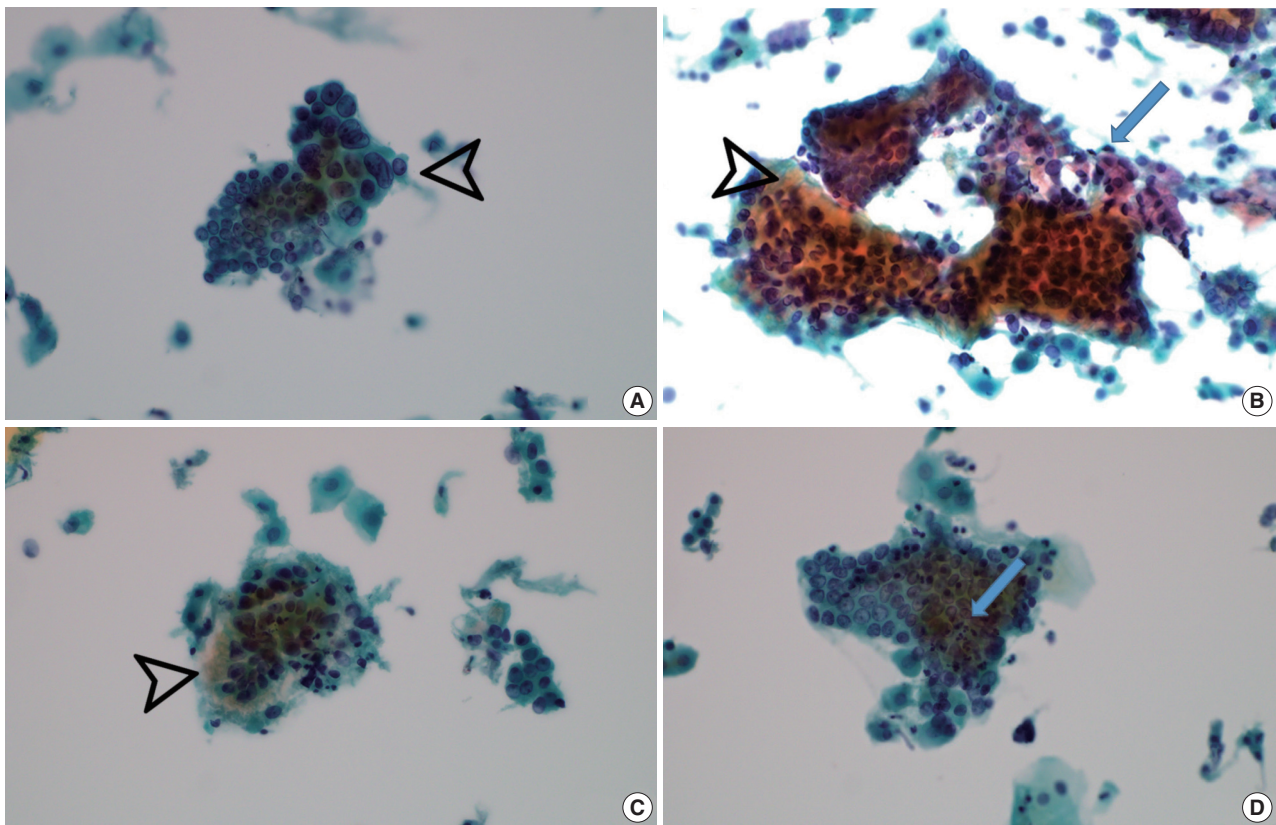


Fig. 4. Human papillomavirus-independent gastric-type endocervical adenocarcinoma. Vesicular nuclei showing nuclear grooves (open arrowhead) (A, liquid-based preparation [LBP]). Atypical tumor cells having abundant vacuolar cytoplasm with golden-brown mucin (open arrowhead) in contrast with pink normal endocervical-type mucin (arrow) (B, conventional smear). Abundant foamy cytoplasm with distinct cell borders and golden-brown mucin (open arrowhead) (C, LBP) and intracytoplasmic neutrophils (arrow) (D, LBP).

