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Panel of Villin, Pro-Ex-C, Estrogen Receptor and Progesterone Receptor Expressions Could Help in Differentiation Between Endocervical and Endometrioid Adenocarcinoma

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ABSTRACT

Objective: Endocervical and endometrioid adenocarcinoma have marked overlapping features and the differentiation between them is important for their accurate management. Villin is an actin-binding protein which has an important role in the maintenance of microvilli in epithelial cells and epithelial cell-specific anti-apoptotic protein processes. Pro-Ex-C is a marker for higher-risk human papilloma virus (HPV) which targets the cell cycle proteins causing their overexpression. The aim of the study was to clarify the diagnostic and predictive role of villin, Pro-Ex-C, estrogen receptor (ER) and progesterone receptor (PR) expression in endocervical and endometrioid adenocarcinoma.

Material and Method: We evaluated villin, Pro-Ex-C, ER and PR expressions in 15 cases of endocervical adenocarcinoma and 30 cases of endometrioid adenocarcinoma. We analyzed the diagnostic and predictive role of that panel in both carcinoma subtypes. Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were calculated.

Results: Positive villin and Pro-Ex-C expressions were positively correlated with the presence and pattern of cervical stromal invasion ($p<0.05$). ER was positive in all cases of endometrioid adenocarcinoma. PR was detected in most cases of endometrioid adenocarcinoma. The differences of villin, Pro-Ex-C, ER and PR expression in endocervical and endometrioid adenocarcinoma was statistically significant ($p<0.05$). This methodology for distinguishing endocervical and endometrioid adenocarcinoma had a sensitivity of 100%, a specificity of 100% and a significant prognostic and predictive role.

Conclusion: In conclusion, villin, Pro-Ex-C, ER and PR expressions have diagnostic and predictive roles in endocervical and endometrioid adenocarcinoma

Key Words: Endocervical adenocarcinoma, Endometrioid adenocarcinoma, Immunohistochemistry, Villin, Pro-Ex-C, ER, PR

INTRODUCTION

Endocervical adenocarcinoma (ECA) is the 4th most common cancer in females globally (1) and it has been ranked the 14th most common cancer in Egyptian women between fifteen and forty-four years old (2). Endometrioid adenocarcinoma (EMC) is the 6th most common cancer in females globally (3), and the 3rd most common cause of female cancer-related mortality after cancers of the ovary and cervix (4). EMC had accounted for 2.6% of total Egyptian female cancers (5). ECA and EMC have significantly overlapping histopathological criteria and the differentiation between them both is important for their accurate management, as surgical excision of cancer and postoperative adjuvant chemo- and radio-therapy would depend on the primary site of origin of the carcinoma. ECA could be divided HPV-related ECAs which include mucinous carcinoma with presence or absence of intestinal differentiation, serous and endometrioid carcinoma and HPV-unrelated subtypes which include mesonephric, clear cell, and gastric carcinomas (6-8). Recent World Health Organization (WHO) Tumors of Female Reproductive System Classification 2014 has included gastric subtype adenocarcinomas as a rare aggressive ECA subtype (9).

The differentiation between ECA and EMC could be difficult if the cancer involved the lower uterine segment or the upper endocervix. Even if hysterectomy specimens were taken, it would be difficult to detect the original site of the carcinoma (10). Villin is an actin-binding protein which plays an important role in the maintenance of microvilli in epithelial cells and also has an essential role in the regulation of cell morphology and cell-specific epithelial anti-apoptotic mechanisms (11). Villin is also expressed in intestinal metaplasia and is associated with Barrett's esophagus and chronic atrophic gastritis, but it

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was not expressed in normal gastric and esophageal tissues (12). Villin is expressed in some adenocarcinomas but the absence of expression in normal epithelial tissues indicates a potential role in epithelial cell hyperplasia, dysplasia, or carcinogenesis (13). Pro-Ex-C is a marker for HPV related cervical cancer and it targets cell cycle proteins like mini-chromosome maintenance protein-2 (MCM2), and topoisomerase II-a (TOP2A) which are overexpressed when viral DNA integrates into the host genome, leading to aberrant S-phase induction (14). Many researchers have evaluated the use of estrogen receptor (ER) and progesterone receptor (PR) in distinguishing between ECA and EMC but the results have not been conclusive (15).

The aim of this study was to clarify the diagnostic panel of villin, Pro-Ex-C, ER and PR immunohistochemical expression in the differentiation between ECA and EMC and also to detect the predictive role of marker expression.

MATERIALS and METHODS

Patients Selection

Formalin-fixed, paraffin-embedded tissue blocks containing 15 cases of ECA and 30 cases of EMC were obtained retrospectively from the archives of the Department of Pathology, Faculty of Medicine, Zagazig University during the period between November 2010 and November 2015. Patient data such as age, tumor size, grade and stage were acquired from the archives. We used the International Federation of Gynecology and Obstetrics' (FIGO) staging system for staging our cases (16). We determined whether there was stromal invasion by carcinoma and classified such invasion by using recent patterns of stromal invasion (17,18).

Immunohistochemical Staining

Villin, Pro-Ex-C, ER and PR expressions were assessed using immunohistochemistry in sections from all the 45 blocks of paraffin. We analyzed correlations between the levels of Villin, Pro-Ex-C, ER and PR expressions and ability of panel of both markers in differentiation between ECA and EMC; we also analyzed the predictive role of marker expression. The streptavidine-biotin technique was used for immunohistochemical staining (19). We incubated sections with primary; rabbit monoclonal anti-Pro-Ex-C-antibody (MCM2 26H6.19), mouse monoclonal anti-Villin-antibody (clone 1D2C3, ab739 , dilution 1:100) at 4°C overnight (Abcam, Cambridge, MA, USA), anti-estrogen receptor (clone GF11, dilution 1:50, Novocastra Laboratories, Newcastle upon Tyne, United Kingdom) and anti-progesterone receptor (clone 16, dilution 1:200, Novocastra), followed by incubation with secondary antibodies. Sections from small intestine were used as a positive control for villin, and sections from EMC were used as the positive control for ER and PR receptor expression (20,21). For negative controls, the primary antibodies were removed but replaced with phosphate-buffered saline. We evaluated the stained slides without previous identification of the clinical and pathological parameters.

