

Histomorphological features and IHC expression in endometrial and endocervical adenocarcinoma



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ABSTRACT

Background: The distinction of endometrial adenocarcinoma (EMA) from endocervical adenocarcinoma (ECA) has great significance preoperatively, since the therapeutic plan depends on the primary site of tumor. Immunohistochemistry (IHC) is an ancillary method in distinguishing primary EMA and ECA, especially in cases of histomorphological overlap.

Aims and Objectives: Primary objective of this study was to describe the expression of IHC markers—estrogen receptor (ER), vimentin, monoclonal carcinoembryonic antigen (CEA), P16 in EMA and ECA. Secondary objective of this study was to describe the histomorphological features of EMA and ECA. **Materials and Methods:** This is a descriptive study done at the Department of Pathology, Government Medical College, Kottayam between November 2019 and May 2021. The sample size was 54 which included primary adenocarcinoma diagnosed on curettage biopsies. IHC was performed using ER, Vimentin, P16, and mCEA. A semiquantitative scoring was done using the German IHC scoring system. **Results:** Histomorphological features and IHC expression in EMA and ECA were studied. Percentage of cases with expression of IHC markers is as follows. In endometrioid EMA-ER in 100% of cases, Vimentin 88.4%, P16 0%, and CEA in 7.3% cases. About 80% of serous carcinomas showed P16 and vimentin positivity with negative staining for ER and CEA. In ECA-ER in 0% cases, Vimentin 33.3%, P16. 83.3%, and CEA in 66.7% of cases. Based on histomorphologic appearance, the common histologic subtype of EMA was endometrioid EMA followed by serous carcinoma. In ECA, the usual type was more common than gastric type. **Conclusion:** IHC markers-ER, vimentin, P16, and CEA are useful in distinguishing endometrioid EMA and ECA.

Key words: Endometrial adenocarcinoma; Endocervical adenocarcinoma; Serous carcinoma; Estrogen receptor; Vimentin; P16; Carcinoembryonic antigen

INTRODUCTION

Endometrial carcinomas and cervical carcinomas are the most common gynecological malignancy in developed and developing countries respectively.¹ Both endometrial adenocarcinoma (EMA) and endocervical adenocarcinoma (ECA) are heterogeneous in its morphology, etiology, and clinical outcome. However, in practice, a small proportion of EMA and ECA show histomorphological overlap making it impossible to determine the primary site of tumor. Identifying the primary site of tumor, in small biopsy specimens, is of

utmost significance since the therapeutic plan, to a large extent, is predominantly based on the primary tumor. Surgical treatment of EMA is usually simple hysterectomy with or without resection of para-aortic lymph nodes. In contrast, ECA is usually managed by radical hysterectomy and pelvic lymphadenectomy or radiation.² Immunohistochemistry (IHC) staining is widely used to differentiate primary endometrial and ECA in cases of histomorphological overlap to identify the primary site of tumor. Several western studies have reported on the use of a panel of IHC markers-estrogen receptor (ER), vimentin, P16, and carcinoembryonic antigen (CEA)

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to distinguish between adenocarcinoma of endometrial and endocervical origin. In study conducted by Yanaranop et al., and McCluggage et al., EMA is characterized by ER positivity, vimentin positivity, monoclonal CEA negativity, and P16 negativity, whereas ECA usually exhibits converse pattern of staining-ER negativity, vimentin negativity, mCEA positivity, and P16 positivity.^{2,3} However, in India, studies with a panel of similar IHC markers were few on literature review and the present study may serve as a database for future comparison. The present study is aimed at describing the expression of IHC markers-ER, vimentin, mCEA, P16 in EMA, and ECA and to describe the histomorphological features of EMA and ECA.

MATERIALS AND METHODS

The present study was a descriptive study done at Government Medical College, Kottayam (study period of 18 months-November 2019–May 2021). The study was approved by the Institutional Ethics Committee (IRB no 109/2019). The sample size was 54. The sampling method was continuous sampling.

$$\text{Sample size was, } N = \frac{Z\alpha^2 pq}{d^2} = \frac{(1.96)^2 \times 93 \times 7}{(7 \times 7)} = 54$$

