ORIGINAL ARTICLE

Evaluation of Morphological and Immunohistochemical Patterns Associated with MELF Type of Myoinvasion in Type I Endometrial Carcinomas

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Received on: 21 April 2022; *Accepted on*: 23 August 2022; *Published on*: 16 November 2022

Abstract

Introduction: Endometrial carcinomas constitute a variety of tumors with varied morphology and clinical outcome. Though type I carcinomas were found to have better prognosis, subsequent studies have shown that 5–10% of type-I carcinomas may recur or metastasize, thus shortening the overall survival. This has led to the search of additional factors which might predict adverse outcomes in these otherwise low-grade tumors. Several histological and immunohistochemical markers were studied and some of them were found to be significantly associated with lymph node metastasis, recurrence, and poor outcome.

Methods: In total, 50 cases of type-I, grade-1/2 endometrioid carcinomas were studied for microcystic, elongated, and fragmented (MELF) type of myoinvasion. About 23 cases showed MELF myoinvasion. The morphological and immunohistochemistry (IHC) pattern associated with MELF were studied. Their association with lymph node metastasis and survival was also noted. IHC done were cytokeratin (CK), CD44, progesterone receptor (PR), E-cadherin, and smooth muscle actin.

Results: Large tumor size, papillary pattern, and lymphovascular emboli (LVE) were associated with MELF. Among the IHC, expression of CD44 and loss of expression of PR and E-cadherin were found to be statistically significant. None of the cases showed lymph node metastasis on routine sections, however, ultrastaging was not done.

Conclusion: Morphological and IHC features differ between MELF-positive and -negative cases. There was no significant difference in survival between the MELF-positive and -negative cases.

Keywords: Endometrial cancer, Histopathology, Malignancy.

Journal of South Asian Federation of Obstetrics and Gynaecology (2022): 10.5005/jp-journals-10006-2122

Introduction

Endometrial carcinomas constitute a variety of tumors with varied morphology and clinical outcome.[1] The earlier classification was based on morphological features. Later it was found that these carcinomas have two distinct mechanisms of carcinogenesis, based on which these were categorized as type-I tumors, which are low-grade endometrioid, and mucinous tumors and type II, which included serous, grade-3 endometrioid, undifferentiated carcinomas, and carcinosarcomas. Though type-I carcinomas were found to have better prognosis, subsequent studies have shown that 5–10% of type-I carcinomas may recur or metastasize, thus shortening the overall survival.[2] This has led to the search of additional factors which might predict adverse outcomes in these otherwise low-grade tumors. Several histological and immunohistochemical markers were studied, and some of them were found to be significantly associated with lymph node metastasis, recurrence, and poor outcome.[1]

Papillary patterns with grade-2 nuclei, mucinous differentiation, microglandular, and villoglandular pattern are the histological patterns previously studied. Microcystic, elongated, and fragmented and single cell type of myoinvasion are other features which were found to be associated with lymphovascular (LV) emboli and lymph node metastasis.[3] Of these morphological features, several studies have shown MELF to be an important predictor of prognosis. The cells in the tumors showing MELF pattern of invasion are found to have a different immune profile when compared with the neoplastic cells in the MELF negative tumors. So also, cells in the invasive front with the MELF pattern may exhibit a different IHC expression when matched with the main tumor.[4] Among the markers studied in MELF are CD44 and CD133, for stem cell marking, estrogen receptor (ER), PR as hormone receptors, CK, epithelial membrane antigen (EMA) as epithelial markers and cadherin, fascin, galactin 3, cyclin D1 and p16 as epithelial–mesenchymal transition (EMT) markers.[5] We chose to study a panel including one epithelial marker CK, one mesenchymal marker smooth muscle actin (SMA),

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How to cite this article: Nair IR, Rajanbabu A, Ambikakumari S, *et al*. Evaluation of Morphological and Immunohistochemical Patterns Associated with MELF Type of Myoinvasion in Type I Endometrial Carcinomas. J South Asian Feder Obst Gynae 2022;14(5):505–509.

Source of support: Nil

Conflict of interest: None

one hormone receptor PR, one stem cell marker CD44, and also E-cadherin as EMT marker.[6]

Current standard of care for endometrial cancer pathology reporting does not include reporting of the invasive front pattern. But if the MELF pattern is found to be associated with lymph node metastasis and increased tumor recurrence, despite having an early stage at presentation, it should be searched for and included in the pathology reporting for endometrial carcinoma. Similarly, the morphological patterns significantly associated with MELF may also be incorporated in the pathology report. The decision regarding adjuvant treatment may also be changed considering the aggressive nature of these tumors.