Evaluation of Immunohistochemical Expression of Villin

We evaluated villin with either cytoplasmic and/or membranous staining, and scoring was according to the following criteria: 0 (not stained); one (membranous staining at less than 50% of the tumor cell); 2 (cytoplasmic staining and/or membranous expression at more than 50% of tumor tissues) (12).

Evaluation of Immunohistochemical Expression of Pro-Ex-C

Pro-Ex-C staining was scored as negative when <5% nuclei were stained, diffuse positive when >80% of nuclei were stained (2), focal positive when 5% to 80% of nuclei were stained (22).

Evaluation of Immunohistochemical Expression of ER and PR Receptors

Nuclear staining was scored by combining both the stain intensity and extent in tumor cells. Stain intensity was graded from zero (negative) to three (strong). The stain extent was graded as zero (negative expression), one (positive expression in less than ten percent of the tumor cells), two (positive expression in ten to fifty percent of the tumor cells) and three (positive expression in greater than 50% of the tumor cells). The final score (zero to nine) was reached by multiplying the staining intensity and extent scores. A final cut off staining score of less than four was interpreted as low expression and a score equal to or more than four was interpreted as high expression (23).

Statistical Analysis

Validity of immunohistochemical markers in diagnosis of histopathological type was calculated using diagnostic performance depending on sample 2x2 contingency tables generation. Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were calculated. All tests were two sided. A p-value <0.05 was considered significant. We used SPSS 22.0 Windows (SPSS Inc., Chicago, USA) and MedCalc Windows (MedCalc Software bvba 13, Ostend, Belgium) for statistical analysis. Continuous variables were designated as mean ± SD and median (range); categorical variables were designated as numbers (percentage). We used Chi-square test for trend

and the Independent samples Student's t-test for comparing the age between groups.

RESULTS

A total of 45 cases were studied; the cases included: 15 cases of ECA, NOS and 30 cases of EMC. The age of all patients ranged from 39 to 72 years; from 44 to 72 years for EMC cases and from 39 to 65 for ECA cases (Table I). There was one case with pattern A, two cases with pattern B (both patterns A and B were Stage I) and thirty-two cases with pattern C (stage II-IV). The types of stromal pattern invasion are detailed in Table I.

Immunohistochemical Findings

The detailed immunohistochemical characteristics that were observed in ECA and EMC using villin, Pro-Ex-C, ER and PR are summarized in Tables II-IV and illustrated in Figures 1A-E, 2A-D.

Villin Expression

Positive staining for villin was observed in 93.3% (14/15) of ECA cases; 12 showed diffuse expression, while the remaining 2 cases showed focal expression. On the other hand, focal positivity was found in 6 cases (20%) of EMC. The difference between villin expression in the two groups

Table I: The clinicopathological features of patients

Characteristics	Number	%
Age (year)		
Mean ± SD	56.84 ± 7.84	
Median (Range)	57 (39 - 72)	
≤ 55 years	18	40
> 55 years	27	60
Histopathology		
Endometrioid carcinoma	30	66.7
Endocervical adenocarcinoma	15	33.3
Size		
<4 cm	17	37.8
>4 cm	28	62.2
Grade		
Grade I	18	40
Grade II	20	44.4
Grade III	7	15.6
Myometrial invasion		
Absent	4	8.9
<50%	17	37.8
>50%	9	20
N/A	15	33.3
LVSI		
Absent	37	82.2
Present	8	17.8
Endocervical gland involvment		
Absent	26	57.8
Present	19	42.2
Cervical stromal invasion		
Absent	10	22.2
Present	35	77.8
Pattern A	1	Stage I
Pattern B	2	Stage I
Pattern C	32	Stages [II-IV]

Characteristics	Number	%
Parametrial invasion		
Absent	20	44.4
Present	10	22.2
N/A	15	33.3
Serosal invasion		
Absent	20	44.4
Present	10	22.2
N/A	15	33.3
Adnexal invasion		
Absent	20	44.4
Present	10	22.2
N/A	15	33.3
Peritoneal cytology		
Negative	23	51.1
Positive	7	15.6
N/A	15	33.3
Lymph node		
Negative	27	60
Positive	18	40
Distant metastasis		
Negative	38	84.4
Positive	7	15.6
Stage		
Stage I	9	20
Stage II	18	40
Stage III	11	24.4
Stage IV	7	15.6

Continuous variables were expressed as mean ± SD and median (range); categorical variables were expressed as number (percentage).

of gynecological malignancy was statistically significant (p<0.001). Positive villin expression in ECA was statistically significantly associated with the size of the tumor, the presence of cervical stromal invasion (p<0.001) and pattern of stromal invasion (p=0.002) and the presence of lymph node metastases (p=0.012). No significant correlations were found between villin expression, FIGO clinical stage and the presence of distant metastases.