All study specimens were fixed in 10% neutral buffered formalin, processed, and embedded in paraffin. 3–4 micron thin sections were made and stained with hematoxylin and eosin stain. IHC was performed using ER (Rabbit monoclonal-ER-PR042), vimentin (Rabbit monoclonal-vimentin-PR075), P16 (mouse monoclonal-P16-MM1240), and CEA (mouse monoclonal-CEA-PM086) on these sections manually. Immunostaining was scored semiquantitatively using the German IHC scoring system, where the final immunoreactive score equals the product of percentage of positive cells times the average staining intensity. Percentage of positive cells were graded as: 0=negative, 1=up to 10% positive cells, 2=11–50%, 3=51–80%, and 4≥80%. Staining intensity was graded as: 0=negative, 1=weakly positive, 2=moderately positive, and 3=strongly positive (focally positive means 1–80% area staining, diffusely positive means 81–100% area staining). Score ranges from 0 to 12. An immune-reactive score of 4–was considered positive (at least moderately positive in at least 11–50% cells) for ER, vimentin, and CEA. Only a score of 12 was considered positive for P16. The data were entered in Microsoft Excel and further statistical analysis done using SPSS software 26. The following parameters were analyzed – age, diagnosis, subtype, grade, IHC expression of ER, vimentin, P16, and CEA and described as percentage and frequency.

Inclusion criteria

Cases diagnosed histologically as adenocarcinoma in curettage biopsies were included in the study.

Exclusion criteria

Cases diagnosed as metastatic adenocarcinoma, inadequate tissue sample for IHC study were excluded from the study.

RESULTS

Among the 54 cases, 88.9% (48/54) cases were EMAs and 11.1% (6/54) were ECAs. Cases ranged from ages of 42 to 85 years with mean age of presentation being 57 years for EMA and 60 years for ECA. About 89.6% (43/48) cases of EMAs were endometrioid type and 10.4% (5/48) were serous type. About 69.8% (30/43) cases of endometrioid EMA were Grade I, 20.9% (9/43) Grade II, and 9.3% (4/43) Grade III. The most common histological pattern seen in Grades I and II endometrioid EMA was back to back glandular pattern (80%, n=39). Twelve cases of glandular pattern also showed squamous differentiation. About 83% (5/6) cases of ECA were usual type and the rest 17% (1/6) was gastric type. About 66.6% (4/6) cases of ECAs were Grade I, 16.7% (1/6) Grade II, and 16.7% (1/6) Grade III. Endometrioid EMA expressed 100% ER, 88.4% vimentin, 0% P16, and 7.3% CEA positivity (Figure 1). ECA showed 0% ER, Vimentin 33.3%, 83.3% P16, and 66.7% CEA positivity (Figure 2). About 80% serous carcinoma showed P16 and vimentin positivity. None of them expressed ER and CEA (Figure 3).

DISCUSSION

The distinction of EMA from ECA is clinically significant especially in pre-operative small biopsies because the definite treatment of EMAs and ECAs is different. Immunohistochemistry is as an ancillary method to distinguish EMA from ECA in cases of histomorphological overlap.² The use of a panel of markers, rather than a single marker, is encouraged. This is because IHC analysis can produce non-specific positive and negative staining reaction

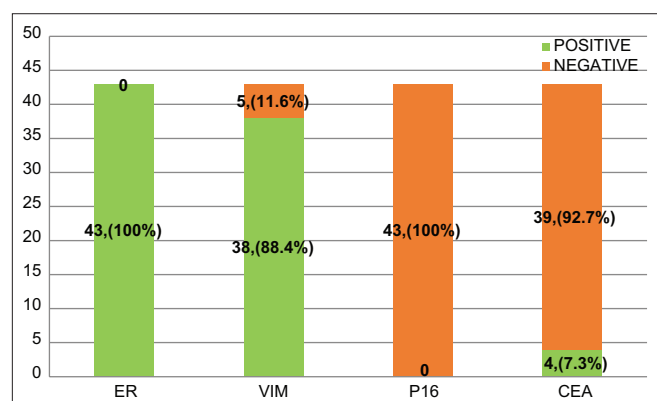


Figure 1: Immunohistochemistry expression in endometrioid endometrial adenocarcinoma

usually with an individual marker, but use of a panel of markers will be helpful in reaching a conclusive diagnosis.