This study attempts to compare the immunoprofile of MELF-positive and negative type-I endometrial carcinomas and to find out whether MELF-type invasion is associated with lymph node metastasis or recurrence in these patients. We also studied the association of morphological features like papillary pattern, mucinous differentiation, and grade-3 nuclei in endometrial cancer with MELF pattern and with disease-free survival.

Materials and Methods

This is a single-institution retrospective study conducted at an Oncology Center in South India. All patients with type-I grade-1 or -2 endometrial cancer, with myoinvasion, whose representative tumor tissue blocks and slides were available, were included. Clinical records of these patients diagnosed from June 2014 to May 2017, were reviewed to record the age, stage at presentation, and the type of surgery. On follow-up, any events in the form of lymph node/distant metastasis or local recurrence were noted. All the histological slides with endomyometrium showing tumor (mean 5 slides per case, range 3–8) were reviewed for myoinvasion. MELF pattern of invasion (Fig. 1), tumor size, papillary pattern within the tumor, grade 3 nuclei, mucinous differentiation, and LVE, if present, were recorded.

The representative sections of tumor with MELF pattern were subjected to immunohistochemical staining using the IHC markers – CK (DAKO, prediluted, clone-AE1/AE3), PR[Master Diagnostica(MD), Spain-prediluted, clone 16], SMA(DAKO-prediluted, clone-1A4), CD44(MD-prediluted, clone-SP37), and E-cadherin(DAKO-prediluted, clone-NCH 38). Cytoplasmic staining in 1% or more of cells was taken as positivity for CK and SMA. Unequivocal membranous staining was taken as positivity for E-cadherin (Fig. 2), CD44 (Fig. 3), and nuclear staining for PR (Fig. 4).