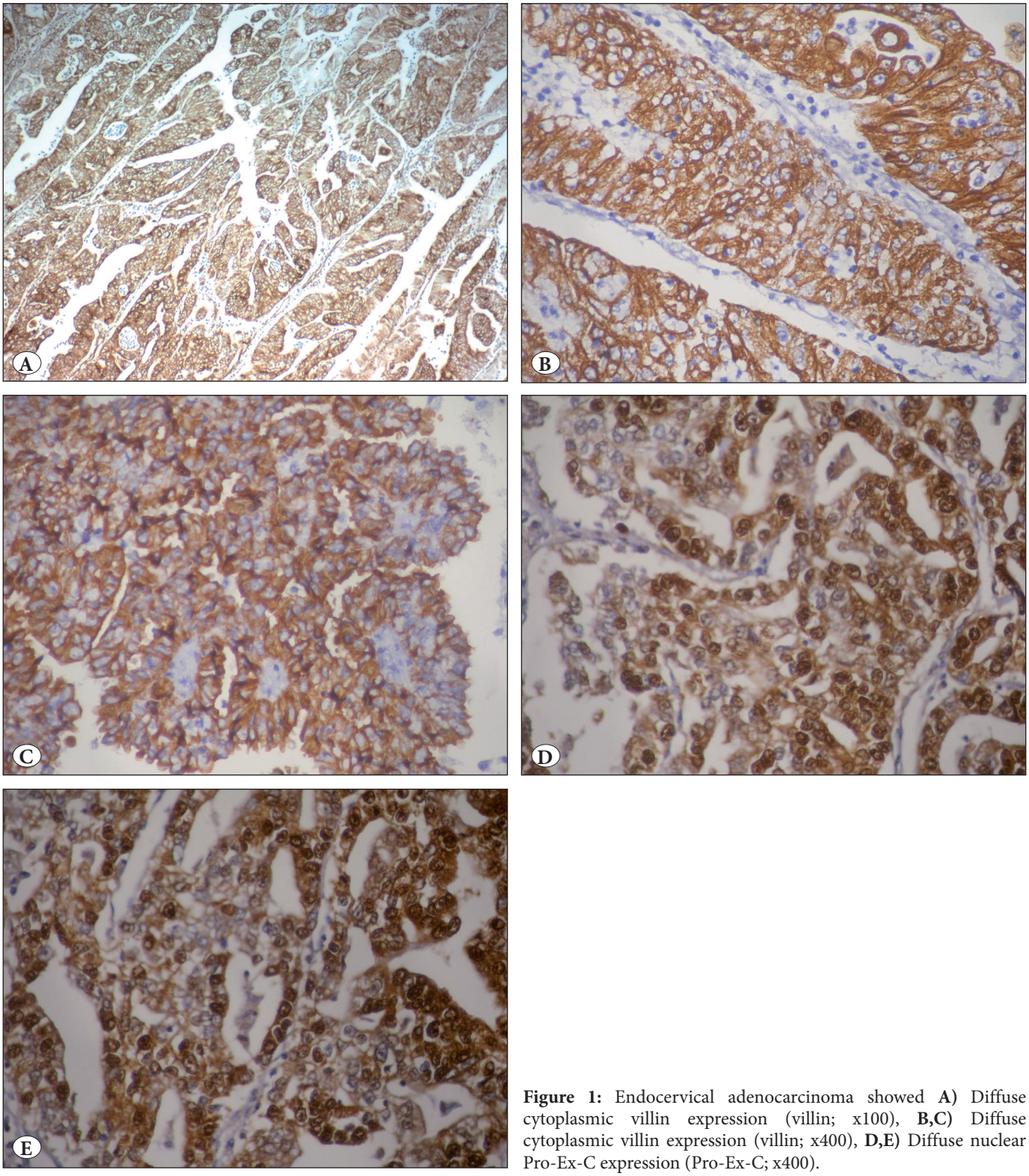

Figure 1: Endocervical adenocarcinoma showed **A)** Diffuse cytoplasmic villin expression (villin; x100), **B,C)** Diffuse cytoplasmic villin expression (villin; x400), **D,E)** Diffuse nuclear Pro-Ex-C expression (Pro-Ex-C; x400).

Pro-Ex-C Expression

Positive nuclear staining for Pro-Ex-C was observed in 86.7% (13/15) of ECA cases; 10 was diffuse expression, while the remaining 3 cases showed focal expression On the other hand, no diffuse positivity was found in EMC and focal positivity was found in 4 EMC cases (13.3%). The difference of Pro-Ex-C expression in the two groups of gynecological malignancy was statistically significant ($p<0.001$). Positive Pro-Ex-C expression in ECA was statistically significantly associate with the size of the tumor ($p=0.008$), the presence of lymph node metastases ($p=0.003$), the presence of cervical stromal invasion ($p<0.001$) and the pattern of stromal invasion ($p=0.002$). No significant correlations were found between Pro-Ex-C expression, grade FIGO clinical stage and the presence of distant metastases. The detailed IHC scoring of different patterns of stromal pattern invasion was as follows: pattern

Table II: The correlation between clinicopathological features and villin expression

	All (n=45)	Villin			p-value
		Negative (n=25)	Focal +ve (n=8)	Diffuse +ve (n=12)	
Characteristics	**No. (%)**	**No. (%)**	**No. (%)**	**No. (%)**	
Age (years)					
Mean ± SD	56.84 ±7.84	61.28 ±5.84	51.87 ±5.27	50.91 ±7.26	<0.001*
Median (Range)	57 (39-72)	60 (50-72)	51.50 (44-61)	50.50 (39-65)	
≤ 55 years	18 (40)	2 (11.1)	6 (33.3)	10 (55.6)	<0.001‡
> 55 years	27 (60)	23 (85.2)	2 (7.4)	2(7.4)	
Histopathology					
Endometrioid carcinoma	30 (66.7)	24 (80)	6 (20)	0(0)	<0.001
Endocervical adenocarcinoma	15 (33.3)	1(6.7)	2 (13.3)	12 (80)	
Size					
<4 cm	17 (37.8)	9 (52.9)	8 (47.1)	0(0)	<0.001‡
>4 cm	28 (62.2)	16 (57.1)	0 (0)	12 (42.9)	
Endocervical gland involvement					
Absent	26 (57.8)	20 (76.9)	6 (23.1)	0(0)	<0.001‡
Present	19 (42.2)	5 (26.3)	2 (10.5)	12 (63.2)	
Cervical stromal invasion					
Absent	10 (22.2)	4 (40)	6 (60)	0(0)	<0.001‡
Present	35 (77.8)	21 (60)	2 (5.7)	12 (34.3)	
Pattern A	1 (stage I)	1	0 (0)	0(0)	
Pattern B	2 (stage I)	2	0 (0)	0(0)	
Pattern C	32 (stage II-IV)	8	10	12 0.002‡	
Lymph node					
Negative	27 (60)	15 (55.6)	8 (29.6)	4 (14.8)	0.012‡
Positive	18 (40)	10 (55.6)	0 (0)	8 (44.4)	
Distant metastasis					
Negative	38 (84.4)	21 (55.3)	8 (21.1)	9 (23.7)	0.318‡
Positive	7 (15.6)	4 (57.1)	0 (0)	3 (42.9)	
Stage					
Stage I	9 (20)	1 (11.1)	8 (88.9)	0 (0)	0.643§
Stage II	18 (40)	14 (77.8)	0 (0)	4 (22.2)	
Stage III	11 (24.4)	6 (54.5)	0 (0)	5 (45.5)	
Stage IV	7 (15.6)	4 (57.1)	0 (0)	3 (42.9)	

Categorical variables were expressed as number (percentage). * Independent samples Student's t-test. ‡ Chi-square test. § Chi-square test for trend.

A and B cases were negative for both villin and Pro-Ex-C while pattern C cases showed more diffuse positivity for both markers and these results were statistically significant (p =0.002 and 0.003, respectively) (Tables III,IV).