In the present study, endometrioid EMA expressed 100% ER and 88.4% vimentin positivity (Figure 4). Being a sex hormone dependent malignancy, endometrioid EMA expressed ER. The pattern of expression being nuclear. Vimentin is an intermediate filament present in normal proliferative endometrial cells and in majority of endometrioid carcinomas. Its expression is cytoplasmic with basolateral accentuation. In vimentin negative cases, the diagnosis of endometrioid adenocarcinoma was merely based on ER status.⁴ None of the endometrioid EMA expressed P16 as they are etiologically unrelated to high-risk human papillomavirus (HPV) and display heterogenous/ patchy P16 expression. To quantify as positive, P16 must be expressed in more than 80% of the cells with a strong nuclear and cytoplasmic positivity.⁵ CEA was negative in 92.7% cases of endometrioid EMA (Figure 1). Endometrial columnar cells contain small amount of mucosubstances located chiefly at their apical border and as a result in EMA, CEA have a focal, apical expression.⁶ Findings in the present study were comparable to study conducted by Marut et al., and McCluggage et al.^{2,3} (Table 1).

About 83.3% cases of ECAs were P16 positive (Figure 5). P16 known as INK4a or cyclin-dependent kinase inhibitor 2A is a tumor suppressor protein. P16 inhibits cyclin-dependent kinase, involved in cell cycle regulation and progression. The expression of P16 is regulated by retinoblastoma (Rb) gene. About 90% of all adenocarcinoma of cervix are HPV associated. The HPV viral E7 oncoprotein inactivates Rb. Therefore, P16 is overexpressed in HPV-associated intraepithelial dysplasia and malignancies. In routine immunohistochemistry, P16 reveals cytoplasmic and nuclear staining pattern and the intensity of stain correlates with grade of HPV infection.⁵ In the present study, out of the total six cases of ECA, five cases were usual type and one case was gastric type. Usual type is HPV dependent and shows overexpression of P16. Gastric type being HPV independent is p16 negative.

None of the ECAs expressed ER as they are ER independent.⁷ About 66.7% CEA positivity seen in ECAs (Figure 2). CEA is normally detected in the glycocalyx of fetal epithelial cells. Normal endocervical epithelium contains large amount of intracellular acidic mucosubstances. Neoplastic endocervical epithelium retains many of the properties of normal epithelium. Thus, majority of ECA show diffuse cytoplasmic CEA expression.⁶ In the present study, 33.3% vimentin positivity seen in ECA. Findings in the present study were in concordance with the study of Marut et al., and McCluggage et al. (Table 2).