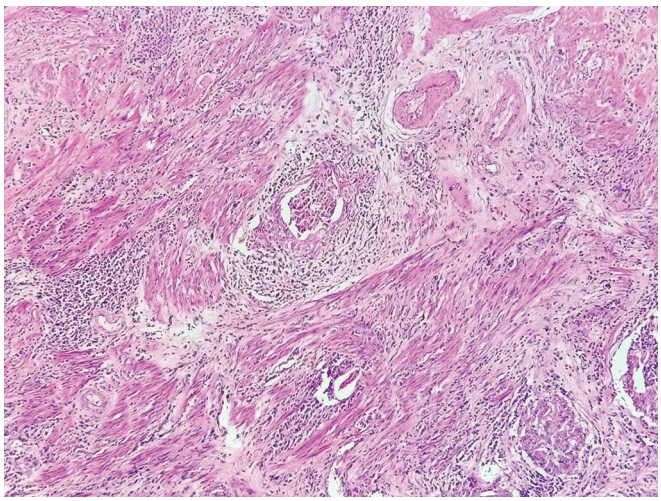

Fig. 1: MELF pattern H&E × 100

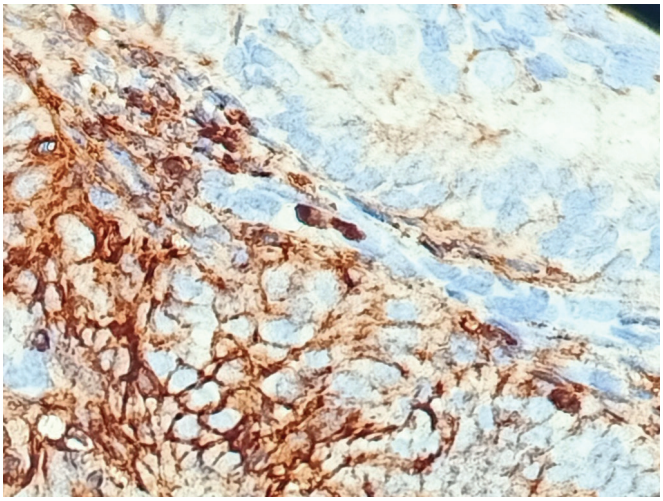

Fig. 2: E-cadherin IHC

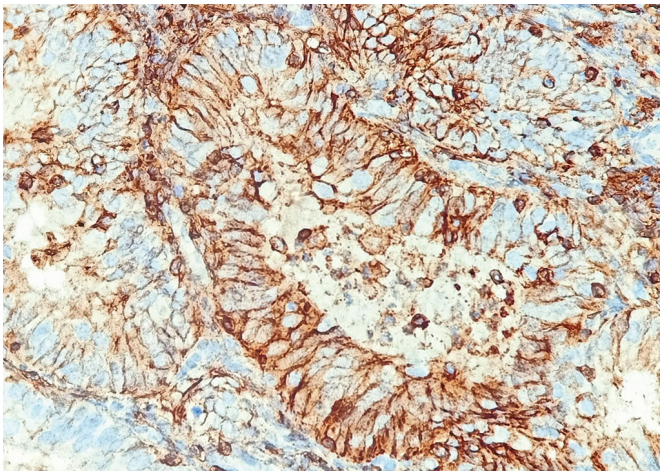

Fig. 3: CD44 IHC

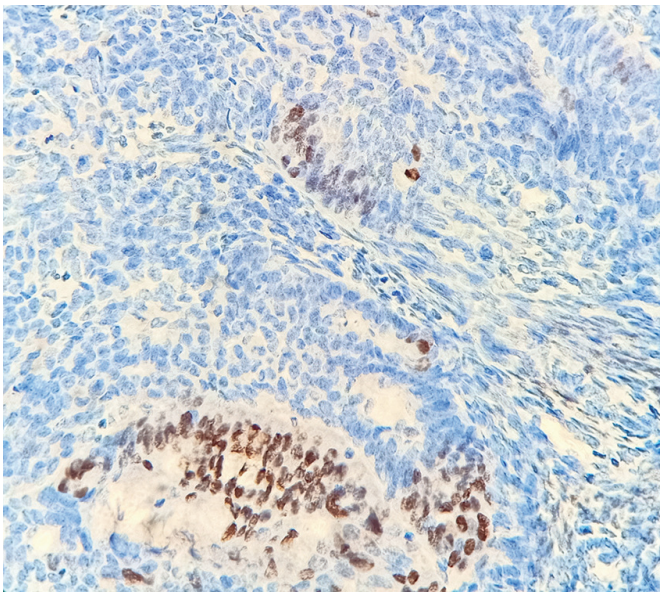

Fig. 4: PR IHC

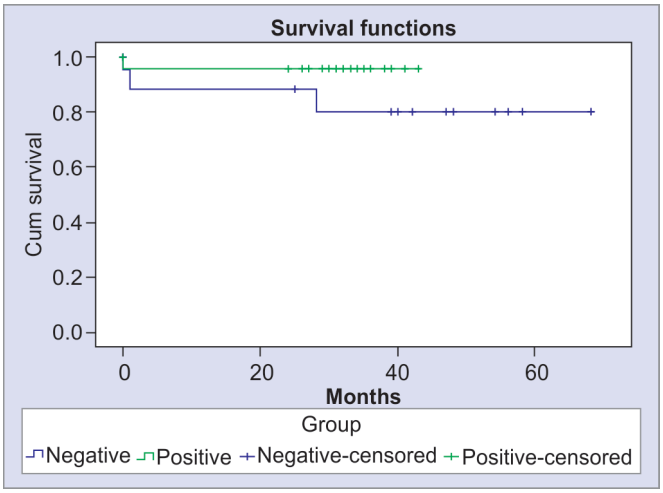

Fig. 5: Kaplan–Meier curve

A total of five high power field (HPF) were counted, and the mean percentage of positive cells was recorded. Intensity of staining was not taken into account.

The association of each of the morphological features with MELF pattern was analyzed using Chi-square test. The differences in IHC expression between MELF positive and negative cases were analyzed using Student's *t*-test. Survival curves of the two groups were plotted using Kaplan–Meier and compared using the log-rank test (Fig. 5).

The study was approved by the Institutional Ethics Committee, IEC-2018-077.