ER and PR Expression

ER was positive in all cases (100%) of EMC where 26 cases showed diffuse positive expression while the remaining 4 cases showed focal positive expression. ER was not detected in any of the ECA cases although immunostaining for ER is usually positive in ECA but our results may be related to testing a small number of cases (n=15). PR was detected in 26 out of 30 cases (86.7%) of EMC, while it was positive in only two cases (13.3%) of ECA. PR was recommended in recent publications in the differential diagnosis of endometrioid adenocarcinoma cases. We found highly significant positive correlations between villin and Pro-Ex-C expression in ECA (p<0.001) and ER and PR expression in EMC (p<0.001). This methodology for distinguishing EMC and ECA had a sensitivity of 100% and a specificity of 100% (Table V).

DISCUSSION

There is a marked morphologic similarity between ECA and EMC which leads to difficult differentiation on hematoxylin&eosin stained sections especially in small pre-operative or D&C samples. The differentiation between these two malignancies is essential for adequate management (24).

Villin is an important component in the structure of cytoskeleton and can bind actin in a calcium-dependent manner (25). It is an anti-apoptotic epithelial protein that plays an essential role in regulating cellular morphology, survival and migration (26). We found in this study that positive staining for villin was observed in 93.3% (14/15) of ECA cases, while in EMC cases focal positivity was found in only 6 cases (20%) and the difference of villin expression between the two groups of gynecological malignancy was statistically significant (p <0.001). These findings were in agreement with Nakamura et al. who have conducted immunohistochemical analysis of 14 villin-positive tumors and revealed that thirteen of such positive cases

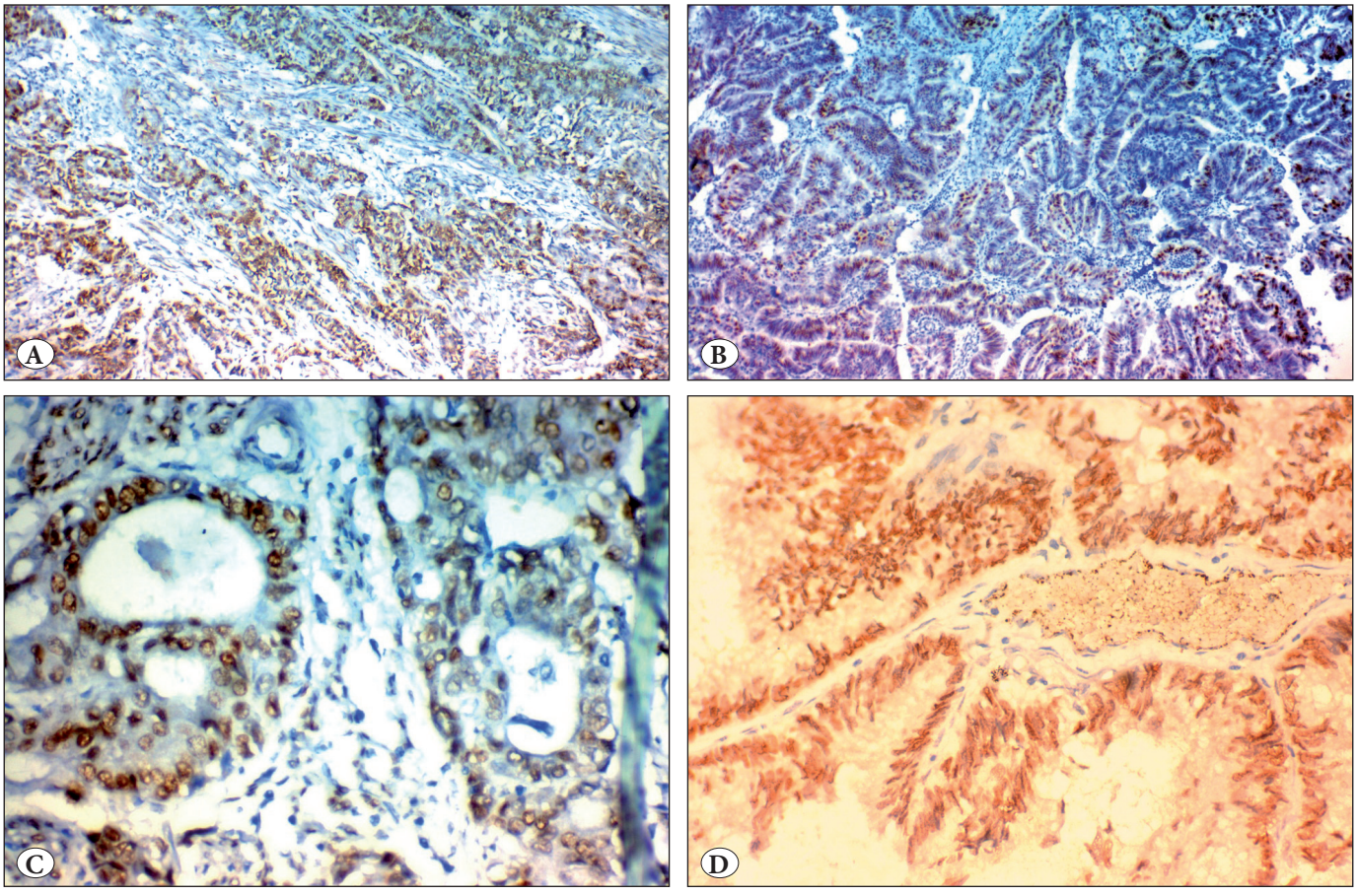

Figure 2: Endometrial adenocarcinoma showed **A)** Diffuse nuclear ER expression (ER; x100), **B)** Diffuse nuclear ER expression (ER; x200) **C,D)** Diffuse nuclear PR expression (PR; x400).

were ECA and the remaining one case was diagnosed as small cell carcinoma of cervix, while no case of EMC was villin-positive (11). Another study by Moll et al. showed results similar to ours in that positive villin staining was found in only in 4/11 EMC (27). The predictive role of villin as well as correlation between villin expression and clinicopathological characteristics has been addressed in our study, and we found a significant association between villin expression, cancer size, presence and pattern of stromal invasion, and presence of nodal metastasis, therefore

Table III: The correlation between clinicopathological features and Pro-Ex-C expression