Table 4. Literature review summary of the pretreatment cytologic diagnosis of GEA cases

	Kawakami et al. [12]	Lu et al. [11]	Schwock et al. [13]	Ryu et al. [15]	This study	Total, n (%)
No. of cases	14	11	15	8	8	56 (100)
Preparation type	CS	LBC	CS and LBC	CS and LBC	CS and/or LBC	CS and/or LBC
TBS classification						
Unsatisfactory	0	1	0	0	0	1 (1.8)
NILM	0	5	5	0	0	10 (17.9)
AGC	3	4	2	4	3	16 (28.6)
AIS	0	0	1	1	1	3 (5.4)
HSIL	0	1	0	0	0	1 (1.8)
Adenocarcinoma	11	0	7	3	4	25 (44.6)

GEA, gastric-type endocervical adenocarcinoma; CS, conventional smear; LBC, liquid-based cytology; TBS, The Bethesda System; NILM, negative for intraepithelial lesion or malignancy; AGC, atypical glandular cells, not otherwise specified and favor adenocarcinoma; AIS, adenocarcinoma in situ; HSIL, high-grade squamous intraepithelial lesion.

cin was frequently present in both LEGH and MDA; however, INCIs were found in 22/24 LEGH cases and not found in either MDA or adenocarcinoma cells associated with LEGH [18]. INCIs were identified in a minority (20%) of GEA cases and not in any UEA case [13]. In the present study, INCIs were identified in 1 GEA case and 1 UEA case ($p = .761$) (Fig. 3D).

Cytologic diagnosis of GEA is problematic and may be diffi-

cult to recognize in cytologic specimens. In the present study, preoperative cytologic diagnoses included AGCs, favor neoplastic in three cases, AIS in one case, and adenocarcinoma in four cases. Preoperative cytologic diagnoses of the previously reported GEA cases were reviewed [11-13,15] including the eight cases used in this study (Table 4). Among a total of 56 cases, the original cytologic interpretations were the following: negative for intraepi-

thelial lesion or malignancy (n = 10, 17.9%); AGC, not otherwise specified/favor neoplastic (n = 16, 28.6%); AIS (n = 3, 5.4%); high-grade squamous intraepithelial lesion (n = 1, 1.8%); adenocarcinoma (n = 25, 44.6%); unsatisfactory for evaluation (n = 1, 1.8%) (Table 4).

In cytologic differential diagnosis of GEA, non-neoplastic atypical glandular changes and syncytial aggregates/hyperchromatic crowded groups (HCGs) should also be considered. In patients with an intrauterine device, the endocervical cells are large and have smooth nuclei with smudged chromatin, prominent nucleoli, and occasional nuclear clefts. Large degenerative cytoplasmic vacuoles push the nucleus toward the periphery [7]. In a reparative process, sheets of atypical endocervical cells have enlarged nuclei with increased nuclear to cytoplasmic ratios, sometimes multiple nucleoli, and mitotic activity. A regular honeycomb arrangement of reactive endocervical cells is also a cytologic feature of non-neoplastic HCGs [19]. Endocervical or endometrial cells presenting as HCGs may mimic glandular high-grade precancers. Atypical endocervical cells associated with tubal metaplasia can be challenging due to nuclear overlapping and crowding of enlarged, variably sized nuclei; however, identification of cilia and pseudostratified nuclei can be helpful [7].

In conclusion, GEA can be recognized based on cytologic features of monolayered honeycomb sheets of atypical endocervical cells with abundant vacuolar cytoplasm and some golden-brown intracytoplasmic mucin. In contrast, three-dimensional clusters, feathering, and nuclear hyperchromasia tended to be extensive in UEA. Awareness of the cytomorphologic features of GEA will allow pathologists to recognize and accurately diagnose this rare and aggressive entity.

Ethics Statement

Ethical approval for the study was obtained from the ethics committee of Chungnam National University Hospital (CNUH-IRB 2021-12-001) (Daejeon, Republic of Korea). An exemption from informed consent was also approved.

Availability of Data and Material

The datasets generated or analyzed during the study are available from the corresponding author on reasonable request.

Code Availability

Not applicable.

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Conflicts of Interest

The authors declare that they have no potential conflicts of interest.

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