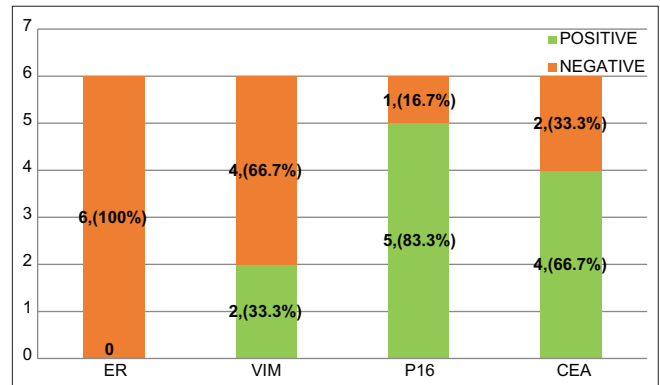


Figure 2: Immunohistochemistry expression in endocervical adenocarcinoma

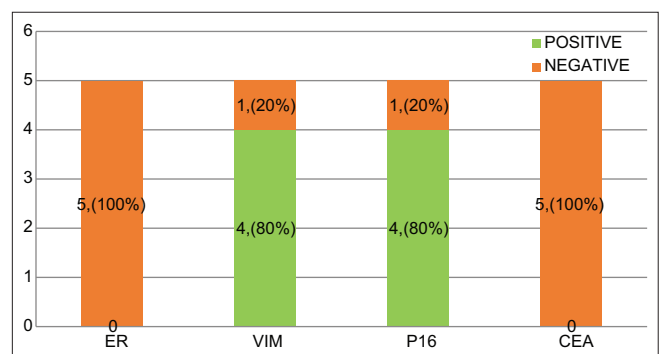


Figure 3: Immunohistochemistry expression in serous carcinoma

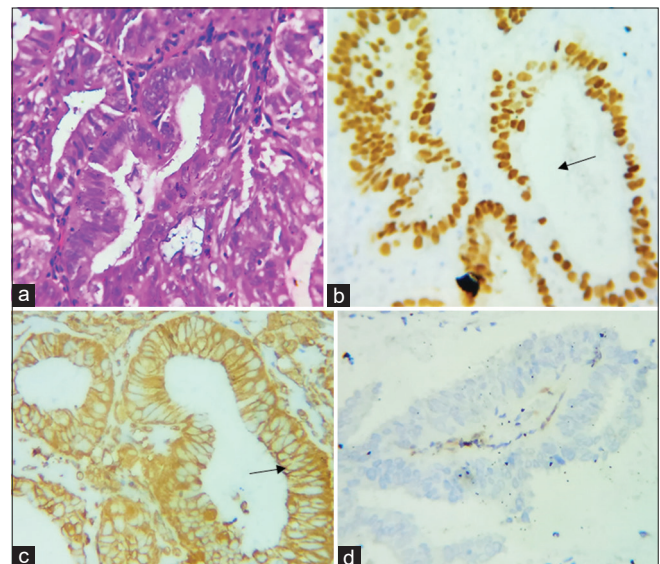


Figure 4: (a) Grade I Endometrioid EMA (H and E, $\times 40$), (b) immunohistochemistry (IHC) ER-positive nuclear staining, $\times 40$, (c) IHC vimentin-positive cytoplasmic staining with basolateral membrane accentuation, $\times 40$, and (d) IHC P16- Negative staining

For the distinction of EMA from ECA, the most useful marker panel depends on which subtypes of EMA and ECA are being considered in the differential diagnosis. The commonly encountered difficulty in histopathology is in the distinction of endometrial EMA from high-risk

Table 1: Immunohistochemistry expression of estrogen receptor, vimentin, P16, and carcinoembryonic antigen in endometrioid endometrial adenocarcinoma with other studies

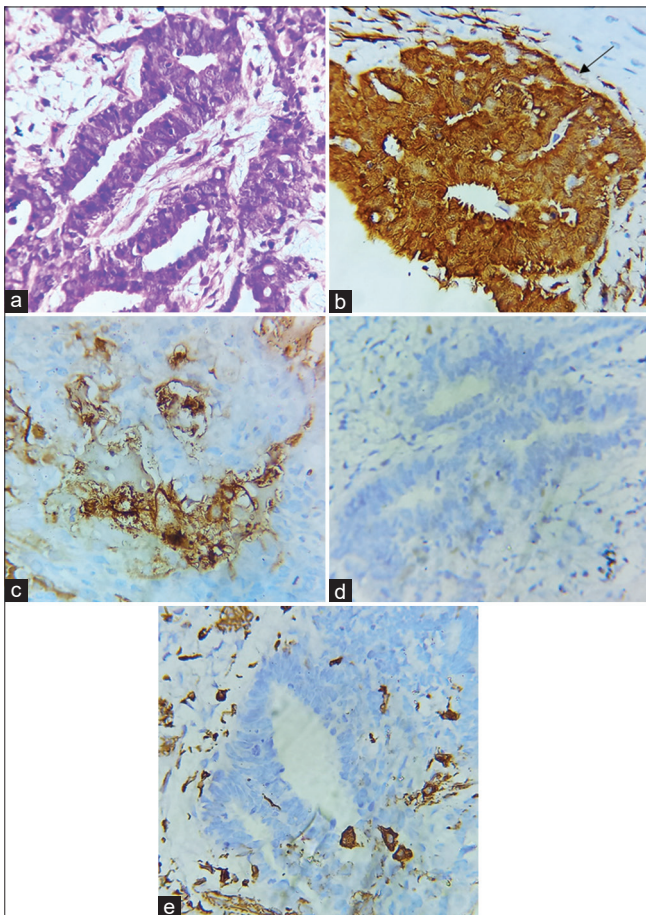
IHC expression	Present study 2019, n=54 (%)	McCluggage et al. ³ 2002, n=52 (%)	Marut et al. ² 2016, n=110 (%)
ER positive	100	93	79.3
Vimentin positive	88.4	97	84.5
P16 negative	100	-	87.9
CEA negative	92.7	30	79.3

IHC: Immunohistochemistry, ER: Estrogen receptor, CEA: Carcinoembryonic antigen

Table 2: Immunohistochemistry expression of estrogen receptor, vimentin, P16, and carcinoembryonic antigen in endocervical adenocarcinoma with other studies

IHC expression	Present study 2019, n=54 (%)	McCluggage et al. ³ 2002, n=52 (%)	Marut et al. ² 2016, n=110 (%)
ER negative	100	62	97.4
Vimentin negative	66.7	92	97.4
P16 positive	83.3	-	94.9
CEA positive	66.7	96	89.7