	All (n=45)	Pro-Ex-C			p-value
		Negative (n=28)	Focal +ve (n=7)	Diffuse +ve (n=10)	
Characteristics	**No. (%)**	**No. (%)**	**No. (%)**	**No. (%)**	
Age (years)					
Mean ± SD	56.84 ±7.84	60.39 ±6.52	54.42 ±6.29	48.60 ±5.23	<0.001*
Median (Range)	57 (39-72)	60 (44-72)	53 (48-65)	49.50 (39-55)	
≤ 55 years	18 (40)	4 (22.2)	4 (22.2)	10 (55.6)	<0.001‡
> 55 years	27 (60)	24 (88.9)	3 (11.1)	0 (0)	
Histopathology					
Endometrioid carcinoma	30 (66.7)	26 (86.7)	4 (13.3)	0 (0)	<0.001‡
Endocervical adenocarcinoma	15 (33.3)	2 (13.3)	3 (20)	10 (66.7)	
Size					
<4 cm	17 (37.8)	12 (70.6)	5 (29.4)	0 (0)	0.008‡
>4 cm	28 (62.2)	16 (57.1)	2(7.1)	10 (35.7)	
Grade					
Grade I	18 (40)	10 (55.6)	5 (27.8)	3 (16.7)	0.544§
Grade II	20 (44.4)	13 (65)	0(0)	7 (35)	
Grade III	7(15.6)	5 (71.4)	2 (28.6)	0 (0)	
LVSI					
Absent	37 (82.2)	23 (62.2)	7 (18.9)	7 (18.9)	0.281‡
Present	8 (17.8)	5 (62.5)	0(0)	3 (37.5)	
Endocervical gland involvement					
Absent	26 (57.8)	22 (84.6)	4 (15.4)	0 (0)	<0.001‡
Present	19 (42.2)	6 (31.6)	3 (15.8)	10 (52.6)	
Lymph node					
Negative	27 (60)	18 (66.7)	7 (25.9)	2 (7.4)	0.003‡
Positive	18 (40)	10 (55.6)	0(0)	8 (44.4)	
Stage					
Stage I	9 (20)	4 (44.4)	5 (55.6)	0 (0)	0.174§
Stage II	18 (40)	14 (77.8)	2 (11.1)	2 (11.1)	
Stage III	11 (24.4)	6 (54.5)	0(0)	5 (45.5)	
Stage IV	7 (15.6)	4 (57.18)	0(0)	3 (42.9)	
Cervical stromal invasion					
Absent	10 (22.2)	6 (60)	4 (40)	0(0)	0.020‡
Present	35 (77.8)	22 (62.9)	3(8.6)	10 (28.6)	
Pattern A	1 (stage I)	1	0(0)	0 (0)	0.003‡
Pattern B	2 (stage I)	2	0(0)	0 (0)	
Pattern C	32 (stage II-IV)	10	12	10	

Categorical variables were expressed as number (percentage). ‡ Chi-square test. § Chi-square test for trend.

demonstrating the prognostic role of villin in addition to the studied role in differentiation between EAC and ECA in our study. However, the absence of significant correlations between villin expression, FIGO stage and presence of distant metastases in our study may be due to the small sample size and was in agreement with the report by Wang et al. that cells expressing villin migrate and form distant metastases more commonly than villin-negative cells (28). Khurana and George reported that villin may be modified during metastasis (26). However, Al-Maghrabi et al. could not establish any association between villin expression and nodal metastasis and stated that villin expression was not able to predict nodal metastasis. In addition, there was no significant correlation between villin expression and tumor stage (29). In our results, villin expression was significantly positively correlated with size in tumors with a size less than 4 cm in diameter compared to those of 4 cm or more, and this finding was in line with results of Al-Maghrabi et al. in colorectal carcinoma (29). All previous data that were in line with ours have highlighted the possible predictive and prognostic role of villin in addition to its diagnostic role that we studied here in our study.

Fletcher et al. introduced Pro-Ex-C as a new marker for cervical dysplasia and neoplasia (30). In our current study, we have explored the diagnostic role of Pro-Ex-C in the differentiation between ECA and EMC. We found that positive nuclear staining for Pro-Ex-C was observed in 86.7% (13/15) of ECA cases and only focal positivity was found in 4 cases (13.3%) of EMC. The difference of Pro-Ex-C expression in the two groups of gynecological malignancy was statistically significant. These findings were in line with results of Esheba et al. who reported that 80% of ECA cases exhibited positive nuclear staining for Pro-Ex-C (25,31). On the other hand, we proved that only 10% of EMC cases showed Pro-Ex-C positive expression. Similar results were obtained by Aximu et al. and Guo et al., who reported that Pro-Ex-C was more sensitive than p16 in detecting ECA as Pro-Ex-C was positive in 93% (27/29) of ECA cases while p16 was over-expressed in 90% (26/29) of ECA cases (32,33). It was not clear why Pro-Ex-C expression was present in some cases of ECA. Kong et al. explained such positivity had resulted from HPV-independent mechanisms (22), but Semczuk et al. have identified HPV-independent mechanisms resulting in Pro-Ex-C positivity in a small number of EMC cases (34).

We found that positive Pro-Ex-C expression in ECA was significantly correlated with larger size of the tumor, the presence of lymph node metastases, and the presence and pattern of cervical stromal invasion which clarified that Pro-Ex-C expression had a predictive and prognostic role

Table IV: The comparison between endocervical and endometrial adenocarcinoma as regard Pro-Ex-C, villin, ER and PR expressions

Characteristics	All (n=45)	Endometrioid carcinoma (n=30)	Endocervical adenocarcinoma (n=15)	p-value
	No. (%)	No. (%)	No. (%)	
Villin				
Negative	25 (55.6)	24 (80)	1 (6.7)	
Focal positive	8 (17.8)	6 (20)	2 (13.3)	<0.001‡
Diffuse positive	12 (26.7)	0 (0)	12 (80)	
Pro-Ex-C				
Negative	28 (62.2)	26 (86.7)	2 (13.3)	
Focal positive	7 (15.6)	4 (13.3)	3 (20)	<0.001‡
Diffuse positive	10 (22.2)	0 (0)	10 (66.7)	
ER				
Negative	15 (33.3)	0 (0)	15 (100)	
Focal positive	4 (8.9)	4 (13.3)	0 (0)	<0.001‡
Diffuse positive	26 (57.8)	26 (86.7)	0 (0)	
PR				
Negative	17 (37.8)	4 (13.3)	13 (86.7)	
Focal positive	9 (20)	7 (23.3)	2 (13.3)	<0.001‡
Diffuse positive	19 (42.2)	19 (63.3)	0 (0)	