Results

A total of 50 cases whose representative tissue blocks and clinical follow-up were available, were included in the study. On morphological assessment and subsequent confirmation with the IHC for CK, 23 of them were found to have MELF pattern of invasion. All patients underwent hysterectomy with bilateral salpingo-oophorectomy. Sentinel node mapping with lymphadenectomy was done in all cases except 2, one 86-year-old lady where node could not be mapped and another patient who underwent primary surgery outside. None of the studied cases had lymph node metastasis on routine staging; however, ultra staging of the nodes was not done. Mean age at presentation was 52 years (range 29–83). We grouped the cases as MELF-positive and -negative ones. Morphological features studied included tumor size, papillary pattern, mucinous differentiation, LVE, and nuclear grade. Tumor size ranged from 1 to 4.6 (mean-2.7) cm in MELF-negative patients, whereas tumors were significantly larger in those associated with MELF (2–7.5, mean-3.8 cm) as indicated by a *p*-value of 0.009 in Student's *t*-test. Papillary pattern was found in 13 out of 23 MELF-positive cases and 7 out of 27 MELF-negative cases (Chi-square test, *p*-value = 0.027). Mucinous differentiation was noted only in 2 cases, each in both categories. Similarly grade 3 nuclei were found only in 3 cases with MELF pattern and 2 cases of the other category. Lymphovascular emboli was seen in 6 MELF-positive cases and 1 MELF-negative cases, thus exhibiting a statistically significant association (Chi-square value 0.023). Thus, the presence of large tumor, papillary pattern, and LV emboli showed a significant association with MELF pattern. None of the other morphological features showed a significant difference between the MELF-positive and -negative cases (Table 1).

Table 1: Difference in morphological characteristics between MELF-positive and -negative groups

Characteristics	*MELF positive (23)*	*MELF negative (27)*	*p-value Chi-square test*
Papillary pattern	13	7	0.027
Mucinous differentiation	2	2	>0.05
Grade 3 nuclei	3	2	>0.05
LV emboli	6	1	0.023

Table 2: Differences in clinical characteristics between MELF positive and negative groups

	MELF positive (23)	*MELF negative (27)*	*p-value Chi-square test*
Stage more than 1A	18	3	0.005
Events	1	2	

Table 3: Immunohistochemical markers in MELF positive and negative cases

IHC (as mean percentage)	*MELF positive*	*MELF negative*	*p-value Student's t-test*
CD44	49.8	12.3	0.002
PR	49.5	71.9	0.002
E-cadherin	58.3	73.9	0.028

In total, 18 out of 23 patients with MELF presented at a stage higher than IA, whereas only 3 of the 27 MELF-negative patients had a higher stage. Thus, MELF positivity bestowed a substantial association with higher stage at clinical presentation (Chi-square test, *p*-value <0.05) (Table 2).

Among the IHC markers done, CK showed uniform expression in carcinoma cells of the main tumor and also the cells in MELF pattern. There was no SMA expression in the tumor cells in both categories. For the other markers, E-cadherin, PR, and CD44, the percentage of positive cells in tumor were recorded. The pattern of expression in MELF area mimicked that of the main tumor cells.

In total, 10–90% (mean 49.8%) of cells showed CD44 positivity in the cases with MELF, while 0–60% of cells showed the expression in MELF-negative group, with an average of 12.3%. There is a significant overexpression of CD44 in MELF-positive group (*p*-value 0.002).

In MELF-negative group, PR expression ranged from 30 to 100%, with a mean of 71.9%. Among the MELF-positive group, it had a mean of 49.5% (range 0–90%). There is a significant loss of PR expression (*t*-test value is 0.002) in the MELF-positive group compared to the negative group.

E-cadherin-positive cells ranged from 10 to 100% with a mean of 73.9 in MELF-negative cases, whereas, it ranged from 0 to 90% in the MELF-positive cases with an average of 58.3% (Fig. 3). Thus, there was a significant loss of E-cadherin expression in the MELF-positive cases (Student's *t*-test value is 0.028) (Table 3).

Patients were followed up for a minimum period of 2 years (24–72, mean 41.5 months). Among the MELF-positive patients, 95.6% were disease-free, with one patient recurring at 22 months

with liver metastasis. About 92.5% of MELF-negative patients were free of disease (2 patients had events, one patient recurred at 10 months, omentum, liver, and the other at 16 months with metastasis in lung), with no significant difference in disease-free survival between the 2 groups (log-rank test, Fig. 5).