IHC: Immunohistochemistry, ER: Estrogen receptor, CEA: Carcinoembryonic antigen

**Figure 5:** (a) Grade I Endocervical adenocarcinoma H and E, $\times 40$, (b) immunohistochemistry (IHC) P16-strong and diffuse nuclear and cytoplasmic staining, $\times 40$ (c) IHC CEA-Focal cytoplasmic staining, (d) IHC ER-negative staining, and (e) IHC vimentin- negative staining

HPV-related ECA. A panel of IHC markers comprising P16, ER, vimentin, and CEA has been shown to be helpful in supporting the diagnosis in this context.⁸ The vast

majority (approximately 90%) of ECA are HPV-related and exhibit diffuse strong p16 expression due to molecular mechanisms by which high-risk HPV transforming proteins (E6 and E7) interact with cell cycle regulatory proteins (p53, Rb) to generate a futile feedback loop resulting in p16 overexpression. These high-risk HPV-related ECA (usual type) typically lose hormone receptor expression. Some have retained ER expression but is weaker and patchy when compared with the typically strong expression in normal endometrial glands.⁸

Yemelyanova et al., found CEA and vimentin to be of some value in the distinction of high-risk HPV-related ECA from endometrioid EMA. Most endometrioid EMA is vimentin positive and CEA negative and most HPV-related usual type ECA are vimentin negative and CEA positive.⁹

Distinction of high-risk HPV-related ECA from high-grade EMA (high-grade endometrioid and serous carcinoma) is based on hormone receptor, P16, and P53 expression. High-grade HPV-related ECA shows P16 overexpression, negative hormone expression, and wild type P53 expression. High-grade endometrioid EMA is usually ER positive, P16 variably positive (non-diffuse), and P53 wild type positive. Serous carcinomas are typically ER negative and show diffuse strong p16 expression and mutant type P53 expression.^{10,11}

In the present study, 80% serous carcinoma showed P16 and vimentin positivity. None of them expressed ER and CEA (Figure 3). Typical serous carcinoma lacks diffuse ER expression. Diffuse/strong p16 and mutant type p53 expression is characteristic of serous carcinoma. Like endometrioid carcinomas, endometrial serous carcinomas commonly express vimentin and lack diffuse, strong cytoplasmic expression

Table 3: Comparison of immunohistochemistry expression in serous carcinoma with similar study

Comparison	ER expression		Vimentin expression		P16 expression		CEA expression	
	ER positive	ER negative	Vimentin positive	Vimentin negative	P16 positive	P16 negative	CEA positive	CEA negative
Present study 2016, n=5 (%)	0	100	80	20	80	20	0	100
Michelle et al., ¹¹ 2006, n=24 (%)	54	46	83	17	92	8	13	87

ER: Estrogen receptor, CEA: Carcinoembryonic antigen

of CEA.¹² Findings in the study were comparable to study conducted by Reid-Nicholson et al.¹¹ (Table 3).

Limitations of the study

Small sample size especially in case of ECA when compared to EMA, HPV status in P16 positive cases of ECA could not be confirmed by deoxyribonucleic acid-polymerase chain reaction or *in situ* hybridization. In the present study, even though p53 was not included in the IHC panel, the diagnosis of serous carcinoma was confirmed by performing IHC for p53. Rare histological types of endometrial carcinoma such as clear cell carcinoma and carcinosarcoma were not studied due to small sample size. Since some IHC markers showed aberrant expression, molecular genetic study is required for definite typing.

CONCLUSION

The present study was done to describe the expression of a panel of four IHC markers-ER, vimentin, P16, and mCEA in EMA and ECA and to study the histomorphological features seen in each carcinoma. The endometrioid EMA expressed 100% ER positivity, 88.4% vimentin positivity in contrast to 0% ER and 33.3% vimentin positivity of ECA. On the other hand, ECA showed 83.3% P16 positivity, 66.7% CEA positivity in contrast to 0% P16, and 7.3% CEA positivity of endometrioid EMA. Hence, IHC markers-ER, vimentin, P16, and CEA are useful in distinguishing endometrioid EMA and ECA in cases of histomorphological overlap to identify the site of origin. This distinction is significant as the treatment of EMA and ECA are different.

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Authors' Contributions:


AMS- Concept and design of study, literature survey, data collection, result analysis and interpretation, manuscript preparation and submission of article; **SMV-** Concept and design of study, reviewed the literature, result analysis and interpretation, manuscript preparation; **LV-** Concept and design of the study, guiding the progress of the study, reviewed result analysis and interpretation; **SS-** Concept and design of the study, reviewed statistical analysis and interpretation, guidance and support.

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