Continuous variables were expressed as mean ± SD and median (range).‡ Chi-square test.

in patients with that cancer in addition to its diagnostic role and this was in agreement with previous researchers that have suggested that Pro-Ex-C facilitates the detection of atypical cells that have developed from normal, reactive or other nonmalignant cells within a Pap cytology specimen (35), and also that Pro-Ex-C is an essential marker for high-grade CIN that can confirm the diagnosis of high-grade CIN and detect cases of atypical squamous metaplasia (36). Thus, the use of Pro-Ex-C to select female patients at risk of cancer progression and who need treatment could improve patient outcome, help early diagnosis, and decrease patient anxiety (35). The absence of significant correlations between Pro-Ex-C expression, FIGO stage and presence of distant metastases in our study may be due to the small sample size.

ER positive expression was found in all cases (100%) of EMC in this study but it was not detected in any of the ECA cases although immunostaining for ER is usually positive in ECA but our results may be related to testing a small number of cases (n=15). PR positive expression was found in 26 out of 30 cases (86.7%) of EMC, while it was positive in only two cases (13.3%) of ECA and PR has been recommended in the differential diagnosis of endometrial carcinoma in recent publications and these finding were in agreement with Esheba who reported that ER positive expression was found in 95% of EMC while it was completely absent in ECA (31). On the other hand, PR positive expression was detected in 80% of EMC and in 20% of ECA. Konishi et al. suggested that reduced ER expression and increased PR expression in ECA were related to the

Table V: Diagnostic performance of Pro-Ex-C, villin, ER and PR expressions in the differentiation between endocervical and endometrioid adenocarcinoma

Markers	SN % (95% CI)	SP % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Accuracy % (95% CI)
Villin (- or focal +)	100	80 (59.8-100.2)	90.9 (81.1-100)	100	93.3 (86.1-100)
Pro-Ex-C (- or focal +)	86.7 (74.5-98.8)	86.7 (69.5-100)	92.9 (83.3-100)	76.5 (56.3-96.6)	86.7 (76.7-96.6)
ER (diffuse + or focal +)	100	100	100	100	100
PR (diffuse + or focal +)	86.7 (74.5-98.8)	86.7 (69.5-100)	92.9 (83.3-100)	76.5 (56.3-96.6)	86.7 (76.7-96.6)
Villin (- or focal +) & Pro-Ex-C (- or focal +)	100	80 (59.8-100.2)	90.9 (81.1-100)	100	93.3 (86.1-100)
Villin (-or focal +) & ER (diffuse + or focal +)	100	100	100	100	100
Villin (- or focal +) & PR (diffuse + or focal +)	86.7 (74.5-98.8)	100	100	78.9 (60.6-97.3)	91.1 (82.8-99.4)
Pro-Ex-C (- or focal +) & ER (diffuse + or focal +)	100	100	100	100	100
Pro-Ex-C (- or focal +) & PR (diffuse + or focal +)	86.7 (74.5-98.8)	100	100	78.9 (60.6-97.3)	91.1 (82.8-99.4)
ER (diffuse + or focal +) & PR (diffuse + or focal)	100	86.7 (69.5-100)	93.8 (85.4-100)	100	95.6 (89.5-100)
Villin (- or focal +), Pro-Ex-C (- or focal +) & ER (diffuse + or focal +)	100	100	100	100	100
Villin (- or focal +), Pro-Ex-C (- or focal +) & PR (diffuse + or focal +)	86.7 (74.5-98.8)	100	100	78.9 (60.6-97.3)	91.1 (82.8-99.4)
Pro-Ex-C (- or focal +), ER (diffuse + or focal +) & PR (diffuse + or focal +)	86.7 (74.5-98.8)	100	100	78.9 (60.6-97.3)	91.1 (82.8-99.4)
Villin (- or focal +), ER (diffuse + or focal +) & PR (diffuse + or focal +)	86.7 (74.5-98.8)	100	100	78.9 (60.6-97.3)	91.1 (82.8-99.4)
Villin (- or focal +), Pro-Ex-C (- or focal +), ER (diffuse + or focal +) & PR (diffuse + or focal +)	100	100	100	100	100

SN: Sensitivity, **SP:** Specificity, **PPV:** Positive Predictive Value, **NPV:** Negative Predictive Value, 95%, **CI:** 95% Confidence Interval.
We stated the finding of marker expression that we depended on for diagnosis inside the brackets as (- or focal +) or (diffuse + or focal +) according to the method of marker evaluation.

proliferation of normal cervical squamous epithelium, and this proliferation-related receptor status which is probably induced by HPV infection and is usually expressed in neoplastic cervical squamous cells (37). Socolov et al. reported that well-differentiated EMC was ER- and PR-positive, so that ER-positive expression was significantly correlated with PR expression. Well-differentiated EMC (GI) in the studied group also showed a higher content of ER and PR compared to moderately-differentiated EMC (GII) (38). Slightly different results were found by Esheba et al. who have demonstrated that some cases of uterine serous carcinoma showed focal strong staining for Pro-Ex-C (2/4, 50%) and less ER and PR expression than usually found in EMC (31). However, we have studied only EMC and Esheba et al. studied other subtypes so it is important to recognize the morphological pattern and specific subtype to avoid misdiagnosis as an endocervical primary based on strong Pro-Ex-C expression.