Discussion

There is heterogeneity in the clinical behavior of low-grade endometrial carcinomas. Studies have been done to identify the morphological and IHC parameters that could predict the clinical outcome. The pattern of myometrial invasion is considered to play a significant role in this. Among the 50 cases of grade-1 and -2 endometrial carcinomas we studied, 23 showed MELF pattern of invasion. Stewart et al. found that 15.9% of low-grade myoinvasive carcinomas show MELF.[7] This type of myoinvasion is considered to be caused by a transition from epithelial-to-mesenchymal cell type (EMT) and hence the preliminary step in lymph node metastasis and an aggressive behavior.[8]

We found statistically significant higher expression of CD44 in tumor cells in MELF-positive carcinomas as compared to MELF-negative cases. Epithelial-to-mesenchymal cell type refers to a change from the epithelial to mesenchymal phenotype. There is cellular junction disassembly, loss of cell adhesion, and change of shape of epithelial cells to spindle with possible motility. This EMT phenomenon is considered to be an initiator for myometrial invasion. Many cell-surface adhesive molecules are present, which cause cell-to-cell and cell-to-matrix adhesion and interactions, which maintain the tissue integrity, architecture, and function. They anchor the cells with each other and to the stroma, control cell mobility, and help in sensing the environmental changes. Among the different families of cell-adhesion molecules, CD44 is a hyaluronic acid receptor, seen in normal and stem cells, which is responsible for adhesion of cells to the extracellular matrix component. It is thought to play a role in the cell proliferation, survival, migration, and even angiogenesis. These functions are also exhibited by this molecule in a subset of cancer cells, also assisting in tumor progression and spread.[9] These are the cancer stem cells which influence the tumor initiation, migration, and chemoresistance in some tumors. CD44, along with CD24, CD133, CD166, and EpCAM are considered to be a reliable cell-surface markers for stem cells.[4] It induces carcinogenesis by varied mechanisms, most importantly, by cell migration and initiation of metastasis. The studies available in literature on CD44 expression in endometrial carcinomas show highly variable results. Overexpression of CD44 was reported by Liu et al. in high-grade carcinomas.[10] Similarly, Leblanc et al. have concluded that expression of CD44 is associated with increased depth of myoinvasion.[11] However, others, like Gun et al., could not find any relation between CD44 and tumor aggressiveness or grade.[12] Yadav et al. studied the role of CSC gene variants in gall bladder cancers. The contradicting results from diverse studies on CD44 expression in cancer aggressiveness may be explained by the possibility that the CD44 gene undergoes alternative splicing, so as to encode different proteins/variants in different cancers.[13]

We also found a diminished expression of the epithelial adhesion molecule cadherin among the tumor cells of MELF-positive cases. In EMT, there is an expression of transcription repressors (like Snail and Slug) of E-cadherin. This causes loss of this junction protein, along with expression of mesenchymal cadherins like N cadherin and others like SMA, vimentin, and fibronectin. Expression of matrix metalloproteinases (MMP2, MMP3, and MMP9) activity is also seen. These alterations cause loss of epithelial cell adhesion enabling the cancer cells to penetrate the stroma and metastasize.[11] However, we could not observe any SMA positivity in any of the tumor cells in both groups.

Similarly, PR expression was seen to be significantly lower in cells of MELF-positive tumors. Progressive loss of ER and PR have also been described, associated with EMT. Researches done in many tumors have shown that loss of progesterone expression correlates with tumor invasion, in concordance with our findings. It is also proved that progesterone can inhibit the Wnt/β-catenin pathway, which initiates tumor progression and invasion.[14]

Among the morphological patterns assessed, large tumor size, papillary pattern, and LV emboli showed statistically significant association with MELF type of invasion. Papillary pattern with grade-2 nuclei has been described to be associated with MELF pattern and myoinvasion by Malpica.[15] Other features like mucinous differentiation and grade-3 nuclei were not found to have any association with MELF in the present study.

An analysis of the disease-free survival at the end of 2 years did not reveal any significant difference between the MELF-positive and -negative groups. Several studies have shown myoinvasive patterns like MELF to be associated with higher rates of lymph node metastasis and disease recurrence.[16] However, we could not find any significant reduction in disease-free survival in cases with MELF myoinvasion. This is in concordance with the findings of Sanci et al.[17] This could be attributed to the lesser period of follow-up done. Ultrastaging of lymph nodes might also be required to reveal the cases with micro metastasis.[18]

Conclusion

We studied the morphological and IHC pattern of low-grade, type-I endometrioid carcinomas associated with MELF pattern of invasion. Large tumor size, papillary pattern, LV emboli, and higher stage (more than 1A) showed a significant association with MELF pattern of myoinvasion. The immunoprofile of tumor cells in cases with MELF showed higher expression of CD44, indicating stem cell characteristics in these cells. We also found diminished expression of E-cadherin in these patients denoting EMT. Significant loss of PR expression was also found. However, no lymph node metastasis or significant reduction in survival was found in MELF-positive cases. A longer follow-up with ultrastaging of lymph nodes would yield a better assessment of behavior of these carcinomas.

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