We have chosen such recent markers because they were found to be more sensitive than conventional markers, e.g. p16 and vimentin (32, 33). Previous studies found that p16INK4a had failed in distinguishing ECA and its expression was also related to a carcinogenesis mechanism involving HPV infection. p16 expression was observed in (55%) 80% of ECA while it was positive in 20% of EMC, and was correlated with HPV infection These results were in keeping with the published data which have shown that p16 immunostaining has a sensitivity for HPV infection-related ECA (range 82-100%) (39), but was less specific than villin and Pro-Ex-C expressions. Conflicting observations on the sensitivity of vimentin in EMC and ECA have been reported. Khoury et al. reported that vimentin was positive in 1 of 14 (7%) ECA, and 9 of 18 (50%) EMC (10), while McCluggage et al. found that vimentin was detected in 29/30 (96.7%) of EMC, and in 2/26 (7.7%) of ECA (40)

In conclusion, highly specific biomarkers such as villin and Pro-Ex-C have the potential to improve the diagnostic accuracy in differentiating between EMC and ECA, NOS. Based on the above mentioned data, the optimal approach to distinguishing between ECA and endocervical adenocarcinomas, NOS would be to use Pro-Ex-C, a hormone receptor marker (ER or PR), and villin as this marker panel's expression had a sensitivity of 100% and a specificity of 100% which is higher than conventional markers like p16 and vimentin that lead to many conflicting results, lower sensitivity and specificity. We also found significant positive correlations between villin and Pro-Ex-C expression, tumor size and grade, and presence and pattern of stromal invasion which highlighted the possible predictive roles of these markers in ECA and EMC. On the other hand we found no significant correlations between villin and Pro-Ex-C expression, FIGO stage and the presence of distant metastases, possibly due to the small sample size and inclusion of only ECA and EMC. We recommend conducting another study that will include a large number of patients to prove and highlight the predictive roles of marker expression in both carcinoma types. Due to the rarity of cervical adenocarcinoma subtypes, we have included only cases of cervical adenocarcinoma, NOS in our study and we have recommended conducting a future study on such subtypes to assess the relations between our marker expression pattern and aggressiveness of such subtypes. It is recommended to perform another study adding the more conventional markers such as p16 and vimentin to compare our results, sensitivity and specificity using larger sample size and all subtypes of ECA and EMC for adequate interpretation.

CONFLICT of INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin. 2014;64: 9-29.
2. WHO/ICO Information Centre on HPV and Cervical Cancer (HPV Information Centre). Human papillomavirus and related cancers in Egypt. Summary Report 2010. http://www.apps. who. int/hpvcentre/statistics/dynamic/ico/country_pdf/EGY.pdf?CFID=4005139&CFTOKEN=50211239.
3. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer. 2010; 127:2893-917.
4. Hoffman BL, Schorge JO, Schaffer JI, Halvorson L, Bradshaw K, Cunningham F. Endometrial cancer. Williams Gynecology. 2nd ed. McGraw-Hill, 2012. 817-23.
5. Salim EI, Moore MA, Al-Lawati JA, Al-Sayyad J, Bazawir A, Bener A, Corbex M, El-Saghir N, Habib OS, Maziak W, Mokhtar HC, Seif-Eldrin IA, Sobue T. Cancer epidemiology and control in the Arab World-past, present and future. Asian Pacific Journal of Cancer Prevention 2009;10:3-16.
6. Park KJ, Kiyokawa T, Soslow RA, Lamb CA, Oliva E, Zivanovic O, Juretzka MM, Pirog EC. Unusual endocervical adenocarcinomas: An immunohistochemical analysis with molecular detection of human papillomavirus. Am J Surg Pathol. 2011;35:633-46.
7. Houghton O, Jamison J, Wilson R, Carson J, McCluggage WG. p16 immunoreactivity in unusual types of cervical adenocarcinoma does not reflect human papillomavirus infection. Histopathology. 2010;57:342-50.
8. Pirog EC, Kleter B, Olgac S, Bobkiewicz P, Lindeman J, Quint WG, Richart RM, Isacson C. Prevalence of human papillomavirus DNA in different histological subtypes of cervical adenocarcinoma. Am J Pathol. 2000; 157:1055-62.

9. Kurman R, Carcangiu ML, Herrington CS, Young RH. WHO Classification of Tumours of Female Reproductive Organs. Lyon:IARC Press, 2014.
10. Khoury T, Tan D, Wang J, Intengan M, Yang J, Alrawi S, Yan P, Byrd J. Inclusion of MUC1 (Ma695) in a panel of immunohistochemical markers is useful for distinguishing between endocervical and endometrial mucinous adenocarcinoma. BMC Clin Pathol 2006; 12(6):1-9.
11. Nakamura E, Iwakawa M, Furuta R , Ohno T, Satoh T, Nakawatari M, Ishikawa K, Imadome K, Michikawa Y, Tamaki T, Katoh S, Kitagawa T, Imai T. Villin1, a novel diagnostic marker for cervical adenocarcinoma. Cancer Biol Ther. 2009;8:1146-53.
12. Patnaik S, George SP, Pham E, Roy S, Singh K, Mariadason JM, Khurana S. By moonlighting in the nucleus, villin regulates epithelial plasticity.Mol Biol Cell. 2016;27:535-48.
13. Lhocine N, Arena ET, Bomme P, Ubelmann F, Prévost MC, Robine S, Sansonetti PJ. Apical invasion of intestinal epithelial cells by Salmonella typhimurium requires villin to remodel the brush border actin cytoskeleton. Cell Host Microbe. 2015;17:164-77
14. Conesa-Zamora P, Doménech-Peris A, Ortiz-Reina S, Doménech-Peris A, Orantes-Casado FJ, Pérez-Guillermo M, Egea-Cortines M. Immunohistochemical evaluation of ProEx C in human papillomavirus-induced lesions of the cervix. J Clin Pathol. 2009;62:159-62.
15. Kamoi S, AlJuboury MI, Akin MR, Silverberg SG. Immunohistochemical staining in the distinction between primary endometrial and endocervical adenocarcinomas: Another viewpoint. Int J Gynecol Pathol. 2002;21:217-23.
16. Lewin SN, Herzog TJ, Barrena Medel NI, Deutsch I, Burke WM, Sun X, Wright JD. Comparative performance of the 2009 international federation of gynecology and obstetrics' staging system for uterine corpus cancer. Obstet Gynecol. 2010; 116(5):1141-9.
17. Diaz De Vivar A, Roma AA, Park KJ, Alvarado-Cabrero I, Rasty G, Chanona-Vilchis JG, Mikami Y, Hong SR, Arville B, Teramoto N, Ali-Fehmi R, Rutgers JK, Tabassum F, Barbuto D, Aguilera-Barrantes I, Shaye-Brown A, Daya D, Silva EG. Invasive endocervical adenocarcinoma: Proposal for a new pattern-based classification system with significant clinical implications: A multi-institutional study. Int J Gynecol Pathol 2013;32:592-601.
18. Roma AA, Diaz De Vivar A, Park KJ, Alvarado-Cabrero I, Rasty G, Chanona-Vilchis JG, Mikami Y, Hong SR, Teramoto N, Ali-Fehmi R, Rutgers JK, Barbuto D, Silva EG. Invasive endocervical adenocarcinoma: A new pattern-based classification system with important clinical significance. Am J Surg Pathol. 2015;39:667-72.
19. Hsu SM, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem. 1981;29:577-80.
20. Yu H, Liu Q, Xin T, Xing L, Dong G, Jiang Q, Lv Y, Song X, Teng C, Huang D, Li Y, Shen W, Teng C, Jin Y, Zhang F. Elevated expression of hyaluronic acid binding protein 1 (HABP1)/P32/C1QBP is a novel indicator for lymph node and peritoneal metastasis of epithelial ovarian cancer patients. Tumor Biol. 2013;34:3981-7.
21. Zhao J, Liu T, Yu G, Wang J. Overexpression of HABP1 correlated with clinicopathological characteristics and unfavorable prognosis in endometrial cancer. Tumour Biol. 2015; 36(2):1299-1306.
22. Kong CS, Beck AH, Longacre TA. A panel of 3 markers including p16, Pro-Ex-C, or HPV ISH is optimal for distinguishing between primary endometrial and endocervical adenocarcinomas. Am J Surg Pathol.2010;34(7):915-26.
23. Engelsen IB, Stefansson IM, Akslen LA, Salvesen HB. GATA3 expression in estrogen receptor alpha-negative endometrial carcinomas identifies aggressive tumors with high proliferation and poor patient survival. Am J Obstet Gynecol. 2008;199:543.e1-7.
24. Yemelyanova A, Vang R, Seidman JD, Gravitt PE, Ronnett BM. Endocervical adenocarcinomas with prominent endometrial or endomyometrial involvement simulating primary endometrial carcinomas: Utility of HPV DNA detection and immunohistochemical expression of p16 and hormone receptors to confirm the cervical origin of the corpus tumor. Am J Surg Pathol. 2009;33(6):914-24.
25. Xi L, Gooding W, McCarty K, Godfrey TE, Hughes SJ. Identification of mRNA markers for molecular staging of lymph nodes in colorectal cancer. Clin Chem. 2006;52:520-3.
26. Khurana S, George SP. Regulation of cell structure and function by actin-binding proteins: Villin's perspective. FEBS Lett. 2008;582:2128-39.
27. Moll R, Robine S, Dudouet B, Louvard D. Villin: A cytoskeletal protein and a differentiation marker expressed in some human adenocarcinomas. Virchows Arch B Cell Pathol Incl Mol Pathol. 1987;54:155-69.
28. Wang Y, Srinivasan K, Siddiqui MR, George SP, Tomar A, Khurana S. A novel role for villin in intestinal epithelial cell survival and homeostasis. J Biol Chem. 2008; 283:9454-64.
29. Al-Maghrabi J, Gomaa W, Buhmeida A, Al-Qahtani M, Al-Ahwal M. Loss of villin immunoexpression in colorectal carcinoma is associated with poor differentiation and survival. ISRN Gastroenterol. 2013;2013:679724.
30. Fletcher AH, Barklow TA, Murphy NJ, Culbertson LH, Davis AV, Hunter L. Pro-Ex-C triage of atypical glandular cells on liquid-based cervical cytology specimens. J Low Genit Tract Dis. 2011;15:6-10.
31. Esheba G. ProExC is a novel marker for distinguishing between primary endometrial and endocervical adenocarcinomas. J Egypt Natl Canc Inst. 2013;25:87-93.
32. Aximu D, Azad A, Ni R, Colgan T, Colgan T, Nanji S. A pilot evaluation of a novel immunohistochemical assay for topoisomerase II alpha and minichromosome maintenance protein 2 expression (Pro-Ex-C) in cervical adenocarcinoma in situ, adenocarcinoma, and benign glandular mimics. Int J Gynecol Pathol. 2009;28:114-9.
33. Guo M, Baruch AC, Silva EG, Jan YJ, Lin E, Sneige N, Deavers MT. Efficacy of p16 and Pro-Ex-C immunostaining in the detection of high grade cervical intraepithelial neoplasia and cervical carcinoma. Am J Clin Pathol. 2011; 135:212-20.

34. Semczuk A, Stenzel A, Baranowski W, Rozynska K, Cybulski M, Kostuch M. Detection of human papilloma virus types 16 and 18 in human neoplastic endometrium: lack of correlation with established prognostic factors. Oncol Rep. 2000;7:905-10.
35. Brown CA, Bogers J, Sahebali S, Depuydt CE, De Prins F, Malinowski DP. Role of protein biomarkers in the detection of high-grade disease in cervical cancer screening programs. J Oncol. 2012;2012:289315.
36. Badr RE, Walts AE, Chung F, Bose S. BD ProEx C. a sensitive and specific marker of HPV-associated squamous lesions of the cervix. Am J Surg Pathol. 2008;32:899-906.
37. Konishi I, Fujii S, Nonogaki H, Nanbu Y, Iwai T, Mori T. Immunohistochemical analysis of estrogen receptors, progesterone receptors, Ki-67 antigen, and human papillomavirus DNA in normal and neoplastic epithelium of the uterine cervix. Cancer. 1991; 68:1340-50.
38. Socolov D, Socolov R, Lupascu IA, Rugina V, Gabia O, Garauleanu DM, Carauleanu A. immunohistochemistry in endometrial hyperplasia and endometrial adenocarcinoma Rev Med Chir Soc Med Nat Iasi. 2016;120:355-62.
39. Liang J, Mittal KR, Wei JJ, Yee H, Chiriboga L, Shukla P. Utility of p16INK4a, CEA, Ki67, p53 and ER/PR in the differential diagnosis of benign, premalignant and malignant glandular lesions of the uterine cervix and their relationship with Silverberg scoring system for endocervical glandular lesions. Int J Gynecol Pathol. 2007;26:71-5.
40. McCluggage WG, Jenkins D. P16 immunoreactivity may assist in the distinction between endometrial and endocervical adenocarcinoma. Int J Gynecol Pathol. 2003;22:231-5.